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## **Biomass Yield Potential and Herbage Quality of Alfalfa (*Medicago Sativa* L.) Genotypes in the Central Highland of Ethiopia**

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**Abstract:** Five alfalfa genotypes were evaluated for herbage yield potential and nutritional quality under supplemental irrigation condition at Holetta Agricultural Research Center during 2010-2014. The experiment was conducted in randomized complete block design with four replications. Diammonium phosphate (DAP) fertilizer at the rate of 100 kg/ha was uniformly applied at sowing. Combined analysis revealed that the tested genotypes varied significantly ( $P < 0.05$ ) for dry matter yield and plant height at forage harvesting. Accordingly, the highest mean dry matter yield was recorded for hairy peruvian (8.7 t/ha) followed by FG-10-09(F) (6.3 t/ha), Magna 801-FG(F) (6.2 t/ha), Magna 788 (5.6 t/ha) and FG-9-09 (F) (5.5 t/ha) genotypes. Similarly, hairy peruvian, FG-10-09 (F), Magna 801 FG (F), Magna 788 and FG-9-09(F) genotypes produced 77.7, 60.1, 55.6, 53.3 and 52.7cm plant height at forage harvesting respectively. Hairy peruvian gave the highest crude protein yield (1.7 t/ha) and digestible yield (6.5 t/ha) followed by FG-10-09(F) genotype, which produced 1.4 t/ha and 4.8 t/ha crude protein yield and digestible yield respectively. On the other hand, the lowest crude protein yield (1.2 t/ha) and digestible yield (4.0 t/ha) was recorded for FG-9-09(F) genotype. The highest relative feed value was recorded for Magna 788 (143.5% DM) genotype followed by FG-9-09(F) (137.6%DM), FG-10-09(F) (134.6%DM), Magna 801-FG(F) (132.9% DM) and hairy peruvian (122.3% DM) genotypes. Except in-vitro dry matter digestibility (IVDMD), the tested genotypes did not show significant ( $P > 0.05$ ) difference for all measured herbage quality traits. The result showed that Magna 801-FG(F) genotype had the highest ash (115.0 g/kg DM), IVDMD (757.7 g/kg DM) and hemicellulose (104.5 g/kg DM) contents. On the other hand, FG-9-09(F) genotype had the lowest NDF (416.9 g/kg DM), ADL (86.1g/kg DM) and hemicellulose (65.2g/kg DM) contents. The highest CP (226.6 g/kg DM) and the lowest ADF (319.9 g/kg DM) and cellulose (229.4 g/kg DM) contents were recorded for Magna 788 genotype. Similarly, the highest NDF (454.6 g/kg DM), ADF (376.6 g/kg DM), ADL (103.7 g/kg DM) and cellulose (279.0 g/kg DM) contents and the lowest ash (102.6 g/kg DM) and CP (199.2 g/kg DM) contents were recorded for hairy peruvian. Generally, alfalfa genotypes showed variations in terms of biomass yield potential and nutritional quality under supplemental irrigation condition at Holetta, in the central highland of Ethiopia.

**Keywords:** alfalfa genotypes, biomass yield, nutritional quality, plant height, relative feed value.

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### **INTRODUCTION**

Alfalfa (*Medicago sativa* L.) is often recognized as one of the most important perennial forage legumes worldwide and is widely known as the “queen of the forages” due to its ability to consistently produce high forage yield and forage quality and adaptability to different climatic conditions (Kamalak *et al.*, 2005; Turan *et al.*, 2009). Alfalfa is a drought tolerant forage crop because it has a deep root system that reaches down to 4 m and to 7-9 m in well drained soils. The plant survive long periods of water stress by impeding its vegetative growth (Annicchiarico *et al.*, 2010) and accessing water from deep layers through its long root system (Volaire, 2008). The optimum growing air and soil temperatures for alfalfa are 27°C and 12°C respectively, but it is tolerant of air temperatures above and below 27°C (McKenzie *et al.*, 1988). This forage legume is also known as an effective source of biological nitrogen fixation, an energy-efficient crop to grow and an important source of protein yield. The optimum soil pH range for alfalfa is 6.5 to 7.5 and tolerates relative salinity. Alfalfa grows best on well-drained, deep soils but it thrives on sandy soils with adequate moisture and fertility (Barnhart, 1997). Alfalfa does not grow well on soils where root growth is limited such as shallow hardpans, high water tables, bedrock or acidic sub soils (Lacefield *et al.*, 1987). Poorly

drained or waterlogged soils are strongly discouraged for alfalfa because root and crown diseases reduce stand longevity under these conditions. The crop needs very frequent irrigation during its early growth period at an interval of about one week but once the plants are established, subsequent irrigations are provided at an interval of 10-15 days during dry season. Alfalfa is one of the few cultivated forage crops that can produce high level of biomass with minimum inputs. Low quality crop residues need nitrogen supplementation, often provided by forage legumes to become productive diets (Anderson, 1985).

Climate, cultivation practices, feed technologies and genetic variations are the main factors affecting the nutritional value of feed for livestock. Forage legumes contribute significantly to livestock production in crop livestock systems. Legume forages generally lead to higher intakes and animal production than grass silages of comparable digestibility (Dewhurst *et al.*, 2003). Alfalfa nutritive value is identified with protein content which depends on the share of leaves in dry matter yield which in turn is positively correlated with protein content. The proportion of leaves and stems in alfalfa hay can vary greatly, depending on maturity at harvest, cultivars, handling and rain damage (Katic *et al.*, 2006). Protein content in alfalfa dry matter varies from 18 to 25% depending on the growth stage, cultivar difference and other factors. Alfalfa is one of the most important forage legumes of the world as major source of protein for livestock and it is a basic component in rations for all classes of domestic animals (Barnes *et al.*, 1988). Where alfalfa can easily be grown, it is regarded as key forage for high-producing ruminants because of its richness in protein, palatability, high calcium and vitamin content. In many cases animals feeding on alfalfa do not require supplements. Alemayehu (2002) noted that because of its very high feed value, alfalfa should be used as a supplement for crop residues and natural hay in mixture of 30 percent alfalfa and 70 percent other roughages. Alfalfa produces more protein per hectare than other legume and grasses; therefore, it is widely used for hay production and as pasture for livestock, especially to ruminants (Monteros and Bouton, 2009). Ruminants fed on alfalfa have higher nutrient intake and digestibility than when fed on other forage legumes and grasses (Frame, 2005). To improve availability of livestock feed in terms of quantity and quality, it is better to cultivate alfalfa forage that have better biomass yield and nutritional quality. Therefore, the objective of the present study was to evaluate the biomass yield potential and nutritional quality of alfalfa genotypes grown at Holetta under supplemental irrigation condition.

## **MATERIALS AND METHODS**

### **Descriptions of the Test Environment**

The experiment was conducted at Holetta Agricultural Research Center (HARC) under supplementary irrigation condition. HARC is located at 9°00'N latitude, 38°30'E longitude at an altitude of 2400 m above sea level. It is 34 km west of Addis Ababa on the road to Ambo and is characterized with the long term (30 years) average annual rainfall of 1055.0 mm, average relative humidity of 60.6% and average maximum and minimum air temperature of 22.2°C and 6.1°C respectively. The rainfall is bimodal and about 70% of the precipitation falls in the period from June to September, while the remaining thirty percent falls in the period from March to May (EIAR, 2005). The soil type of the area is predominantly red nitosol, which is characterized by an average organic matter content of 1.8%, total nitrogen 0.17%, pH 5.24 and available phosphorus 4.55ppm (Gemechu, 2007). The farming system of the study area is mixed crop livestock production where tef is the main staple crop complemented by other cereals such as barley and Wheat. In addition, faba bean, field pea and horticultural crops such as potato are the major crops growing in the area. The main feed resources in the area are natural pasture, crop residues and cultivated forage crops mainly oats/vetch mixture are grown by some farmers for their cross-bred dairy cows (HARC, 2009).

### **Experimental Design and Data Collection**

Four alfalfa genotypes, namely, FG10-09(F), FG9-09(F), Magna 801-FG(F), Magna 788 recent introductions from USA and hairy peruvian genotype were evaluated for five consecutive years of 2010-2015 under supplemental irrigation condition at Holetta in the central highland of Ethiopia. The plots were uniformly irrigated at field capacity in every 15 days during dry season of the year. The experiment was laid out in RCBD design replicated four times. Plot size was 4m x 1.6m= 6.4m<sup>2</sup> consisted of 8 rows with 1m inter-plot and inter-block space. The seed of the five alfalfa genotypes were sown on well-prepared seedbed in rows spaced 20 cm apart using a seed rate of 20 kg per hectare. Diammonium phosphate (DAP) fertilizer was applied at the rate 100 kg per hectare at planting. Plots were hand-weeded during the establishment and subsequent years. Plant height was measured using a steel tape from the ground level to the highest leaf at forage harvesting stage. For

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plant height determination, mean height of three randomly selected plants was recorded for each plot. At full bloom stage, described as a stage when open flowers emerge on average of 2 or more nodes and no seed pods present (Ball, 1998), six interior rows were clipped at 5cm above the ground level to determine the biomass yield. Weight of the total fresh biomass yield was recorded from each plot in the field and the estimated 500 g sample was taken from each plot to the laboratory. The sample taken from each plot was weighed to know the total sample fresh weight using sensitive table balance and oven dried for 24 hours at a temperature of 105°C for herbage DM yield determination. The herbage sample taken from each plot was weighed to know the total sample fresh weight using sensitive table balance and manually fractionated in to leaf and stem. The morphological parts were separately weighed to know their sample fresh weight, oven dried for 24 hours at a temperature of 105 °c and separately weighed to estimate the proportions of these morphological parts. Accordingly, leaves were separated from stems and the leaf to stem ratio (LSR) was estimated based on the dry matter basis of each component. Generally, maximum care was taken in the experimental plots to reduce the possible yield limiting factors which could affect the performance of yield potential of alfalfa genotypes.

### **Relative Feed Value**

Relative Feed Value (RFV) is an index used for legumes based on potential intake and fiber digestibility (Undersander and Moore, 2002). The index used to rank feeds relative to the typical nutritive value of full bloom alfalfa hay, containing 41% ADF and 53% NDF on a DM basis, and having a RFV of 100, which is considered to be a standard score. This index is widely used to compare the potential of two or more forages on the basis of energy intake (Schroeder, 2013). Accordingly, forages with RFV greater than 100 are considered to have better quality than full bloom alfalfa hay and those with RFV lower than 100 are regarded as of lower quality than the same. Such a single parameter is considered to be of useful practical significance in forage pricing and marketing (Uttam *et al.*, 2010; Schroeder, 2013) and, was calculated as:  $RFV = (DDM \% DM) \times (DMI \% BW) / 1.29$  (Uttam *et al.*, 2010), where DDM (digestible dry matter) and DMI (dry matter intake potential as % of body weight) were calculated from ADF and NDF, respectively as:  $DDM (\% DM) = 88.9 - (0.779 \times \% ADF)$ ;  $DMI (\% BW) = 120 / (\% NDF)$ .

### **Chemical Analysis and *In-Vitro* Organic Matter Digestibility**

Harvesting for chemical analysis was undertaken as the genotypes reached 50% flowering, as recommended for forage harvesting for alfalfa. The fresh weights of the samples were recorded, and they were then oven dried at a temperature of 65°C for 72 hours for dry matter determination and laboratory analysis to determine chemical composition and *in-vitro* organic matter digestibility of the genotypes. The dried samples then ground to pass a 1 mm sieve and used for laboratory analysis. Analysis was made for ash, CP, NDF, ADF, ADL and IVDMD nutritional parameters. Total ash content was determined by oven drying the samples at 105°C overnight and by combusting the samples in a muffle furnace at 550°C for 6 hours (AOAC, 1990). Nitrogen (N) content was determined following the micro-Kjeldahl digestion, distillation and titration procedures (AOAC, 1995) and the crude protein (CP) content was estimated by multiplying the N content by 6.25. The structural plant constituents (NDF, ADF and ADL) were determined according to Van Soest and Robertson procedure (1985). The two stage *in-vitro* fermentation technique of Tilley and Terry as modified by Van Soest and Robertson procedure (1985) was used to determine *in-vitro* organic matter digestibility (IVDMD). Hemicellulose and cellulose contents were estimated from subtracting ADF from NDF and ADL from ADF respectively. The CP yield in t/ha was calculated by multiplying CP with total dry biomass yield and then divided by 100. The digestible yield in t/ha was also determined by multiplying IVDMD with total DM yield and then divided by 100.

### **Statistical Analysis**

Differences among genotypes were tested using analysis of variance (ANOVA) procedures of SAS general linear model (GLM) to compare treatment means (SAS, 2002). Least significance difference (LSD) at 5% significance level was used for comparison of means. The following model was used for combined analysis:  $Y_{ijk} = \mu + G_i + Y_j + (GY)_{ij} + B_{k(j)} + e_{ijk}$ ; Where,  $Y_{ijk}$  = measured response of genotype i in block k of year j;  $\mu$  = grand mean;  $A_i$  = effect of genotype i;  $Y_j$  = effect of year j;  $GY$  = genotype by year interaction;  $B_{k(j)}$  = effect of block k in year j;  $e_{ijk}$  = random error effect of genotype i in block k of year j. For each year analysis, the model was:  $Y_{ij} = \mu + G_i + B_j + e_{ij}$ ; Where,  $Y_{ij}$  =

measured response of genotype  $i$  in block  $j$ ;  $\mu$  = grand mean;  $A_i$  = effect of genotype  $i$ ;  $B_j$  = effect of block  $j$ ;  $e_{ij}$  = random error effect of genotype  $i$  in block  $j$ .

## RESULTS AND DISCUSSION

### Combined Analysis of Variance

The combined analysis of variance over years showed significant differences among the tested genotypes and years for both measured agronomic traits (Table 1). Mean squares of genotypes and years were significantly ( $P < 0.001$ ) different for dry matter yield and plant height at forage harvesting stage. However, dry matter yield and plant height were not significantly ( $P > 0.05$ ) affected by interaction effects that indicates consistency of performance of genotypes over years. This was reflected by no change in ranking order of genotypes over years due to relatively uniform growing conditions (rainfall, irrigation water, temperature etc.) during the experimental years. The yielding ability of a genotypes is the result of its interaction with the environment. Environmental factors such as soil characteristics, moisture and temperature over years and locations have an impact on yield performance. There is strong influence of environmental factors during various stages of crop growth (Bull *et al.*, 1992), thus genotypes differ widely in their response to environments. Some genotypes exhibit highly specific response to a particular environment (soil, rainfall and temperature), others are uniform in performance over a range of environments. When genotypes perform consistently across environments, breeders are able to effectively evaluate genotypes with a minimum cost in a few environments for ultimate use of the resulting genotypes across wider geographic areas (Gemechu, 2012). However, with high genotype by environment interaction effects, genotypes selected for superior performance under one set of environmental conditions may perform poorly under different environmental conditions (Romagosa *et al.*, 1996; Ceccarelli, 1997). Therefore, it could be implicated that selection of better performing genotypes at one environment may not enable the identification of genotypes that can repeat nearly the same performances at another environments.

**Table 1.** Mean DM yield and plant height performance of alfalfa genotypes tested over years at Holetta research center.

Traits	Mean square			Mean
	Genotype	Year	Genotype x Year	
Dry matter yield (t/ha)	***	***	ns	6.46
Plant height (cm)	***	***	ns	59.88

### Plant Height at Forage Harvesting Stage

Plant height was not significantly differ ( $P > 0.05$ ) for the first two consecutive years, however it showed significant ( $P < 0.05$ ) variations for the remaining three consecutive years and for the overall mean at forage harvesting stage (Table 2). The highest mean plant height was recorded in the fourth year while the second year gave the lowest. The overall mean plant height ranged from 52.7 to 77.7 cm with a mean of 59.9 cm. Plant height was consistently the highest for hairy peruvian over years. The highest mean plant height was recorded for hairy peruvian followed by FG-10-09(F) and Magna 801 FG(F), while FG-9-09(F) had the lowest plant height. The significant cultivar differences for plant height concurs with other reports in the literature (Altinok and Karakaya, 2002; Sengul, 2002). In addition to genetic variability, soil fertility and environmental conditions could also contribute to the difference in height over years. Generally, presence of genetic variation among the tested genotypes, response of genotypes to environmental factors and their interactions are the major reason for plant height difference in alfalfa. Ullah *et al.* (2009) also reported variations in plant height to be linked to genotypic differences and explained this trait to be influenced by differential response of genotypes to prevailing site and crop management conditions. The research results indicated that plant height in alfalfa can be influenced by application of fertilizers (Dineshkumar, 2007). Research findings of Mohammadjanloo *et al.* (2009) indicated that, variety and the interaction between variety and fertilizer were an important agronomic factors influencing plant height in alfalfa.

**Table 2.** Mean plant height (cm) of alfalfa genotypes tested over years at Holetta research center

Genotypes	Years					Mean
	2010	2011	2012	2013	2014	
FG-10-09(F)	65.40	40.58	62.38 <sup>ab</sup>	81.43 <sup>ab</sup>	50.93 <sup>b</sup>	60.14 <sup>b</sup>
FG-9-09(F)	49.98	40.43	58.05 <sup>b</sup>	64.00 <sup>b</sup>	51.25 <sup>b</sup>	52.74 <sup>c</sup>
Magna 801 FG(F)	59.15	41.53	57.45 <sup>b</sup>	64.75 <sup>b</sup>	54.85 <sup>b</sup>	55.55 <sup>bc</sup>

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Magna 788	60.83	41.40	54.98 <sup>b</sup>	63.83 <sup>b</sup>	45.45 <sup>b</sup>	53.30 <sup>bc</sup>
Hairy peruvian	75.78	55.13	75.28 <sup>a</sup>	95.73 <sup>a</sup>	86.55 <sup>a</sup>	77.69 <sup>a</sup>
<b>Mean</b>	<b>62.23</b>	<b>43.81</b>	<b>61.63</b>	<b>73.95</b>	<b>57.81</b>	<b>59.88</b>
<b>SEM</b>	<b>0.75</b>	<b>0.88</b>	<b>0.66</b>	<b>0.80</b>	<b>0.72</b>	<b>0.34</b>
<b>P-value</b>	<b>0.0755</b>	<b>0.6186</b>	<b>0.0428</b>	<b>0.0145</b>	<b>0.0009</b>	<b>0.0001</b>

Means followed by a common superscript letters with in a column are not significantly different from each other at  $P < 0.05$ .

### Herbage Dry Matter, Crude Protein and Digestible Yields

Mean values for herbage dry matter (DM) yield of five alfalfa genotypes at forage harvesting stage are presented in Table 3. The herbage DM yield showed non-significant ( $P > 0.05$ ) variation, but the fourth year and the overall mean herbage DM were significantly ( $P < 0.05$ ) vary among the alfalfa genotypes. The overall mean DM yield ranged from 5.5 to 8.7 with a mean of 6.5 t/ha. The overall mean indicated that DM yield was significantly higher ( $P < 0.05$ ) in hairy peruvian followed by FG-10-09 (F) whereas the other three genotypes had intermediate values between the two genotypes. The significant genotypic differences observed for herbage DM yield of alfalfa in this study concurs with reports of other researchers (Marijana *et al.*, 2008; Monirifar, 2011; Sun *et al.*, 2011; Diriba *et al.*, 2014). Significant differences in DM yield was also reported among 16 alfalfa cultivars with overall mean of around 12 t/ha (Hayek *et al.*, 2008). Mean DM yield in the order of 11 t/ha was reported in a study where three alfalfa cultivars were evaluated (Zeinab *et al.*, 2013) indicating the comparatively low herbage yield potential of the cultivars evaluated in the present study. Quite the reverse, DM yield values ranging from 1.78-3.23 t/ha (Afsharmanesh, 2009) and from 0.67-2.16 t/ha were reported (Awad and Bakeri, 2009), which were indeed much lower than those observed in the present study. The wide range of herbage DM yield values observed in different research findings could be attributed to varietal and environmental differences and their interactions. If harvested at the appropriate development stage, alfalfa can provide high yields of quality forage for 3 to 5 years. If it is harvested at later stages produces increased yields of green forage and DM whilst prolonging the productive life of the alfalfa forage (Lioveras *et al.*, 1998). Growth stage, cut number, leaf to stem ratio, 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 and Fritz, 2003). The CP yield showed non-significant ( $P > 0.05$ ) variation while digestible yield showed significant ( $P < 0.05$ ) difference among the tested genotypes (Table 4). The CP and digestible yields ranged from 1.23 to 1.74 with a mean of 1.39 t/ha and from 4.03 to 6.50 with a mean of 4.84 t/ha respectively. The result revealed that hairy peruvian had the highest CP yield and digestible yield followed by FG-10-09 (F) whereas the genotype FG-9-09(F) had the lowest CP and digestible yields.

**Table 3. Mean herbage DM yield (t/ha) of alfalfa genotypes tested over years at Holetta research center**

Genotypes	Years					Mean
	2010	2011	2012	2013	2014	
FG-10-09(F)	4.65	3.54	7.00	10.19 <sup>b</sup>	6.21	6.32 <sup>b</sup>
FG-9-09(F)	4.60	3.63	6.93	7.48 <sup>b</sup>	4.85	5.50 <sup>b</sup>
Magna 801 FG(F)	4.98	3.94	7.33	8.68 <sup>b</sup>	6.05	6.20 <sup>b</sup>
Magna 788	5.29	3.78	6.96	7.01 <sup>b</sup>	4.87	5.58 <sup>b</sup>
Hairy peruvian	4.75	3.88	9.34	15.52 <sup>a</sup>	10.12	8.72 <sup>a</sup>
<b>Mean</b>	<b>4.85</b>	<b>3.76</b>	<b>7.51</b>	<b>9.77</b>	<b>6.42</b>	<b>6.46</b>
<b>SEM</b>	<b>0.22</b>	<b>0.31</b>	<b>0.33</b>	<b>0.41</b>	<b>0.42</b>	<b>0.16</b>
<b>P-value</b>	<b>0.8414</b>	<b>0.9980</b>	<b>0.4658</b>	<b>0.0263</b>	<b>0.2760</b>	<b>0.0003</b>

Means followed by a common superscript letters with in a column are not significantly different from each other at  $P < 0.05$ .

### Leaf to Stem Ratio at Forage Harvesting Stage

The leaf to stem ratio, which ranged from 0.83 to 1.03 with a mean of 0.97, was not significantly different ( $P > 0.05$ ) among alfalfa genotypes at forage harvesting stage (Table 4). The leaf to stem ratio was highest ( $P > 0.05$ ) for Magna-788 and least for hairy peruvian but the remaining genotypes having intermediate values between the two genotypes. Among the others, hairy peruvian showed inferior leaf to stem ratio in the present study is in agreement with others research findings (Diriba *et al.*,

2014; Mekuanint *et al.*, 2015) and this could be attributed to its distinctly higher plant height and stem proportion are correlated positively. The non-significant genotype differences in leaf to stem ratio at forage harvesting stage observed in the present study is in disagreement with other reports (Lamb *et al.*, 2003; Hayek *et al.*, 2008; Monirifar, 2011; Mekuanint *et al.*, 2015), but concurs with others research findings (Afsharamanesh, 2009; Diriba *et al.*, 2014). The leaves to stem ratio, which varied depending on the number of cuts, harvest cycles and harvest stage, is an important quality indicator during evaluation of herbage quality. Leaf to stem ratio is an important trait in the selection of appropriate forage cultivar as it is strongly related to forage quality (Kratchunov and Naydenov, 1995; Julier *et al.*, 2000; Sheaffer *et al.*, 2000). The proportion of leaves and stems in alfalfa hay can vary greatly, depending on maturity at harvest, handling and rain damage. Research results indicated that alfalfa at early bud may have 600 g/kg leaves while at early flower, the leaf share declines to 450 g/kg (Sheaffer *et al.*, 2000). To value alfalfa hay as a source of leaf protein, accurate prediction of the proportion of leaf and stem in the hay is necessary (Halgerson *et al.*, 2004). Decreasing protein content is a dilution effect related with the decreasing leaf to stem ratio; the leaves have stable protein content and their protein level is much higher than the protein content in stems. The proportion of leaves at the time of harvest is a major factor that determines the quality of the forage (Jung, 2005). Percentage of leaves is desirable to be as high as possible, because in the leaves are found a crude protein content better than stem (Mihai *et al.*, 2012).

### Relative Feed Value

The relative feed value (RFV) is an index used for legumes based on potential intake and fiber digestibility (Undersander and Moore, 2002). The index is used to price forage and to allocate forage to appropriate ruminant livestock performance levels. The RFV, which showed non-significant difference ( $P>0.05$ ), for tested alfalfa genotypes is presented in Table 4. The non-significant difference in RFV of alfalfa genotypes in the present study was in agreement with (Mekuanint *et al.*, 2015) and in disparity with (Diriba *et al.*, 2014). The result showed that the RFV ranged from 122.3 to 143.5 with a mean of 134.2. The highest RFV was recorded for Magna 788 followed by FG-9-09(F) and FG-10-09(F) while the lowest value obtained from hairy peruvian. The lowest RFV recorded for hairy peruvian in the present study was in agreement with the others findings (Diriba *et al.*, 2014; Mekuanint *et al.*, 2015). According to Diriba *et al.* (2014), Magna 788 had the highest RFV which concurs with the present study. On the other hand, Magna 801-FG-(F) had the highest RFV according to (Mekuanint *et al.*, 2015) which was in disparity with the present study. The RFV recorded in the present study was much lower than the value revealed by other researchers (Diriba *et al.*, 2014; Mekuanint *et al.*, 2015). Feeds with RFV index higher than 100 are considered to be of higher quality compared to full bloom alfalfa hay and those with a value lower than 100 are of lower value (Dunham, 1998). Other research reports indicated that high quality alfalfa contains more than 151% RFV (Redfearn and Zhang, 2011). In the current study alfalfa genotypes had a RFV ranging from 122-144 and this was apparently above the threshold level of 100 according to the revealed of (Dunham, 1998) but below the threshold level of 151 reported by other researchers (Redfearn and Zhang, 2011). The RFV index observed for alfalfa genotypes was higher than a threshold level of 100, apparently indicating the genotypes to have prime quality standard, with the highest value being for Magna788. Generally, the RFV index was proposed to reflect 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).

**Table 4.** Mean leaf to stem ratio, CP yield (t/ha), digestible yield (t/ha) and relative feed value of alfalfa genotypes tested at Holetta

Genotypes	CP yield (t/ha)	Digestible yield (t/ha)	Leaf to stem ratio	RFV
FG-10-09(F)	1.37	4.78 <sup>b</sup>	1.00	134.64
FG-9-09(F)	1.23	4.03 <sup>b</sup>	1.01	137.62
Magna 801 FG(F)	1.36	4.70 <sup>b</sup>	1.01	132.91
Magna 788	1.25	4.22 <sup>b</sup>	1.03	143.54
Hairy peruvian	1.74	6.50 <sup>a</sup>	0.83	122.32
<b>Mean</b>	<b>1.39</b>	<b>4.84</b>	<b>0.97</b>	<b>134.20</b>
<b>SEM</b>	<b>0.13</b>	<b>0.23</b>	<b>0.31</b>	<b>0.79</b>
<b>P-value</b>	<b>0.3011</b>	<b>0.0440</b>	<b>0.3125</b>	<b>0.2544</b>

Means followed by a common superscript letters with in a column are not significantly different from each other at  $P<0.05$ .

### **Herbage Nutritive Value at Forage Harvesting Stage**

Herbage quality traits of alfalfa genotypes are presented in Table 5. All the traits showed non-significant ( $P>0.05$ ) difference except *in-vitro* dry matter digestibility. The ash content of alfalfa genotypes ranged from 102.6 to 115.0 with a mean of 110.9 g/kg DM. The ash content reported in the present study was higher when compared with others research reports (Diriba *et al.*, 2014; Mekuanint *et al.*, 2015). The ash content was highest for Magna 801-FG (F) followed by FG10-09(F) and Magna-788 and was least for hairy peruvian. The lowest ash content in hairy peruvian in the present study was in agreement with other findings (Diriba *et al.*, 2014). The mineral content is affected by the stage of maturity and the leaf to stem ratio, since alfalfa leaves contain more P, Ca, Mg, Cu, Zn, Fe and Mn while stems contain more K (Markovic *et al.*, 2009). Since the concentration of minerals in forages are affected by stage of maturity, climatic and seasonal changes (Minson, 1990), regular analysis has been recommended for formulating appropriate mineral supplementation schedules (Spears, 1994). Other studies also indicated that concentration of minerals in forage varies due to factors like plant developmental stage, morphological fractions, climatic conditions, soil characteristics and fertilization regime (McDowell and Valle, 2000; Jukenvicius and Sabiene, 2007). Differences in both proportion and composition of the different morphological fractions could explain varietal differences in ash content. Alfalfa is a highly valued animal feed. It is a rich source of proteins, fibers, minerals and vitamins used in the diet of livestock, especially ruminants. The content of minerals in alfalfa fully meets the livestock requirements while the content of fats is low (averaging 3.8 g/kg), and it varies slightly among cultivars (Katic *et al.*, 2009).

The CP content of alfalfa genotypes ranged from 199.2 to 226.3 g/kg DM with a mean of 216.6 g/kg DM. The non-significant CP content in the present study was in agreement with (Mekuanint *et al.*, 2015) and in disparity with (Diriba *et al.*, 2014). The CP content reported in the present study was higher when compared with others research findings (Diriba *et al.*, 2014; Mekuanint *et al.*, 2015). The highest CP content was recorded for Magna 788 followed by FG-9-09(F), Magna 801 FG(F), FG-10-09(F) while the lowest value was recorded for hairy peruvian and the same order was also reported for these genotypes (Diriba *et al.*, 2014). High quality alfalfa was reported to contain >19% CP (Redfearn and Zhang, 2011). On the other hand, alfalfa forage quality values at full bloom stage contain CP >16% (Dunham, 1998). In this study, all tested alfalfa genotypes had CP content greater than the threshold value >19% indicated by other researchers (Redfearn and Zhang, 2011). Alfalfa nutritive value is identified with protein content which depends on the share of leaves in dry matter yield which in its turn is positively correlated with protein content (Julier *et al.*, 2001; Katic *et al.*, 2005). Protein content in alfalfa dry matter varies from 18 to 25% depending on the growth stage, cultivar and storage method (Katic *et al.*, 2006). Harvesting at earlier development stages produces more crude protein and less crude cellulose (Katic *et al.*, 2003). A wider range of values observed in the literature for CP and fiber fractions of alfalfa can be attributed to various factors such as cultivar, climatic and agronomic management practices and/or their interactions (Diriba *et al.*, 2014). The result showed that alfalfa produces more protein per hectare than other legume and grasses; therefore, it is widely used for hay production and as pasture for livestock, especially to ruminants (Monteros and Bouton, 2009). Indeed, all the genotypes had CP values of above 15%, a level suggested for a protein source supplement to be considered optimal to support lactation and growth in dairy cattle (Nsahlai *et al.*, 1996).

The IVDMD, which ranged from 734.8 to 757.7 g/kg DM with a mean of 750.2 g/kg DM, of the five alfalfa genotypes is presented in Table 5. The result revealed that the highest IVDMD value was recorded for Magna 801 FG(F) followed by Magna 788 and FG-10-09(F) while FG-9-09(F) had the lowest value. The highest and lowest values recorded for Magna 801 FG(F) and FG-9-09(F) respectively, in the present study were in agreement with other finding (Mekuanint *et al.*, 2015). The significant difference ( $P<0.05$ ) among the tested genotypes was in agreement with (Diriba *et al.*, 2014) and in disparity with others finding (Mekuanint *et al.*, 2015). Volenec and Cherney (1990) reported significant differences in IVDMD among alfalfa cultivars and these differences were indicated to be associated with variation in digestibility of the stem fraction (Tremblay *et al.*, 2002). A significant difference among 14 alfalfa varieties was also reported for IVDMD, with values ranging from 59 to 66% (Kamalak *et al.*, 2005), which indeed were much lower than those recorded in the present work. Previous research has demonstrated variability among alfalfa germplasm for ruminal

degradation of total crude protein (Tremblay *et al.*, 2003). Digestibility of alfalfa organic matter depends on the contents of cellulose and lignin. As lignin is virtually indigestible, intensive lignifications of cell wall in late stages of alfalfa development tends to reduce the coefficient of digestibility. Organic matter digestibility ranges from 55% to 77% and depends on growth stage, leaf to stem ratio, cutting frequency, harvesting conditions and processing (INRA, 2007). Digestibility of alfalfa decrease with maturity as a result of increased concentration of cell wall material in stems, decreased stem digestibility and decreased leaf weight ratio (Albrecht *et al.*, 1987). Soil fertility, cultivar, climatic conditions, harvesting stage and preservation method are some of the factors affecting alfalfa hay quality (Stancheva *et al.*, 2008).

The non-significant ( $P>0.05$ ) differences for NDF and ADF contents of alfalfa genotypes are indicated in Table 5. Mekuanint *et al.*, (2015) also reported non-significant differences for NDF and ADF contents of alfalfa cultivars. The result revealed that the highest NDF and ADF contents recorded for hairy peruvian, indicating low quality alfalfa genotype when compared with others. Diriba *et al.*, (2014) also reported that hairy peruvian had the highest NDF and ADF contents when compared with others alfalfa cultivars. High quality alfalfa was reported to contain NDF <400 g/kg DM and ADF <310 g/kg DM (Ball *et al.*, 1997; Redfearn and Zhang, 2011; Kazemi *et al.*, 2012). On the other hand, alfalfa forage quality values at full bloom stage contain NDF <530 g/kg DM and ADF <410 g/kg DM reported as better quality (Dunham, 1998). The NDF content of all the genotypes was below the critical level (530 g/kg DM) reported in alfalfa (Dunham, 1998) could indicate that it has better digestibility. However, the NDF values reported in the present study was much higher than the threshold level (<400 g/kg DM) reported for high quality alfalfa in literature (Ball *et al.*, 1997; Redfearn and Zhang, 2011; Kazemi *et al.*, 2012). The ADF values reported in the present study was lower than the threshold level (<410 g/kg DM) reported (Dunham, 1998) but much higher than threshold level (<310 g/kg DM) reported in the literature (Ball *et al.*, 1997; Redfearn and Zhang, 2011; Kazemi *et al.*, 2012). Significant differences were registered in the contents of ADF and NDF that were caused by genetic factors (Katić *et al.*, 2008). Furthermore, Sheaffer *et al.* (1998) obtained significant differences in the contents of NDF and ADF between low, medium and high quality alfalfa cultivars. In general, a wider range of values observed for fiber fractions of alfalfa in the literature can be attributed to factors such as cultivar, climatic factors and agronomic management practices or their interactions.

The ADL content showed non- significant ( $P>0.05$ ) difference among the tested alfalfa genotypes and this also reported by other researchers (Diriba *et al.*, 2014; Mekuanint *et al.*, 2015). The ADL content ranged from 86.1 to 103.7 g/kg DM with a mean of 95.6 g/kg DM. The highest ADL content was recorded for hairy peruvian, indicating low quality when compared with the others genotypes. The lignin component contributes erectivity, strength and resistance to plant tissue, thereby limiting the ability of rumen microbes to digest cell wall polysaccharides, cellulose and hemicellulose (Reed *et al.*, 1988). Hence, alfalfa genotypes with lower lignin content should have better digestibility. High lignin content in alfalfa plants increases their resistance to lodging; however, lignin is a major factor that limits cell wall digestibility because it inhibits the digestibility of polysaccharides (Katic *et al.*, 2008). The hemicellulose and cellulose contents in the present study were non-significant ( $P>0.05$ ) and this was in agreement with other researchers (Mekuanint *et al.*, 2015). The hemicellulose content of alfalfa genotypes ranged from 65.2 g/kg DM in FG-9-09(F) to 104.5 g/kg DM in Magna 801 FG(F) with a mean of 88.7 g/kg DM. On the other hand, the cellulose content ranged from 229.4 g/kg DM in Magna 788 to 279.0 g/kg DM in hairy peruvian with a mean of 248.6 g/kg DM. The structural polysaccharides composed primarily of cellulose and hemicelluloses are primary restrictive determinants of nutrient intake and digestibility. The higher hemicellulose content in the feed limits forage intake and digestibility (Lundvall *et al.*, 1994; Wolf *et al.*, 1993).

**Table 5.** Mean chemical composition and in-vitro dry matter digestibility of alfalfa genotypes tested at Holetta

Genotypes	g/kg DM							
	Ash	CP	IVDMD	NDF	ADF	ADL	HECLL	CELL
FG-10-09(F)	114.4	215.8	756.3 <sup>a</sup>	433.7	335.4	97.9	98.3	237.5
FG-9-09(F)	109.4	221.6	734.8 <sup>b</sup>	416.9	351.7	86.1	65.2	265.7
Magna 801 FG(F)	115.0	220.1	757.7 <sup>a</sup>	440.3	335.8	98.5	104.5	237.3
Magna 788	113.2	226.3	757.0 <sup>a</sup>	417.3	319.9	92.1	97.5	229.4
Hairy peruvian	102.6	199.2	745.3 <sup>ab</sup>	454.6	376.6	103.7	78.0	279.0
<b>Mean</b>	<b>110.9</b>	<b>216.6</b>	<b>750.2</b>	<b>432.6</b>	<b>343.9</b>	<b>95.6</b>	<b>88.7</b>	<b>248.6</b>



## Biomass Yield Potential and Herbage Quality of Alfalfa (*Medicago Sativa L.*) Genotypes in the Central Highland of Ethiopia

SEM	0.19	0.34	0.22	0.37	0.37	0.26	0.33	0.36
P-value	0.1377	0.5522	0.0230	0.3243	0.1117	0.4501	0.1307	0.1298

Means followed by a common superscript letters with in a column are not significantly different from each other at  $P < 0.05$ .

### CONCLUSION

The tested five alfalfa genotypes varied in plant height, DM yield, CP and digestible yields, leaf to stem ratio and RFV. The result revealed that the highest mean plant height was recorded for hairy peruvian followed by FG-10-09(F) and Magna 801 FG(F), while FG-9-09(F) had the lowest plant height. The overall mean indicated that DM yield was higher in hairy peruvian followed by FG-10-09(F) whereas the other three genotypes had intermediate values between the two genotypes. Hairy peruvian had the highest CP yield and digestible yield followed by FG-10-09(F) whereas the genotype FG-9-09(F) had the lowest CP and digestible yields. The leaf to stem ratio was highest for Magna-788 and least for hairy peruvian but the remaining genotypes having intermediate values between the two genotypes. The highest RFV was recorded for Magna 788 followed by FG-9-09(F) and FG-10-09(F) while the lowest value obtained from hairy peruvian. The herbage quality traits also varied among the tested alfalfa genotypes. The result showed that the ash content was highest for Magna 801-FG(F) followed by FG10-09(F) and Magna-788 and lowest for hairy peruvian. The highest CP content was recorded for Magna 788 followed by FG-9-09(F), Magna 801 FG(F), FG-10-09(F) while the lowest value was recorded for hairy peruvian. The result revealed that the highest IVDMD value was recorded for Magna 801 FG(F) followed by Magna 788 and FG-10-09(F) while FG-9-09(F) had the lowest value. The highest NDF and ADF contents recorded for hairy peruvian, indicating it had low quality when compared with others. The highest ADL content was also recorded for hairy peruvian, indicating low quality when compared with the others genotypes. Generally, hairy peruvian had the highest mean plant height, mean DM yield, CP and digestible yields and fiber contents (NDF, ADF and ADL). On the other hand, the lowest leaf to stem ratio, RFV, ash content and CP content were recorded for hairy peruvian when compared with others genotypes.

### ACKNOWLEDGEMENT

We would like to thank the Eastern Africa Agricultural Productivity Project (EAAPP) for the financial support of this study.

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