

Registration of New Alfalfa-1086 and Alfalfa-ML-99 (*Medicago sativa* L.) Varieties

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Abstract

Background: Ethiopia has the highest livestock population in Africa. However, the productivity of livestock and the role it plays in the economy of the country is very low. This is mainly because of the inadequate availability of quality feed. Enhancing the production of alfalfa could contribute to overcome the shortage of feed in the country and enhance the productivity of all classes of livestock in the country.

Objective: To evaluate performances of high-yielding and disease-tolerant alfalfa varieties.

Materials and Methods: Six introduced alfalfa varieties were tested against a standard check (DZF-552) and an old introduction of alfalfa (hairy Peruvian) varieties for biomass yield, quality attributes, disease and pest reactions, and other agronomic traits at Holetta, Kulumsa, Debrezeit, Melkassa, Werer, and Pawe Agricultural Research Centers during the 2013 and 2014 cropping seasons. Three superior alfalfa varieties (alfalfa-1086, alfalfa-ML-99 and alfalfa-998) were selected from multi-location evaluation trials and the candidate varieties with recently released alfalfa variety (DZF-552) were evaluated under verification trial at Kulumsa, Melkassa, Werer, and Wondo-genet Agricultural Research Centers in 2015.

Results: Alfalfa-1086, alfalfa-998, and alfalfa-ML-99 had dry matter (DM) yield advantages of 30, 27, and 23% over hairy Peruvian variety, respectively. Alfalfa-1086, alfalfa-ML-99 and alfalfa-998 also had crude protein (CP) yield advantages of 109, 89, and 73% over the hairy Peruvian variety, respectively. More importantly, alfalfa-1086, alfalfa-ML-99, and alfalfa-998 varieties had CP yield advantages of 55, 41, and 28%, respectively, over the recently released (DZF-552) check variety. The *in vitro* dry matter digestibility (IVDMD) yield advantages were 66% and 23% for alfalfa-1086; 58 and 17% for alfalfa-ML-99 and 57 and 16% for alfalfa-998 over the hairy Peruvian and recently released standard check variety, respectively. The results revealed that the candidate varieties had better ash, CP, and IVDMD but lower fiber contents when compared to the check varieties.

Conclusions: The released varieties had advantages of CP and IVDMD yields over hairy Peruvian and standard check varieties. Moreover, the released varieties had advantages of leaf to stem ratio, CP content, and IVDMD over the two checks. Due to the better nutritional quality and yields of CP and IVDMD, the released varieties are very important to improve the livestock production and productivity of smallholder farmers. Therefore, the two alfalfa varieties (alfalfa-1086 and alfalfa-ML-99) were approved for cultivation in the low to high altitude areas of Ethiopia due to their better yield performance, quality attributes, disease and pest reactions, and other agronomic traits.

Keywords: Adaptation; Dry matter yield; Quality attributes; Reaction; Yield stability

1. Introduction

Despite the enormous contribution of livestock to the livelihood of farmers, the availability of poor quality feed resources remains to be the major bottleneck to livestock production in Ethiopia (Seyoum Bediye and Zinash Sileshi, 1995; Zinash Sileshi *et al.*, 1995; Fekede Feyissa *et al.*, 2015a). The traditional livestock

production system mainly depends upon poor pasturelands and crop residues, which are usually inadequate to support reasonable livestock production (Tsigie Yohannes, 2000; Assefa Admassie, 2005). These feed resources are high in fiber, with low to moderate digestibility and low levels of nitrogen (Preston, 1995; Tsigie Yohannes, 2000). Their crude protein and neutral detergent fiber content ranges



between 2.5 to 27.5% and 72.6 to 77.8%, respectively. Such low-quality feeds are associated with a low voluntary intake, thus resulting in insufficient nutrient supply, low productivity, and even weight loss (Hindrichsen *et al.*, 2004). The available feed resources are utilized to support the maintenance requirement of the animals with little surplus left for production. Poor animal nutrition and productivity arising from the inadequate supply and low-quality feed are among the major constraints facing livestock production in developing countries (Fekede Feyissa *et al.*, 2015b). The livestock master plan of Ethiopia indicated an estimated national annual feed availability of 81.3 million tons DM in bad weather conditions (Shapiro *et al.*, 2015). However, the national annual feed requirement is estimated to be 130 million ton of DM, indicating a negative feed balance of 48.7 million ton of DM is recorded in bad weather conditions (Shapiro *et al.*, 2015).

Forage legumes contribute significantly to livestock production in all crop-livestock production systems. They generally lead to higher intakes and animal production than the grass of comparable digestibility (Dewhurst *et al.*, 2003). Alfalfa (*Medicago sativa* L.) is often recognized as one of the most important perennial forage legumes worldwide and used as a feed for all classes of domestic animals (Barnes *et al.*, 1988). It is widely known as the “queen of the forages” due to its ability to consistently produce high forage yield and quality feed as well as its adaptability to different climatic conditions (Kamalak *et al.*, 2005; Turan *et al.*, 2009). Ruminants fed on alfalfa have higher nutrient intake and digestibility than when fed on other forage legumes and grasses (Frame, 2005). Alfalfa also provides higher amounts of minerals (mainly calcium, magnesium, potassium, sulfur, iron, cobalt, manganese, and zinc) and vitamins (beta-carotene) than other fodders (Frame, 2005). Alemayehu Mengistu (2002) noted that because of its very high feed value, alfalfa can be used as a supplement for crop residues and natural hay in a mixture of 30% alfalfa and 70% other roughages.

To improve the availability of livestock feed in terms of quantity and quality, it is better to cultivate alfalfa forage that has better biomass yield and nutritional quality. The number of alfalfa varieties, which produce better yield and quality, is low in Ethiopia. So far, only one alfalfa variety (DZF-552) is officially recommended for cultivation by Debrezeit Agricultural Research Center in 2014 (MoA, 2014).

Therefore, to alleviate the existing feed shortage problems, there is a need to introduce and evaluate alfalfa varieties that produce high biomass yield and quality feed to different agro-ecologies of the country. Accordingly, two alfalfa varieties (alfalfa-1086 and alfalfa-ML-99) with better dry matter yield, crude protein yield, and *in vitro* dry matter digestibility yield

and nutritive value have been selected and recommended for cultivation from six alfalfa varieties introduced by ELFORA Agro-Industries private limited company to bridge the feed shortage problem in Ethiopia. Thus, this paper presented the description of the two newly released varieties and data on their forage yield performance, nutritional quality, agro-ecological adaptation, disease reaction, and other morpho-agronomic traits and management recommendations.

2. Varietal Origin and Evaluation

Six alfalfa varieties (alfalfa-1086, alfalfa ML-99, alfalfa-998, alfalfa-CW-830, alfalfa Mirage, and alfalfa-fast grow II) were introduced from abroad by ELFORA and one adapted but not registered alfalfa variety (hairy *Peruvian*) as a check was used to conduct the national variety trial at Holetta, Kulumsa, Debrezeit, Melkassa, Werer, and Pawe Agricultural Research Centers for two years (2013 and 2014) under supplemental irrigation conditions. But, due to disease and adaptability problems, data from the three varieties (alfalfa-CW-830, alfalfa Mirage, and alfalfa-fast grow II) were not generated for evaluation. So, only three varieties (alfalfa-1086, alfalfa ML-99, and alfalfa-998) were considered. The varieties were uniformly irrigated at field capacity every 15 days during the dry season of the year. The experiment was laid out in randomized complete block design (RCBD) and replicated three times per treatment. The introduced alfalfa varieties with the check (hairy *Peruvian*) were sown on well-prepared seedbeds in rows of 20 cm apart using a seed rate of 20 kg ha⁻¹. Di-ammonium Phosphate (DAP) fertilizer was applied at the rate of 100 kg ha⁻¹ at planting at each location. Plots were hand-weeded during the establishment during the subsequent years of evaluation.

Based on the overall performances, the three better-performing alfalfa varieties (alfalfa-1086, alfalfa-ML-99, and alfalfa-998) were promoted to a variety verification trial with a recently released check (alfalfa-DZF-552) at Holetta, Kulumsa, Melkassa, Werer, and Wondo-Genet Agricultural Research Centers during the main cropping season in 2015 (Table 1). The varieties were planted in rows of 20 cm apart on a plot size of 10 m by 10 m with a seeding rate of 20 kg ha⁻¹. At sowing, the recommended rate of DAP fertilizer was uniformly applied on the plots at each location. Other recommended cultural practices were also applied. The National Variety Release Committee (NVRC) evaluated the varieties at field conditions and based on their evaluation result, the two varieties (alfalfa-1086 and alfalfa-ML-99) were approved for cultivation in April 2016 to be utilized by various end-users.

3. Agronomic and Morphological Characteristics

The hairy Peruvian variety was the tallest plant (71.8 cm) followed by alfalfa-DZF-552 (65.1 cm). The

newly released varieties, alfalfa-1086, and alfalfa-ML-99 had average plant height of 64.2 and 63.1 cm, respectively, while alfalfa-998 was the shortest (61.7 cm) plants over the growing seasons and locations (Table 1).

Table 1. Average plant height of alfalfa varieties tested at Holetta, Kulumsa, Debrezeit, Melkassa, Werer, and Pawe research centers in 2013 to 2015 cropping seasons.

Variety	Location of varietal evaluation						Mean
	Holetta	Kulumsa	Debrezeit	Melkassa	Werer	Pawe	
Alfalfa-1086	70.7 ^b	65.4 ^a	64.1	64.4 ^{ab}	69.0	51.8 ^b	64.2 ^b
Alfalfa-ML-99	72.9 ^b	66.5 ^a	59.1	74.5 ^a	59.6	46.2 ^b	63.1 ^b
Alfalfa-998	76.0 ^{ab}	55.3 ^b	64.1	55.2 ^b	68.7	50.7 ^b	61.7 ^b
Check: hairy <i>Peruvian</i>	81.9 ^a	69.5 ^a	68.0	71.1 ^a	74.0	66.5 ^a	71.8 ^a
Mean	75.4	64.2	63.8	66.3	67.8	53.8	65.2
CV (%)	5.5	4.7	8.4	8.2	8.2	7.1	9.3
LSD (5%)	8.3	6.0	10.7	10.9	11.1	7.7	4.1
Check variety DZF-552	–	–	79.3	45.3	67.8	–	65.1

Note: Means followed by different letters within a column are significantly different from each other at $P < 0.05$. CV = Coefficient of variation (%); and LSD = Least significant difference at 5% probability level.

The released alfalfa varieties are perennial herbaceous legumes characterized by their agro-morphological characteristics. The maximum plant height at the forage harvesting stage (50% flowering) was 75 and 70 cm for alfalfa-1086 and alfalfa-ML-99 varieties, respectively. Alfalfa-1086 variety was relatively early for the forage harvesting stage when compared to the alfalfa-ML-99 variety. The leaf to stem ratio, crude protein yield, digestible yield, and crude protein (CP) were relatively higher for the alfalfa-1086 than the alfalfa-ML-99 variety. On the other hand, the higher ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and *in vitro* dry matter digestibility (IVDMD) were recorded for alfalfa-ML-99 variety when compared to the alfalfa-1086 variety. However, both released varieties per cut produced a mean of 4 t ha⁻¹ dry matter yield at the forage harvest stage. The varieties adapted to lower to higher altitudes ranging from 750-3000 meters above sea level with an annual rainfall ranging from 850-1200 mm. Both varieties had good performance under nitosol and clay loam soil types. The varieties should be sown with a seeding rate of 10-20 kg ha⁻¹ at 20 cm row spacing in early June. At planting, the application of the recommended rate of DAP fertilizer enhances the establishment performance of alfalfa varieties. A summary of agronomical and morphological characteristics of the released two alfalfa varieties is presented in Table 2.

4. Yield Performances of Varieties over Locations

The varieties produced different forage dry matter (DM) yields across the test environments during the experimental years (Table 3). The highest mean DM yield was recorded at Werer (6.1 t ha⁻¹) followed by

Melkassa (4.8 t ha⁻¹), Kulumsa (4.1 t ha⁻¹), and Pawe (3.5 t ha⁻¹) while the lowest yield of 2.2 t ha⁻¹ was obtained from Holetta Agricultural Research Center. The mean DM yields performance of alfalfa varieties ranged from 3.0 to 3.9 t ha⁻¹ with a mean of 3.6 t ha⁻¹ across the test environments. The highest mean DM yield was recorded for alfalfa-1086 followed by alfalfa-998 and alfalfa-ML-99, while hairy *Peruvian* gave the lowest DM yield across the environments. The rank of the varieties for DM yield changed across the test environments indicating the effect of genotype x environment interaction on DM yield performances of the varieties (Figure 1).

Yields advantage of the new varieties over the two check varieties is presented in Table 4. The result showed that the candidate varieties had advantages of DM yield, crude protein yield, and digestible yield over unregistered alfalfa check variety (hairy *Peruvian*). On the other hand, the varieties showed crude protein yield and digestible yield advantages over the recently released alfalfa variety (DZF-552) but the candidate varieties did not have DM yield advantages over the recently released variety. The highest DM yield advantage was recorded for alfalfa-1086 (30%) followed by alfalfa-998 (27%) and alfalfa-ML-99 (23%) over hairy *Peruvian* variety. The highest crude protein yield advantage was obtained from alfalfa-1086 (109%) followed by alfalfa-ML-99 (89%) and alfalfa-998 (73%) over hairy *Peruvian* variety. Similarly, crude protein yield advantages of 55, 41, and 28% were recorded for alfalfa-1086, alfalfa-ML-99, and alfalfa-998 varieties over recently released variety, respectively. The *in-vitro* dry matter digestibility yield advantages were 66 and 23% for alfalfa-1086; 58 and 17% for alfalfa-ML-99 and 57 and 16% for alfalfa-998 over hairy *Peruvian* and recently released check varieties, respectively.

Table 2. Agronomic and morphological characteristics of alfalfa-1086 and alfalfa-ML-99 varieties.

Characteristic	Alfalfa-1086	Alfalfa-ML-99
Species	<i>Medicago sativa</i> L.	<i>Medicago sativa</i> L.
Variety	Alfalfa-1086	Alfalfa-ML-99
Adaptation	Lower to higher altitude	Lower to higher altitude
Altitude (m.a.s.l.)	750–3000	750–3000
Rainfall (mm)	850–1200	850–1200
Soil type	Nitosol and clay loam	Nitosol and clay loam
Seeding rate (kg ha ⁻¹)	10–20	10–20
Inter-row spacing (cm)	20	20
Planting date	Early June	Early June
Fertilizer rate (kg ha ⁻¹)		
DAP	100 DAP	100 DAP
Plant height at forage harvest (cm)	60–75	60–70
Days to harvesting (50% flowering)	80–90	85–95
Leaf to stem ratio	1.10	1.04
Yield per cut (t ha ⁻¹)		
Forage dry matter:	3–5	3–5
Crude protein yield	1.15	1.04
Digestible yield	2.88	2.74
Fodder quality (%)		
Ash	12.61	13.63
Crude protein	29.39	28.19
NDF	44.49	44.56
ADF	33.43	33.75
ADL	4.63	4.87
IVDMD	73.82	74.07
Year of release	2016	2016
Breeder/maintainer	ELFORA/HARC/EIAR	ELFORA/HARC/EIAR

Note: *m.a.s.l.* = Meters above sea level; *DAP* = Diammonium phosphate; *NDF* = Neutral detergent fiber; *ADL* = Acid detergent lignin; *ADF* = Acid detergent fiber; *IVDMD* = In vitro dry matter digestibility; *HARC* = Holetta Agricultural Research Center; and *EIAR* = Ethiopian Institute of Agricultural Research.

Table 3. Average forage dry matter yield (t ha⁻¹) of alfalfa varieties tested at Holetta, Kulumsa, Debrezeit, Melkassa, Werer, and Pawe Research Centers in 2013 to 2014 cropping seasons.

Variety	Location of varietal evaluation						Mean
	Holetta	Kulumsa	Debrezeit	Melkassa	Werer	Pawe	
Alfalfa-1086	2.2	4.6 ^{ab}	3.8 ^a	4.3	4.4 ^b	4.3	3.9 ^a
Alfalfa-ML-99	2.4	4.7 ^a	3.0 ^a	5.9	3.5 ^{bc}	3.0	3.7 ^a
Alfalfa-998	2.4	3.7 ^a	3.1 ^a	4.4	5.9 ^a	3.5	3.8 ^a
Check: hairy <i>Peruvian</i>	1.9	3.5 ^c	2.0 ^b	4.7	2.8 ^c	3.1	3.0 ^b
Mean	2.2	4.1	3.0	4.8	6.1	3.5	3.6
CV (%)	17.0	10.9	17.3	15.3	16.9	21.0	22.0
LSD (5%)	0.8	0.9	1.0	1.5	1.4	1.5	0.5
Check variety DZF-552	–	–	4.4	3.0	4.2	–	3.9

Note: Means followed by different letters within a column are significantly different from each other at $P < 0.05$. *CV* = Coefficient of variation (%); and *LSD* = Least significant difference at 5% probability level.

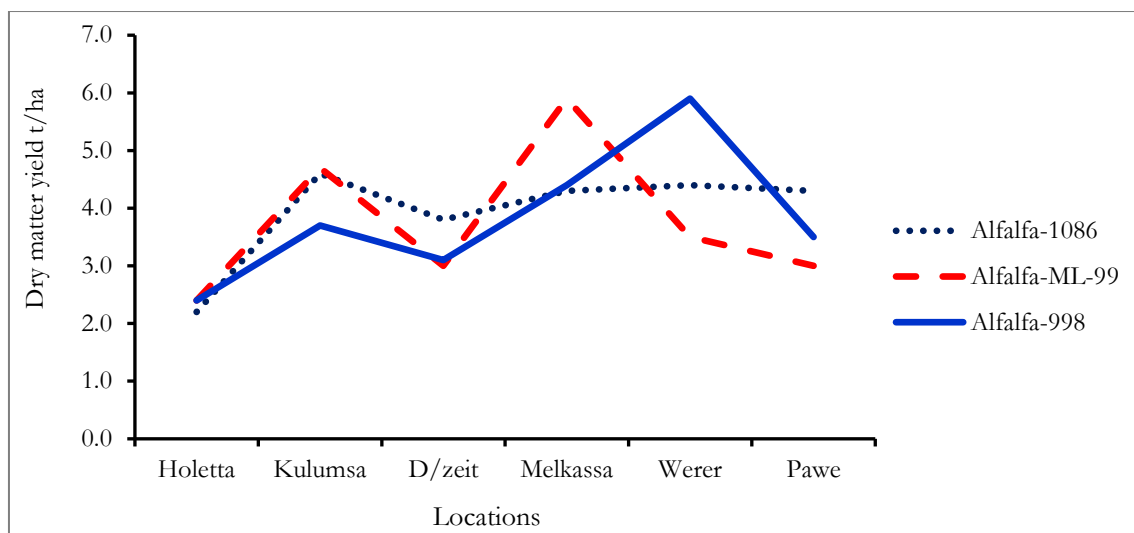


Figure 1. Overall mean dry matter yield performances of the candidate varieties across the test environments.

Table 4. Percent advantages of dry matter, crude protein, and *in vitro* dry matter digestibility yields of alfalfa varieties over unregistered and recently released standard check varieties.

% advantage	Trait	Alfalfa varieties		
		Alfalfa-1086	Alfalfa-ML-99	Alfalfa-998
Over hairy Peruvian	DM yield	30.0	23.3	26.7
	CP yield	109.1	89.1	72.7
	IVDMD yield	65.5	57.5	56.9
Over check variety DZF-552	DM yield	0.0	-5.1	-2.6
	CP yield	55.4	40.5	28.4
	IVDMD yield	22.6	16.6	16.2

Note: DM = Dry matter; CP = Crude protein; and IVDMD = *In vitro* dry matter digestibility.

5. Reaction to Diseases and Pests

The released alfalfa varieties (alfalfa-1086 and alfalfa-ML-99) were tested for their diseases and pests reaction starting from the initial stage of evaluation to verification stage and found to be resistant/moderately resistant to major diseases and pests which can affect the varieties (Figures 2 and 3). The diseases and pest effects on the performance of alfalfa varieties were recorded as 0-10% resistant, 11-30% moderately resistant, 31-60% moderately

susceptible, and 61-100% susceptible. Accordingly, the released varieties were found to be resistant to moderately resistant to the recorded major diseases (downy mildew, common leaf spot, aphanomyces root rot, and bacterial wilt) and pests (aphids) in the test locations during the experimental periods. The resistance reaction of the varieties could be integrated with other diseases and pest management strategies for better results. Generally, the released varieties are superior in tolerance to major diseases and pests to the standard check.

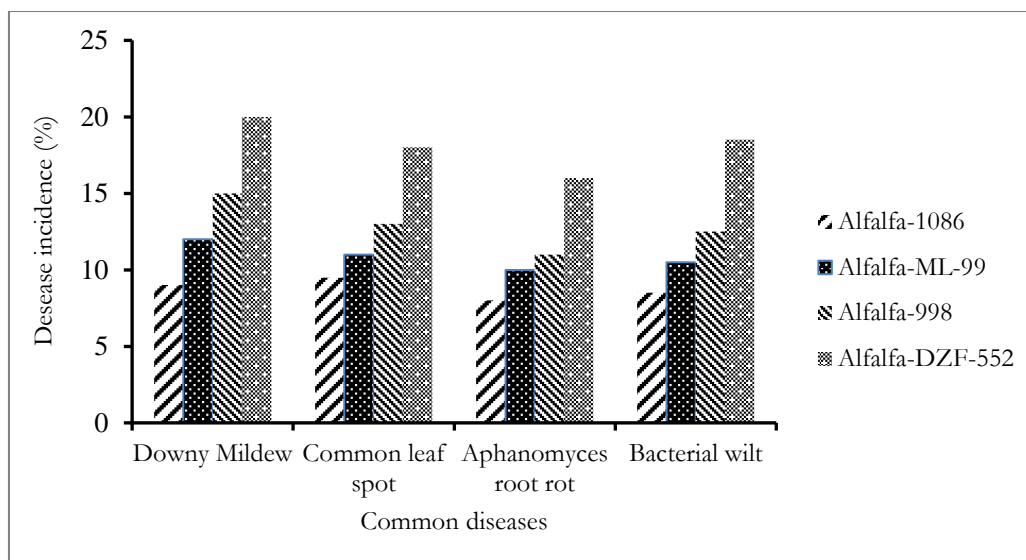


Figure 2. Overall mean response of alfalfa varieties for common diseases.

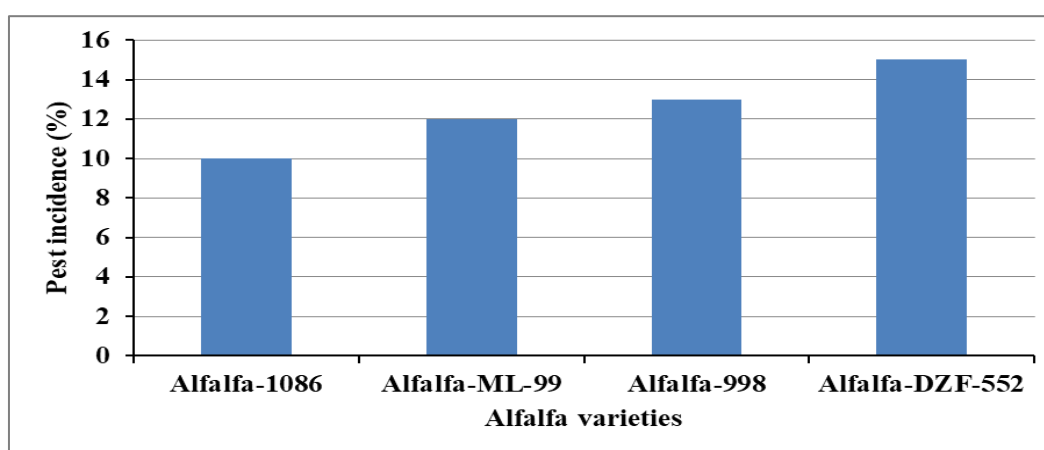


Figure 3. Overall mean response of alfalfa varieties for aphids.

6. Quality Attributes

The chemical composition and *in vitro* dry matter digestibility of alfalfa varieties are presented in Table 5. The candidate varieties had higher ash, CP, and IVDMD but lower fiber contents than the check varieties. The highest ash content (13.63%) was recorded for alfalfa-ML-99 followed by alfalfa-99 (13.49%) and alfalfa-1086 (12.61%). The released alfalfa varieties (alfalfa-1086 and alfalfa-ML-99) produced the highest CP content. The lowest NDF content was recorded for alfalfa-998 while alfalfa-1086 produced the lowest fiber (ADF and ADL) contents when compared to other varieties. The highest IVDMD (74.1%) was obtained from alfalfa-ML-99 followed by alfalfa-1086 (73.8%) and alfalfa-998 (71.8%). The candidate varieties had advantages over the check varieties in terms of leaf to stem ratio (LSR),

CP, and IVDMD (Table 6). Alfalfa-1086, alfalfa-ML-99 and alfalfa-998 varieties had LSR advantages of 9, 3, and 1% over hairy *Peruvian*, respectively. Similarly, LSR advantages of 24, 17, and 15% were recorded for alfalfa-1086, alfalfa-ML-99, and alfalfa-998 varieties, respectively, over the standard check. On the other hand, alfalfa-1086, alfalfa-ML-99, and alfalfa-998 varieties had CP advantages of 60, 54, and 36% over hairy *Peruvian* and 55, 49, and 32% CP advantage over recently released (DZF-552) alfalfa variety, respectively. Alfalfa-ML-99 had the highest IVDMD advantages (28.1 and 22.5%) followed by alfalfa-1086 (27.6 and 22.1%) and alfalfa-998 (24.2 and 18.8%) over hairy *Peruvian* and recently released alfalfa varieties, respectively.

Table 5. Chemical compositions (%) and *in vitro* dry matter digestibility (%) of alfalfa varieties.

Variety	Ash	CP	NDF	ADF	ADL	IVDMD
Alfalfa-1086	12.61	29.39	44.99	33.43	4.63	73.82
Alfalfa-ML-99	13.63	28.19	44.56	33.75	4.87	74.07
Alfalfa-998	13.49	25.00	44.38	33.78	4.86	71.84
Check: hairy Peruvian	11.28	18.37	46.31	38.11	9.44	57.84
Check variety DZF-552	11.18	18.98	41.92	35.66	6.75	60.45

Note: CP = Crude protein; NDF = Neutral detergent fiber; ADL = Acid detergent lignin; ADF = Acid detergent fiber; and IVDMD = In vitro dry matter digestibility.

Table 6. Percent leaf to stem ratio, crude protein, and *in-vitro* dry matter digestibility advantages of alfalfa varieties over unregistered and recently released standard check varieties.

% advantage	Trait	Alfalfa varieties		
		Alfalfa-1086	Alfalfa-ML-99	Alfalfa-998
Over: hairy Peruvian	LSR	8.9	3.0	1.0
	CP	60.0	53.5	36.1
	IVDMD	27.6	28.1	24.2
Over check variety: DZF-552	LSR	23.6	16.9	14.6
	CP	54.8	48.5	31.7
	IVDMD	22.1	22.5	18.8

Note: LSR = Leaf to stem ratio; CP = Crude protein; and IVDMD = In vitro dry matter digestibility.

7. Conclusion

The agro-morphological performance and nutritive value of alfalfa varieties varied across the test environments due to the differential response of the varieties to various biotic and abiotic factors. The released alfalfa varieties (alfalfa-1086, and alfalfa-ML-99) had better dry matter yield and medium plant height compared to the check varieties and other candidate variety included in this study. The released varieties had advantages of dry matter yield, crude protein yield, and *in vitro* dry matter digestibility yield over hairy Peruvian variety. Similarly, the released varieties had advantages of crude protein yield, and *in vitro* dry matter digestibility yield over the standard check. The nutritional qualities indicated that the released varieties had advantages over the two check varieties in terms of leaf to stem ratio, crude protein content, and in vitro dry matter digestibility. Due to the better nutritional quality and yields of CP and IVDMD, the released varieties are very important to improve the livestock production and productivity of Ethiopia. Based on the yield and nutritional quality performances, both alfalfa varieties (alfalfa-1086 and alfalfa-ML-99) have been released for production in the low and high-altitude areas. The breeder and pre-basic seeds of both alfalfa varieties (alfalfa-1086 and alfalfa-ML-99) are maintained by ELFORA Agro-industries Pvt. Ltd. Co. and the feed and nutrition research section of Holetta Agricultural Research Center.

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