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Introducing some Iranian Ecotypes of Alfalfa

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Abstract

The objective of this research was to evaluate forage yield and quality of native alfalfa ecotypes. Correlations among the investigated traits were also determined, since data on relationships are of great importance in selection, especially for traits with low genetic variability. Experimental material consisted of 13 alfalfa germplasms. Investigation was carried out during a three-year period. The field trial was arranged as the randomized complete block design with four replications. There was a statistically significant difference among ecotypes for plant height ranging from 74.50 cm (Moapa) to 96.00 (Alhord), number of internodes from 12.75 (Khosrovanagh) to 16.00 (Moapa), leaf size from 0.45 (Ranger) to 1.0125 (Leghlan), leaf fresh weight to stem fresh weight ratio (LFW/SFW) from 0.44 (Leghlan) to 0.54 (Moapa), leaf dry weight to stem dry weight ratio (LFW/SFW) from 0.3725 (Leghlan) to 0.4750 (Moapa), in vitro dry matter digestibility (IVDMD) from 45.42% (Gara-Yonjeh) to 50.67% (Baftan), acid detergent fiber (ADF) from 38.83% (Khaje) to 44.70% (Gara-Yonjeh), crude fiber (CF) from 25.96% (Sivan) to 32.48% (Ranger) and neutral detergent fiber (NDF) from 47.03% (Khaje) to 57.43% (Moapa). There were positive correlations between plant fresh yield, dry yield, number of stems and plant height. LFW/SFW and LDW/SDW didn't show any significant correlation with quality components (IVDMD, CP, ADF, CF and NDF). There was a negative correlation between CP and CF. Sivan, Dizaj-Safarali, Gara-Baba, Khosrovanagh, Khaje, Alhord and Leghlan ecotypes had the best quality. They can be recommended and considered to make synthetic varieties.

Keywords: Alfalfa; Ecotype; Quality; Yield

Introduction

Alfalfa, queen of forage crops (*Medicago sativa* L.) is the most important forage legume (Michaud *et al*. 1988) and is the most cultivated forage legume due to its ability to fix atmospheric dinitrogen and its high protein content. However, since alfalfa is autotetraploid (2n= 4x= 32), allogamous and seed propagated crop, successful assessment of its genetic diversity has been hampered by the available statistical methods (Flajoulot *et al.* 2005). At the moment, its importance is raising with the increase of public interest in sustainable agriculture because, as reported by McCoy and Echt (1992), alfalfa is a low input energy efficient crop that helps improve soil tilth. Furthermore, it occupies a significant economic position in the animal feed market (i.e. hay, dehydrated forage, pellets and silage products).

The nature of alfalfa, allogamy and autotetraploidy, contribute to large genetic variability among and within alfalfa populations or varieties. The analysis of the genetic variability within and among populations of cultivated alfalfa can assess future risk of genetic erosion and help in the development of sustainable conservation and genetic improvement strategies. High variability for many morphological and agronomical important traits in alfalfa has been registered (Mikic *et al.* 2005; Radovi´c *et al.* 2006). Besides, variability among varieties and variability of individual plants within varieties is high for numerous traits (Julier *et al.* 2000; Annicchiarico 2006; Salvia *et al.* 2006). Variability among and within population were confirmed using molecular analysis (Zaccardeli *et al*. 2003).

Variability for agronomic and morphological traits of alfalfa is frequently used in breeding programs for developing cultivars with a high forage production and better quality. Increasing variability in the selection material could be achieved by introducing distinct alfalfa varieties, as new sources of diversity. Agro-morphological traits have been used to classify and study genetic diversity in alfalfa germplasm collections as well as other crops (Radovi´c *et al.* 2006). Jasmina *et al.* (2010) showed significant differences of alfalfa varieties from USA with domestic varieties for almost all investigated traits. They reported that the highest variability among and within varieties was obtained for green and dry matter yield per plant and number of stems in all cuts. The lower coefficient of variation was noticed for other traits.

Smith *et al*. (1995) classified 41 Middle Eastern alfalfa accessions collected from different elevations in Oman, Yemen and southwestern Saudi Arabia based on morphological and agronomic traits into separate classes with regard to their tolerance to low winter temperature. Fombellida (1998) analyzed 56 ecotypes of alfalfa collected from north Spain and classified them into four groups based on spring growth rate, regrowth rate after cutting, mortality, persistence and precocity rate.

According to Collins and Fritz (2003) digestibility and forage quality are both at the maximum during the vegetative stage and continually decrease as stems and flowers develop. Maturity at harvest is considered to be the most important factor affecting forage quality. With the development of near-infrared reflectance spectroscopy (NIRS), forage quality can now be quickly and inexpensively measured. Many high-quality cultivars have been released with the development of this new technology (Hall *et al.* 2000). Near-infrared reflectance spectroscopy predicts forage quality components {(i.e. acid detergent fiber (ADF), neutral detergent fiber (NDF), protein (CP)} through detection of rotational and vibration amplitudes associated with hydrogen bonding (C-H, O-H and N-H). These bonds absorb a specific band of near-infrared radiation between 800 and 2500 nanometers. Materials high in proteins will absorb more radiation in the N-H region, while materials high in moisture will absorb more in the O-H region. The NIR spectrum for a sample will be a combination of the reflectance from all three regions. This is a very reliable and efficient method for predicting forage quality components for large samples (Halgerson *et al.* 2004). Many studies have found the prediction of CP, ADF, NDF and in vitro dry matter digestibility (IVDMD) by NIRS to be very accurate (Shenk *et al.* 1981; Marten *et al.* 1983), although it cannot directly predict inorganic components such as minerals (Clark *et al.* 1987). Shenk *et al*. (1981) reported R^2 values for CP to be as high as 0.99 between the predicted NIRS value and the known value.

Selection for improved forage quality has been successful for increasing protein concentration, in vitro dry matter digestibility (IVDMD), vigor and levels of resistance to diseases, and decreasing NDF and acid detergent lignin (ADL) concentration of alfalfa herbage (Shenk and Elliot 1971; Hill and Barnes 1977; Hill 1981; Sumberg *et al.* 1983; Coors *et al.* 1986). However, because these selection studies were done on the whole herbage, the observed shifts in forage quality may have resulted from inadvertent selection for altered leaf-to-stem ratio. Quality of alfalfa leaf and stem material are sufficiently different that any shift in relative proportions of leaf and stem results in significant changes in herbage quality (Sheaffer *et al.* 2000). In the case of selection for reduced ADL concentration in alfalfa herbage (Hill 1981), it was shown that the resulting divergence in herbage ADL concentration was due primarily to a higher leaf proportion in the low lignin alfalfa line, although the ADL concentration of stem material did show some responses (Kephart *et al.* 1989, 1990). While a higher leaf-to-stem ratio of the low lignin lines did result in reduced NDF concentration, the increase in IVDMD was minimal and only correlated with NDF concentration (Kephart *et al.* 1990).

Breeding alfalfa with higher forage quality may alter other plant characteristics inadvertently. Correlations between forage quality and morphological and agronomic traits have been reported. Positive correlations of IVDMD with leaf/stem ratio and number of vascular bundles were reported (Shenk and Elliot 1971). Negative correlations of lignin with leaf/stem ratio and stem height were also observed (Kephart *et al.* 1989, 1990). Johnson *et al*. (1994) found positive correlations of CP with leaf/stem ratio and lodging, and negative correlations of CP with regrowth height. However, moderate correlations or no associations of yield with NDF, ADF, lignin, IVDMD and CP were reported (Gil *et al*. 1967; Shenk and Elliot 1971; Hill and Barnes 1977; Hill 1981; Sumberg *et al.* 1983; Coors *et al.* 1986; Kephart *et al.* 1989).

Our research objectives were to determine the diversity for morphology and forage quality traits in a group of alfalfa varieties and ecotypes to identify superior ecotypes for synthetic variety improvement.

Materials and Methods

Alfalfa ecotypes used in this study were identified as part of a breeding project for synthetic variety improvement (Monirifar 2010). Ranger and Moapa cultivars were used as check (Table 1).

The seeds were planted in individual pots containing a mixture of sandy-loam soil, peat and sand with 2:1:1 ratio on March 2009. The field experiment was carried out at the Research Station of Tikmadash, Tabriz, Iran, during 2009 and 2011. The site is located at 37°45´ N latitude, 45°55´ E longitude and altitudes of 1800 m. Before beginning of the experiment, a composite soil sample was taken in order to determine the physical and chemical properties of the soil. Based on the soil analysis, nitrogen, phosphorous and potassium were supplied in the form of super phosphate triple, potassium nitrate and urea (250, 150 and 100 kg ha^{-1}, respectively). The experiment was carried out as randomized complete block design with four replications. The plots were 2.4 m long and consisted of four rows, 0.6 m apart. Between all plots, 1 m alleys were considered. From each ecotype, 96 plants were transplanted into the field. There were 24 plants in each plot. The first irrigation was performed immediately and irrigation interval was as weekly schedule. Weeds were controlled with hand hoeing as needed throughout the growing seasons.

Plants were under irrigated condition for three years. First year was considered for making uniformity. All data were recorded from the two central rows. During the second year plant height (PH), number of shoots (NS), plant fresh weight (PFW), plant dry weight (PDW), leaf size (LS), number of internodes (NIN), leaf fresh weight to stem fresh weight (LFW/SFW) and leaf dry weight to stem dry weight (LDW/SDW) ratios were measured. PFW was obtained by hand cutting of plants at approximately 5 cm above the ground and weighing by the electronic balance. To determine PDW, fresh samples of randomly chosen plants were taken from each plot and placed into paper bags. The samples were then weighted and dried at 105ºC for 24 h to assess average dry matter content (DMC). PDW was then calculated by DMC×PFW/100. Sum of PFW and PDW for each plant were determined from each cut during a year. NS of individual plants was recorded directly following cuts. Prior to cuts, PH was measured from the ground to the top of the inflorescence. The mean of PH, NS, LFW/SFW and LDW/SDW and the sum of PFW and PDW for each year were used for data analysis.

Data for in vitro dry matter digestibility (IVDMD), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude fiber (CF) were estimated using near-infrared reflectance spectroscopy (NIRS) model 8620. Means over the three harvest data for two years were used.

All data were analyzed by the analysis of variance (ANOVA) using the GLM procedure in SAS. The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution with a mean of zero.

Table 1. Names and collection sites of alfalfa ecotypes used for evaluation of quality and agronomic traits

Ecotype name	Collection site	Ecotype name	Collection site
Leghlan	Ahar	Dizaj-Safarali	Varzegan
Sivan	Marand	Khosrovanagh	Varzegan
Sattelou	Tabriz	Alhord	Varzegan
Gara-Baba	Bostan-Abad	Gara-Yonjeh	Khosro-Shah
Baftan	Sarab	Ranger	Improved Variety
Ilan-Jough	Ardabil	Moapa	Improved Variety
Khaje	Heris	------	-----

Results and Discussion

Analysis of variance of the 11 ecotypes and two cultivars of alfalfa for traits under study are shown in Table 2. The lowest coefficient of variation was indicated for IVDMD (3.76%) and ADF (4.19%), and the highest coefficient for plant fresh and dry weight (14.41 and 14.01%). A highly significant ($P<0.01$) effect of ecotype on PH, NIN and LS and significant effect ($P<0.05$) on LFW/SFW and LDW/SDW were obtained. Also significant differences among ecotypes were observed for in vitro dry matter digestibility (IVDMD), acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude fiber (CF), but not for crude protein (CP) content (Table 2).

Table 3 shows large variation for many measured traits indicating the existence of genetic diversity within the investigated alfalfa germplasm. Plant height is regarded as an important yield component in alfalfa and it is often used as a selection criterion when choosing superior genotypes in an early stage of selection (Tucak *et al.* 2008). Alhord, Leghlan and Sattelou ecotypes were the tallest and Moapa cultivar and Sivan ecotype were the shortest in the experiment (96.0, 91.5, 91.0, 74.5 and 76.2 cm, respectively). Although there was no significant difference among ecotypes for numbers of stems, but Gara-Baba, Ilan-Jough and Alhord ecotypes had the highest NS (55.5, 54.0 and 51.25, respectively) (Table 3).

In addition to selection for greater leaf proportion in alfalfa for higher quality, changes in other morphological traits associated with forage quality have been investigated. Kephart *et al*. (1989) found a lower Kalu and Fick maturity index (Kalu and Fick 1981) and shorter stem length, but no difference in internode number, for low lignin alfalfa lines. In contrast, Hall *et al.* (2000) found no difference in maturity index when compared two high-quality alfalfa cultivars with two check cultivars. Furthermore, it was shown that internode length was correlated with length of individual cells of stem tissues in a single alfalfa genotype, where longer internodes exhibited greater degradation of tissues in large particles (Engels and Jung 2005). In our experiment, Moapa, Ilan-Jough and Alhord ecotypes had the highest number of internodes (16.0, 15.0 and 14.2, respectively) and Sivan and Khosrovanagh had the lowest values (12.5 and 12.7, respectively). The highest leaf area among ecotypes belonged to Leghlan, Khaje and Sivan ecotypes (1.0125, 1.0050 and 0.9750, respectively); while Ranger and Moapa cultivars had the lowest leaf area (0.45 and 0.50, respectively). Moapa, Ranger and Sivan ecotypes showed higher leaf fresh and dry weight to stem fresh and dry weight ratio (LFW/SFW and LDW/SDW) than other ecotypes.

No significant differences were observed among ecotypes for fresh and dry yield (PFW and PDW) per plant ($P>0.05$). The average fresh yield per plant of Gara-Baba and Alhord ecotypes were 476.27 and 452.70 gr/plant, respectively (Table 3). The dry yields of these ecotypes were more than other ecotypes, PFW and PDW had positive and highly significant correlation ($P<0.01$), however, PDW for Khaje ecotype was an exception.

Table 2. Summary of analysis of variance for the morphological and qualitative traits of alfalfa germplasm

Source of variation	Mean squares†												
	PH	NS	NIN	LS	PFW	PDW	(LFW/SFW)	(LDW/SDW)	IVDMD	CP	ADF	CF	NDF
Ecotype	156.63**	0.84 ns	3.23**	0.049**	11.61 ns	11.61 ns	0.003*	0.004*	11.24**	7.61 ns	14.67**	17.48**	25.68**
Year	137.14ns	0.36 ns	0.97 ns	0.029 ns	7.10 ns	2.17 ns	0.001 ns	0.002 ns	0.33 ns	6.16 ns	0.57 ns	7.51 ns	8.18 ns
Ecotype× Year	129.3 ns	0.23 ns	0.88 ns	0.017 ns	6.11 ns	2.01 ns	0.001 ns	0.001 ns	0.33 ns	7.01 ns	0.44 ns	6.66 ns	7.31 ns
C.V.%	7.71	10.90	7.71	12.89	14.41	14.41	8.00	10.46	3.76	8.31	4.19	6.89	5.14

*, ** and ns show significance at 5% and 1% probability levels and non-significant, respectively.
†PH= Plant height, NS= Number of shoots, NIN= Number of internodes, LS= Leaf size, PFW= Plant fresh weight, PDW= Plant dry weight, LFW/SFW= Leaf fresh weight to stem fresh weight ratio, LDW/SDW= Leaf dry weight to stem dry weight ratio, IVDMD= In vitro dry matter digestibility, CP= Crude protein, ADF= Acid detergent fiber, CF= Crude fiber, NDF= Neutral detergent fiber.

Table 3. Means for agronomic and quality traits of alfalfa germplasm

Ecotype	PH†	NS	NIN	LS	PFW	PDW	(LFW/SFW)	(LDW/SDW)	IVDMD	CP	ADF	CF	NDF
Ilan-Jough	85.50abcd	48.33	15.00ab	0.5533abc	402.63	112.06	0.4700bc	0.3833ab	47.27ab	23.63	43.97a	29.62abcd	54.52ab
Sivan	76.25cd	38.50	12.50c	0.9750ab	281.67	74.22	0.5050abc	0.4725ab	47.82ab	25.15	43.29ab	25.96d	55.84ab
Gara-Yonje	89.32abc	37.25	13.75bc	0.6700abc	306.08	82.13	0.4925abc	0.4325ab	45.42b	23.01	44.70a	29.70abcd	53.32ab
Dizaj-Safarali	89.60abc	45.75	14.00abc	0.8625abc	400.17	109.48	0.4675bc	0.4125ab	47.38ab	25.35	43.95a	27.35cd	55.43ab
Baftan	90.83abc	48.50	13.75bc	0.9700ab	419.08	115.03	0.4750abc	0.3975ab	50.67a	22.65	39.55bcd	30.36abc	50.79bc
Sattelou	91.05ab	41.00	13.75bc	0.6750abc	339.06	93.73	0.4900abc	0.4125ab	48.13ab	24.72	42.77abc	27.70bcd	54.21ab
Gara-Baba	87.46abcd	55.00	13.33bc	0.7300abc	476.27	114.95	0.4933abc	0.4333ab	49.34a	24.89	42.06abcd	27.90cd	54.31ab
Khosrovanagh	82.75abcd	43.25	12.75c	0.8825abc	372.02	94.60	0.4850abc	0.4400ab	49.58a	24.40	41.26abcd	26.23cd	53.64ab
Khaje	85.00abcd	47.00	13.75bc	1.0050a	443.99	118.62	0.4550bc	0.4125ab	50.53a	21.85	38.83d	26.10cd	47.03c
Leghlan	91.50ab	40.50	13.75bc	1.0125a	386.75	102.71	0.4400c	0.3725b	47.88ab	23.38	42.34abcd	29.14abcd	54.77ab
Alhord	96.00a	51.25	14.25abc	0.7400abc	452.70	120.58	0.4450c	0.3825ab	48.19ab	24.28	42.92abc	28.33abcd	53.63ab
Ranger	81.00bcd	40.00	13.50bc	0.4500c	275.40	72.82	0.5200ab	0.4400ab	50.66a	22.37	39.25cd	32.48a	52.49abc
Moapa	74.50d	37.00	16.00a	0.5000bc	344.92	88.51	0.5400a	0.4750a	46.82ab	21.31	43.02abc	31.71ab	57.43a
Total Mean	86.938	44.26	13.76	0.8026	380.08	101.05	0.4789	0.4178	48.41	23.74	42.20	28.35	53.50

Means with the same letter (s) in each column are not significant at 1% probability level.
†PH= Plant height, NS= Number of shoots, NIN= Number of internodes, LS= Leaf size, PFW= Plant fresh weight, PDW= Plant dry weight, LFW/SFW= Leaf fresh weight to stem fresh weight ratio, LDW/SDW= Leaf dry weight to stem dry weight ratio, IVDMD= In vitro dry matter digestibility, CP= Crude protein, ADF= Acid detergent fiber, CF= Crude fiber, NDF= Neutral detergent fiber.

The knowledge about the correlations among traits is an important issue in plant breeding programs. When choosing superior individuals for a certain trait according to phenotypic expression, other traits may be changed at the same time. The study of correlations provides the possibility of improvement of a larger number of traits simultaneously. Calculations of correlations are also important for traits with low genetic variability; in this case the progress can be achieved by indirect selection. Phenotypic correlation coefficients among the studied traits are shown in Table 4. Plant height was positively correlated with number of stems (0.358*), fresh yield (0.408*) and negatively correlated with leaf fresh (-0.471**) and dry weight (-0.445**) to stem fresh and dry weight ratio. The correlation coefficients of plant height with IVDMD, CP, ADF, CF and NDF (0.038, -0.104, -0.057, 0.153 and -0.051, respectively) were low and non-significant. In addition, number of stems was strongly correlated with PFW and PDW (0.647** and 0.617**, respectively), and significantly and negatively correlated with NDF (-0.412**). PFW showed strong and positive correlation with PDW (0.974**). The correlation coefficient of PFW with PH was 0.624. Annicchiarico (2006) reported similar results for this crop. LFW/SFW and LDW/SDW, as an indicators of forage quality, were negatively and significantly correlated with PFW and PDW (-0.503** and -0.545**, respectively). Leaf ratio was strongly and negatively correlated with green mass yield (–0.78**), dry matter (–0.65**), protein (–0.62**) and plant height (–0.68**). Correlations of plant height with LFW/SFW and LDW/SDW were negative and significant (-0.471** and -0.445**). Kephart *et al*. (1989, 1990) reported negative correlation of leaf/stem ratio with stem height.

Buxton *et al*. (1987) measured a range of morphological and growth traits, in vitro digestible dry matter (IVDMD) and crude protein concentration for 64 alfalfa introductions and recorded significant variation for these traits. They suggested that improvement of forage quality can be made by selection through nutritive value of the stem. Shukla and Malviya (1988) carried out path analysis on green fodder yield (GFY) and four related traits in alfalfa and found positive relationship of stem number and weight with GFY. Leaf and stem weight were suggested important parameters for improving GFY through selection.

Smith and Hamel (2005) believed that alfalfa forage yield depends upon three factors including plant number/unit area, stem number/plant and single-stem yield. However, Sengul (2002) considered plant height, stem number/plant and single-stem yield as the forage yield components. Johnson *et al*. (1994) found positive correlations of CP with leaf/stem ratio and lodging, and negative correlations of CP with regrowth height. Moderate or no associations of yield with NDF, ADF, lignin, IVDMD and CP was reported (Hill 1981; Sumberg *et al.* 1983; Coors *et al.* 1986; Kephart *et al*. 1989).

Ranger and Moapa cultivars were superior in LFW/SFW and LDW/SDW than the other ecotypes and in contrast they had the lowest leaf area. The correlation coefficients of these traits were negative and non-significant (P>0.05). Leaf number was higher in these cultivars, so the leaf fresh and dry weight to stem fresh and dry weight ratio were higher too. Leghlan and Alhord ecotypes had the lowest LDW/SDW ratios (0.3725 and 0.3825, respectively). Leaf to green mass ratio is regarded as an important trait in alfalfa breeding regarding the forage quality. The selection of plants with a high leaf : green mass ratio would probably increase protein content indirectly, i.e. improve its quality, but, on the other hand, it would decrease total green mass yield. Therefore, selection on quality traits alone without yield is not recommended.

Baftan, Ranger and Khaje had the IVDMD of 50.67, 50.66 and 50.53, respectively, and were superior to other ecotypes. On the other hand Gara-Yonjeh and Moapa had the lowest IVDMD content. Gara-Yonjeh, Ilan-Jough and Dizaj-Safarali ecotypes had the highest ADF percentage and the lowest CF was belonged to Sivan and Khaje ecotypes. Moapa cultivar and Sivan and Dizaj-Safarali ecotypes were superior to other ecotypes in terms of NDF content.

Forage yield and its quality are complex traits whose expression are influenced by genetic constitution of a plant as well as environmental factors, as stated by Julier *et al*. (2000). For this reason, determining the genetic potential of the alfalfa ecotypes and the interrelation among traits, are of utmost important. Plant height may be regarded as the most reliable and stable selection criterion due to its relatively large variability which makes it a successful trait for the improvement of alfalfa yield. Considering plant height, Alhord, Leghlan and Sattelou were the best ecotypes. In addition, CP and CF are regarded as the most important quality traits (Rotili *et al.* 2001). Rotili *et al.* (2001) pointed out that successful improvement in alfalfa protein content may be achieved indirectly by selection of the following traits: high tolerance of early cutting (green bud), resistance to diseases and insects, delay of leaf senescence and modification of stem morphology (a larger number of short internodes). There was no significant correlation between CP and other traits, but it had negative and strong correlation with CF. Sivan, Dizaj-Safarali, Gara-Baba, Khosrovanagh, Khaje, Alhord and Leghlan ecotypes had the highest quality. Thus, they can be recommended for producing synthetic varieties.

Table 4. Phenotypic correlation coefficients among studied traits in alfalfa ecotypes

Traits†	NS	NIN	LS	PFW	PDW	(LFW/SFW)	(LDW/SDW)	IVDMD	CP	ADF	CF	NDF
PH	0.358*	0.170	0.188	0.387**	0.408**	-0.471**	-0.445**	0.038	-0.104	-0.057	0.153	-0.051
NS	1.000	0.161	0.078	0.647**	0.617**	-0.259	-0.231	0.290	0.099	-0.172	-0.101	-0.412**
NIN		1.000	0.228	0.144	0.195	0.008	-0.016	-0.179	-0.104	0.154	0.303*	0.078
LS			1.000	-0.004	-0.025	-0.080	0.110	0.085	-0.011	-0.133	-0.219	-0.166
PFW				1.000	0.974**	-0.503**	-0.545**	0.254	0.022	-0.183	-0.075	-0.362*
PDW					1.000	-0.547	-0.600**	0.229	-0.011	-0.176	-0.020	-0.352*
(LFW/SFW)						1.000	0.817**	-0.057	0.148	0.079	-0.058	0.163
(LDW/SDW)							1.000	-0.061	0.192	0.094	-0.244	0.137
IVDMD								1.000	0.075	-0.924**	-0.355*	-0.565**
CP									1.000	0.251	-0.679**	-0.012
ADF										1.000	0.152	0.581**
CF											1.000	0.354**
NDF												1.000

*, ** and [ns] show significance at 5% and 1% probability levels and non-significance, respectively.

†PH= Plant height, NS= Number of shoots, NIN= Number of internodes, LS= Leaf size, PFW= Plant fresh weight, PDW= Plant dry weight, LFW/SFW= Leaf fresh weight to stem fresh weight ratio, LDW/SDW= Leaf dry weight to stem dry weight ratio, IVDMD= In vitro dry matter digestibility, CP= Crude protein, ADF= Acid detergent fiber, CF= Crude fiber, NDF= Neutral detergent fiber.

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