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DETERMINATION OF LIGHT ABSORPTION PATTERNS WITHIN ALFALFA VARIETIES IN GHERGHITA PLAIN

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Abstract

The paper presents the assessment of light interception and absorption patterns, canopy structure and aerial dry matter allocation in 27 alfalfa varieties grown in the eco-climatic conditions of Gherghita Plain, Romania, and whether the results can lead to the improvement of cropping technologies for obtaining superior forage yields and better winter hardiness. Alfalfa is a perennial crop that produces its highest yields during the second year of growth and the paper emphasizes the experimental results obtained during the last harvest cycle of the second cropping year. This period was selected to establish the ecophysiological patterns of the alfalfa plants before entering the winter. The morphological variables were the leaf/stem ratio L/S (g DM leaf m^{-2}/ g DM stem m^{-2}), average height of the canopy (cm) and forage yield (t DM ha^{-1}). The radiation variables comprised the incident and transmitted light, zenith angle, probe's spread, and leaf area index (LAI). PAR profile was assessed before cutting using a canopy analyzer (SunScan Canopy Analysis System, Delta-T Devices Ltd., Cambridge, UK).The mean values for the 27 varieties were as follows: LAI of 2.69 (CV=28.76%), canopy height of 41.44 cm (CV=18.41%), leaf/stem ratio of 0.73 (CV=17.19%), and a forage yield of 4.23 t DM ha^{-1} (CV=21.66%). The relative low coefficients of variation related to the ecophysiological response of alfalfa plants to the abiotic factors suggest a moderate variability between varieties despite their geographical region of origin. A positive correlation was found between canopy height and L/S Ratio ($r = 0.52$; $p<0.01$), and with forage yield ($r = 0.54$; $p<0.01$). L/S Ratio was positevely correlated with forage dry matter yield (0.63; $p<0.001$). The interactions between vertical light absorption - absorbing medium - morphological and yield characteristics of the variety have been accurately assessed using the factor analysis. These three factors have explained 79.4% of the variance in the dataset. The obtained indicators are useful to characterize the winter hardiness and to facilitate the selection of the most suitable varieties to be cultivated in equivalent eco-climatic conditions.

***Key words**: alfalfa, canopy analyzer, leaf area index, photosynthetically active radiation, vertical light absorption.*

INTRODUCTION

Alfalfa or lucerne (*Medicago sativa* L.) is an important forage species in temperate regions improving the soil characteristics through the symbiosis with *Rhizobium* strains (Bărbulescu et al., 1991). It was found that yield and growing range of alfalfa are limited by abiotic stress (Motcă et al., 1994). Recent studies on physiology, genetic and molecular aspects have underlined complex processes integrated at various levels that regulate stress adaptation and tolerance (Song et al., 2019).

Understanding the fluxes between atmosphere, canopy and soil, and the relationship with sources and sinks within the carbon cycle are becoming more important for the global climate change research. In New Zealand, irrigated alfalfa was C-neutral despite two harvests and losses following conversion from grassland. In the 2nd and 3rd years combined, the biomass-C removal exceeded net CO_2 uptake, causing net losses of 450 g C m^{-2} and 210 g C m^{-2} for irrigated and non-irrigated lucerne, respectively. Irrigation made no difference to the photosynthetic water-use efficiency at field scale, but enhanced production water-use efficiency (biomass/water input). Irrigation increased both the absolute amount of drainage and the fraction of water inputs lost by drainage (Laubach et al., 2019).

Assessment of the light absorption by leaves within canopy is an important task in modeling CO_2 assimilation (Dunea and Dincă, 2014).

In central Oklahoma, USA, rainfed alfalfa yields were regulated by amount and timing of rainfall resulting in cumulative dry forage yield up to 7.5 t ha^{-1} (four harvests) in 2016 (dry year) and up to 10 t ha^{-1} (five harvests) in 2017 (wet year). The response of gross primary production to photosynthetically active radiation (PAR) varied with growth stage of alfalfa and climatic conditions (i.e., dry or normal/wet periods) (Wagle et al., 2019).

In Romania, Romanian varieties showed valuable biological characteristics in rainfed conditions providing good annual water use efficiencies(WUE) ranging from 13-14 kg dry matter (DM) mm^{-1} ha^{-1} (1^{st} year of cropping), 15-16 (2^{nd} year), and 18-19 (3^{rd} year), respectively (Dincă et al., 2017). The multiannual average of Radiation Use Efficiency (RUE) ranged between 1.3 and 1.4 g MJ m^{-2}. It was found that increasing of RUE determines the decreasing of crude protein content (% DM) of the alfalfa silage. More efficient bioconversion processes may occur with advancing in maturity and crop aging that increase the DM content, but the quality of DM may decrease, pointing out that stand management is a key factor to insure optimal nutritional value of the resulted fodder (Dunea et al., 2018). In Germany, under water stress, the mean RUE of cup plant (*Silphium perfoliatum* L.) (1.3 g MJ^{-1}) was significantly lower than that of maize (2.9 g MJ^{-1}) and lucerne-grass mixture (1.4 g MJ^{-1}) - *Medicago sativa* L., *Festuca pratensis* Huds. and *Phleum pratense* L. (Schoo et al., 2017).

In Argentina, RUE values ranged from 2.0 to 1.6 g DM MJ^{-1} for 15 and 30 cm row spacing, respectively. Plant density was most affected by row spacing and it increased with narrow spacing, while the leaf area index (LAI) was also affected by row spacing. Aerial DM responses to reduced row spacing were positive due to a linear increase in PAR radiation interception and an optimal pattern in RUE (Mattera et al., 2013). In growth chamber conditions, PAR had a strong effect on alfalfa growth with significant effects on the size and mass of all yield components, and relative growth rates. Humidity effects were less evident, although still significantly increased total alfalfa leaf area (24-30%), stem mass (17-42%), shoot mass (13-33%), and height (23-24%) (Powell and Bork, 2005). Extinction coefficients for average transmittance were found to differ for alfalfa and tall fescue canopies. Canopy structure, LAI, and, to a lesser degree, the extent of direct and diffuse radiant energy were found to influence penetration more than sun angle. Leaf area altered the average ratios of above-canopy UV-B/UV-A and UV-B/PAR ratios (Shulski et al., 2004). In Romania, the maximum LAI at the first cutting ranged between 5.1 and 5.3 depending on the variety. The ELADP varied between 1.47 and 2.36 (a typical ELADP of alfalfa is 1.54 - Campbell and van Evert, 1994), while light extinction coefficient (*k*) reached 0.8. The energy transmitted to the canopy ranged between 7.4 and 22.3 μmol m^{-2} s^{-1} on average. The coefficient of absorption varied between 0.75 and 0.83 depending on variety and experimental year. RUE of alfalfa ranged between 0.23 and 0.27 g $moles^{-1}$ m^{-2} day^{-1} (Dincă and Dunea, 2018).

In this context, the rationale of the study was to assess the light interception and absorption patterns, canopy structure and aerial dry matter allocation in 27 alfalfa varieties grown in the eco-climatic conditions of Gherghita Plain, Romania, and whether the results can lead to the improvement of cropping technologies for obtaining superior forage yields. Alfalfa is a perennial crop that produces its highest yields during the second year of growth (FAO, 2019).

In this regard, the paper presents the experimental results obtained during the last harvest cycle of the second cropping year, a period that was selected to establish the growth and development patterns of the alfalfa plants before entering the winter. The obtained variables will be useful to characterize the winter hardiness of the tested varieties.

MATERIALS AND METHODS

The experiments were carried out on plots in Gherghita Plain, Puchenii Mari village (N44.824060, E26.092660). Each of the 27 varieties had three replicates and several variables have been determined for each harvest cycle: Relative Growth Rate, Tillering Rate and type (vegetative and generative), Leaf/Stem Ratio, net yield, Forage quality, canopy height, RUE, and LAI. The collection comprises varieties from Romania, other European countries, U.S.A., and Russian Federation. All plots have been maintained in the same conditions of fertilization and irrigation.

On 16 October 2019, the PAR profile was assessed before cutting using a canopy analyzer (SunScan Canopy Analysis System, Delta-T Devices Ltd., Cambridge, UK) by introducing the 1-m probe at the bottom layer of the canopy, near the ground, and positioning a beam fraction sensor (BF5 reference PAR sunshine sensor) outside the canopy (*beam fraction* is the fraction of the total incident PAR in the direct beam). The BF5 sensor consists of an array of 7 photodiodes under a specially shaped shadow mask, used for measuring direct and diffuse light above the canopy (https://www.delta-t.co.uk/wp-content/uploads/2017/02/SSI-UM_v3.3.pdf).

PAR measurements were repeated 3 times in each alfalfa variety to improve the accuracy of the measurements (Figure 1).

Figure 1. Monitoring of light interception and absorption in alfalfa canopy using a Delta-T Devices SunScan SS1 canopy analyzer and beam fraction sensors; 27 varieties have been screened for solar radiation patterns

The radiation dataset presented in this paper comprises the incident and transmitted light, zenith angle, spread, and leaf area index. Zenith angle is estimated between the center of the sun and the point directly overhead. Spread is a measure of the relative variation in light intensity along the SunScan probe that contains 64 photodiodes equally spaced along its length, being calculated as the standard deviation divided by the mean. An absorption coefficient of 0.83 and an ELADP of 1.5 were considered based on previous studies performed in Romania.

The morphological dataset contains the leaf/stem ratio – L/S (g DM leaf m^{-2}/g DM stem m^{-2}), average height of the canopy (in cm, measured in 5 points of the plot) and potential forage yield (t DM ha^{-1}).

The aboveground part of the harvested plants, separated in morphological components (stems, leaves, buds/flowers), was dried in the oven at 80°C for 24 hours to determine the accumulated aerial dry matter (ADM). The dried material was weighed with a Sartorius precision electronic balance. LAI values (m^{-2} leaves m^{-2} soil surface) were provided directly by the canopy analyzer system and they have been checked randomly using measurements of leaf area samples in a planimeter.

The fourth harvest cycle lasted 42 days from September 4 to October 16, 2019.

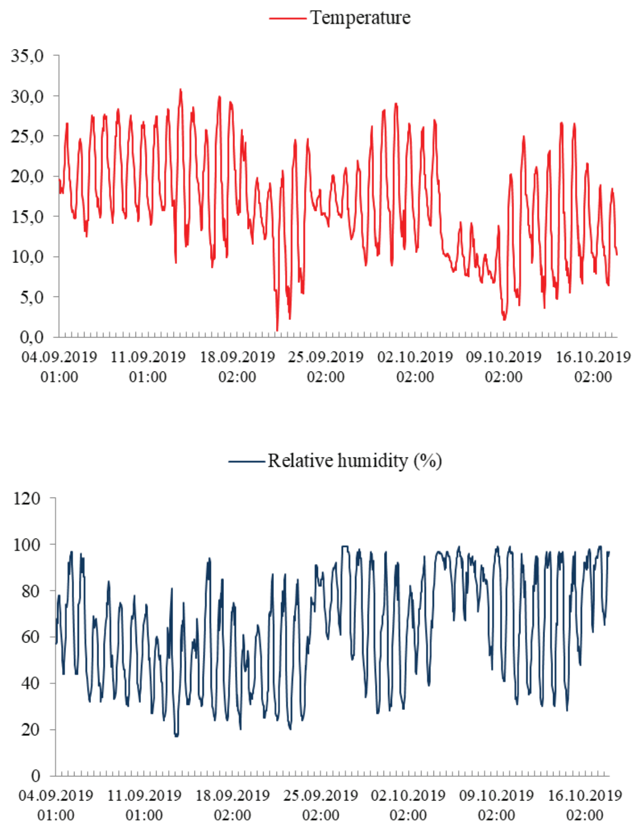

Figure 2. Air temperature (°C) and relative humidity (%) hourly time series recorded during the fourth growth cycle of alfalfa varieties

Figure 2 presents the hourly evolution of air temperature and relative humidity (RH) recorded during the fourth growth cycle in 2019. The average temperature of the period was 16.4°C (Coeff. of variation - CV = 39.02%), accumulating 720°C, while the average RH was 63.9% (CV = 36.18%). During the last day before cutting, when the radiation measurements have been performed, the daily average air temperature was 14.1°C, and RH = 82.7%.

STATISTICA program (Statsoft. Inc., Tulsa, OK, USA, 2007) was used to perform the statistical analysis of the results, i.e. Pearson's correlation to identify the strength of the linear relationship between the variables. Factor analysis (FA) using principal component analysis (PCA) based on normalized Varimax was applied to reduce the number of factors that explains the variability in the dataset of the 27 alfalfa varieties. The rotation of the factor axes (dimensions) made the structure of loadings more explicit. The input matrix began with 81 objects (variables associated to each variety × 3 replicates) by 8 variables (LAI, incident light, zenith angle, transmitted light, spread, canopy height, L/S ratio, and aerial dry matter). Based on the eigenvalues that satisfied the Kaiser criterion (>1), three factors were selected. Eigenvalues define the variance within the dataset accounted for by each factor. A factor with a low eigenvalue has a diminished contribution to the explanation of variances within variables and may be ignored (Acquaah et al., 1992).

RESULTS AND DISCUSSIONS

The measurements of the radiative conditions showed an averageDiffuse/Total PAR ratioof 0.91, with a total PAR of 347.79 μmol m^{-2} s^{-1}, and diffuse PAR of 316.9, respectively. Figure 3 presents the time series of PAR evolution during field assessments performed on 16 October 2019 between 11 a.m. and 1 p.m.

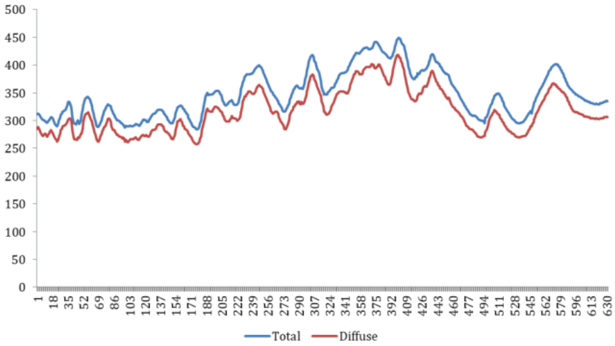

Figure 3. PAR measurements (μmol m^{-2} s^{-1}) performed on 16 October 2019 between 11 a.m. and 1 p.m. (total and diffuse time series)

Table 1 provides an overview of the statistical indicators for each variable aggregated from the measurements performed in each variety and in the surrounding radiative environment.

Table 1. Descriptive statistics of the variables measured in Puchenii Mari, Prahova County; aggregated results for 27 alfalfa varieties × 3 replicates representative for the last harvest cycle in 2019

Variables	LAI	Incident light	Zenith angle	Trans-mitted light	Spread	Height	L/S ratio	ADM	Total PAR	Diffuse PAR	Diffuse/ Total ratio
Unit	(m^2 m^{-2})	(μmol m^{-2} s^{-1})	(°)	(μmol m^{-2} s^{-1})	-	(cm)	-	(t ha^{-1})	(μmol m^{-2} s^{-1})	(μmol m^{-2} s^{-1})	-
Average	2.69	297.25	57.82	52.42	0.49	41.44	0.73	4.23	347.79	316.90	0.91
Median	2.70	295.40	57.70	48.30	0.50	40.00	0.75	4.25	338.70	310.10	0.91
Min.	0.80	242.90	56.90	24.20	0.27	30.00	0.50	2.54	284.40	257.60	0.88
Max.	3.80	344.20	58.80	136.90	0.85	60.00	0.92	5.59	449.20	418.70	0.94
Range	3.00	101.30	1.90	112.70	0.58	30.00	0.43	3.05	164.80	161.10	0.06
St. dev.	0.77	29.59	0.54	27.39	0.17	7.63	0.13	0.92	42.82	39.26	0.01
Coeff. of var. (%)	28.76	9.95	0.94	52.26	34.08	18.41	17.19	21.66	12.31	12.39	1.19
Skewness	-0.62	-0.08	0.13	1.64	0.53	0.53	-0.26	-0.27	0.50	0.55	-0.24
Kurtosis	0.33	-0.96	-1.15	3.46	-0.70	-0.50	-0.90	-0.79	-0.82	-0.67	0.02
Q1	2.25	276.45	57.40	33.65	0.34	35.00	0.62	3.80	311.00	283.20	0.90
Q3	3.20	323.50	58.25	62.40	0.62	46.00	0.82	4.98	383.30	347.90	0.92
IQR	0.95	47.05	0.85	28.75	0.28	11.00	0.20	1.18	72.30	64.70	0.01

Table 2. Pearson correlations and *p* values (2-tailed) for the interactions between the variables; ** -significant correlation at the 0.01 level (2-tailed), and *-significant correlation at the 0.05 level (2-tailed)

Variable	LAI	Inc.Light	Zenith angle	Tr.Light	Spread	Height	L/S Ratio	ADM
LAI	1	0.181	0.141	**-0.955****	0.122	0.100	0.205	0.157
		0.367	*0.484*	*0.000*	*0.544*	*0.618*	*0.306*	*0.433*
Incident Light	-	1	**-0.499****	-0.054	**0.646****	**-0.439***	-0.118	-0.188
			0.008	*0.789*	*0.000*	*0.022*	*0.557*	*0.349*
Zenith angle	-	-	1	-0.181	**-0.444***	0.265	0.294	-0.009
				0.366	*0.020*	*0.181*	*0.137*	*0.966*
Transmitted Light	-	-	-	1	-0.020	-0.118	-0.197	-0.208
					0.922	*0.558*	*0.324*	*0.297*
Spread	-	-	-	-	1	**-0.479***	-0.241	-0.339
						0.011	*0.226*	*0.084*
Height	-	-	-	-	-	1	**0.526****	**0.546****
							0.005	*0.003*
L/S Ratio	-	-	-	-	-	-	1	**0.631****
								0.000
ADM	-	-	-	-	-	-	-	1

Overall, the average results of the 27 varieties provided an LAI of 2.69 (range = 3; IQR = 0.95), canopy height of 41.44 cm (range = 30; IQR = 11), leaf/stem ratio of 0.73 (range = 0.43; IQR =0.20) and a forage yield of 4.23 t DM ha^{-1} (range = 3.05; IQR = 1.18). Forage yield reached a variation coefficient of 21.66%, while LAI had a higher coefficient (28.76%). Height and L/S ratio showed CVs of 18.41% and 17.19%, respectively. These relative reduced CV values related to the ecophysiological response of alfalfa plants to the abiotic factors suggest a moderate variability between varieties despite their originating geographical region (Table 1).

Regarding the data distribution, LAI (-0.62) and height (0.53) showed moderately skewed data, while L/S ratio (-0.27) and forage yield (-0.26) had fairly symmetrical data. Kurtosis as a measure of outliers present in the data distribution was platikurtic for all the morphological variables (kurtosis < 3), which defines light-tailed data or lack of outliers.

The radiative environment was characterized by the following means: incident light of 297.25 μmol m^{-2} s^{-1} (CV=9.95%), zenith angle of 57.82º (CV=0.94%), transmitted light of 52.42 μmol m^{-2} s^{-1} (CV=52.26%) and the spread of 0.49 (CV=34.08%). According to the Beer's law, which describes the transmission through an absorbing medium, a higher variability occurred between varieties regarding the transmitted light in correlation with LAI. The intensity has fallen off exponentially with distance through the canopy.

Table 2 presents the Pearson correlations and *p* values (2-tailed) for the interactions between the variables. There is a close negative relationship between LAI and transmitted light, (r = -0.95; $p<0.001$) suggesting that the amount of transmitted light is diminishing together with a higher LAI. Incident light is correlated with zenith angle (-0.49; $p<0.01$), spread (0.64; $p<0.001$), and canopy height (0.43; $p<0.05$).

Zenith angle is negatively correlated with spread (r = -0.44; $p<0.05$), while spread is also negatively correlated with canopy height (r = -0.48; $p<0.05$).

Canopy height shows a positive correlation with L/S Ratio (r = 0.52; $p<0.01$) and with accumulated aerial dry matter (r = 0.54; $p<0.01$). L/S Ratio is positevely correlated with accumulated aerial dry matter (0.63; $p<0.001$).

The established correlations pointed out the intensity of processes occurring in the canopies of the 27 varieties. Normally, the alfalfa canopy intercepts 95% of the incoming PAR at an LAI of 3.5 (Dincă and Dunea, 2018). From the tested collection, only five varieties reached or overpassed this threshold at the last harvest cycle.

The response of the alfalfa plants from various varieties to the radiative regime was further elucidated by the factor analysis reducing the number of variables into a few interpretable underlying factors.

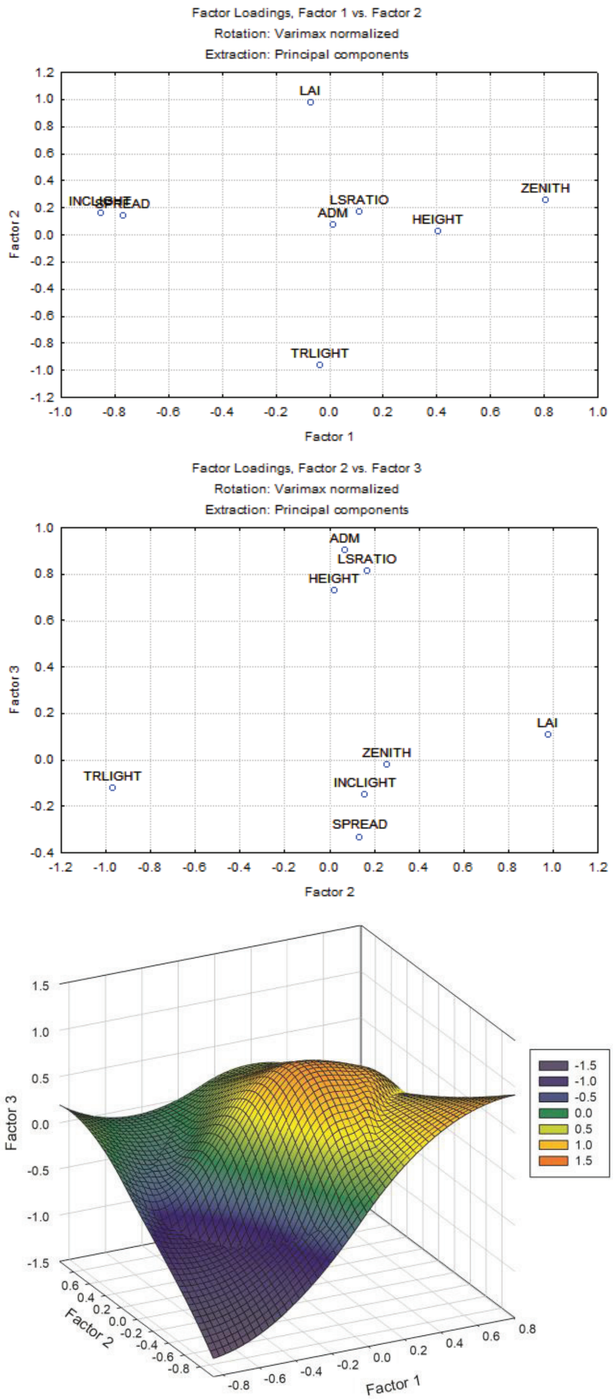

Figure 4. Plots of factor loadings resulted from the factor analysis using Varimax normalized rotation and the surface 3D plot

The response of the alfalfa plants from various varieties to the radiative regime was further elucidated by the factor analysis reducing the number of variables into a few interpretable underlying factors. Each extracted factor describes an amount of the overall variance in the observed variables (Table 3) based on the eigenvalue. Three factors with an eigenvalue ≥1 were observed explaining more variance in the dataset (79.4%) compared to a single observed variable.

Table 3. Factor extraction based on Eigenvalues: Principal components (Kaiser criterion >1)

Factor	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.98	37.25	2.98	37.25
2	2.09	26.09	5.07	63.33
3	1.29	16.11	6.36	79.44

The factor loading explains the relationship of each variable to the underlying factor. Table 4 presents the grouping of variables for each factor based on factor loadings >0.70.

Table 4. Factor Loadings (Varimax normalized) Extraction: Principal components (marked loadings are >0.70)

Variable	Factor - 1	Factor - 2	Factor - 3
LAI	-0.069	**0.978**	0.106
INC. LIGHT	**-0.853**	0.160	-0.150
ZENITH	**0.808**	0.258	-0.024
TR. LIGHT	-0.033	**-0.966**	-0.121
SPREAD	**-0.768**	0.137	-0.332
HEIGHT	0.405	0.024	**0.730**
L/S RATIO	0.113	0.167	**0.812**
ADM	0.013	0.069	**0.904**
Explained Variance	2.153	2.034	2.168
Proportion of total explained variance	0.269	0.254	0.271

The first factor is related to the "*vertical light absorption*" containing 3 variables i.e., incident light, zenith angle and SunScan probe's spread. The second factor comprises LAI and transmitted light being related to the "*absorbing medium*".
The third factor gathers three morphological variables such as canopy height, L/S ratio and forage yield. This factor is related to "*morphological and yield characteristics of the variety*". Figure 4 highlights in more detail the relationships occurring between the three factors extracted from the factor analysis. The 3D surface plot cumulates the interactions between *vertical light absorption* - *absorbing*

medium-variety's morphological and yield characteristics.

By monitoring and relating the intercepted and absorbed radiation to the incident radiation, the optical properties of the alfalfa canopy can be characterized more accurately including processes such as soil reflectance, reflectance of soil-canopy ensemble, and canopy transmittance (Brown et al., 2006). This approach optimizes the estimation of the solar radiation use efficiencies for light absorption and interception, respectively.

Light interception in uniform canopies, such as alfalfa pure crop, is mainly influenced by the LAI, the leaf area distribution on canopy layers, and leaf absorption characteristics of the species (Campbell and van Evert, 1994). Seasonal growth variations in alfalfa can be explained by seasonal incident radiation and seasonal actual evapotranspiration (Druille et al., 2017). Radiation interception increases exponentially against LAI with a mean critical value of 3.6 (Varella, 2002). Canopy architecture has an important role regarding the light interception process and in alfalfa it has an extinction coefficient ranging from 0.81 to 0.88 (Gosse et al., 1986), and a mean foliage angle of 40º in irrigated and 42º in non-irrigated conditions, respectively (Varella, 2002).

Based on the field measurements performed in the uniform canopies of 27 geographically-different varieties, the resulted variables can provide useful indicators for the parameterization of the alfalfa canopy regarding the growth and development in relationship with the radiative conditions. These experimental setup and monitoring approach will be continued in the next growth season for each harvest cycle.

CONCLUSIONS

The main findings of the experiments performed in the fourth harvest cycle of the second cropping year considering 27 alfalfa varieties were related to the plant ecophysiological and agronomic responses to the radiative environment in view of the parameterization of the alfalfa canopy in the eco-pedoclimatic conditions of Gherghita Plain considered to have a potential impact on the growth and development of the plants' canopy. The interactions between *vertical light absorption - absorbing medium - morphological and yield characteristics of the variety* have been accurately assessed using the factor analysis. These three factors have explained 79.4% of variance in the dataset of alfalfa varieties.

The mean values for the 27 varieties were as follows: LAI of 2.69 (CV=28.76%), canopy height of 41.44 cm (CV=18.41%), leaf/stem ratio of 0.73 (CV=17.19%), and a forage yield of 4.23 t DM ha^{-1}(CV=21.66%). The relative low coefficients of variation related to the ecophysiological response of alfalfa plants to the abiotic factors suggest a moderate variability between varieties despite their geographical region of origin. A positive correlation was found between canopy height and L/S Ratio (r = 0.52; p<0.01) and with forage yield (r = 0.54; p<0.01). L/S Ratio was positevely correlated with forage yield dry matter (0.63; p<0.001). The obtained indicators will be useful to characterize the winter hardiness of the tested varieties.

The screening of the alfalfa varieties collection will also provide important information regarding the selection of the most suitable varieties to be cultivated in equivalent eco-climatic conditions.

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