EVOLUTION AND RELATIONSHIP OF SOME MACRO MINERALS IN *Medicago sativa* L. PLANTS

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Abstract

Research conducted between 2013-2014, at Ezăreni Farm, Iaşi (47°05' - 47°10' north latitude and 27°28' - 27°33' east longitude) investigated at alfalfa (*Medicago sativa* L.), in the second, third, fourth and fifth vegetation year at the first cut, the influence of alfalfa plant phenophase at harvest on plant production of leaves, stems and whole plant (DM - dry matter) per hectare and leaves, stems and whole plant N (nitrogen), P (phosphorus), Ca (calcium) and Mg (magnesium) content. The experiment was laid by randomized block method in three replicates. Graduations of the studied factor were represented by the development phenophases: early bud, mid bud, late bud; early bloom, 10% bloom and full bloom. The results showed that the total production of DM was between 3.20 $Mg \cdot ha^{-1}$ (control variant) and 4.73 $Mg \cdot ha^{-1}$ (full bloom variant). At a more detailed analysis of interdependencies between mineral elements studied were highlighted some important ideas. Thus, from mineral elements studied in whole plants, between Ca and P there is the slightest interdependent relationship. The relationships N-P/P-N (R^2=0.739-0.841) and N-Mg/Mg-N (R^2=0.613-0.661) are positive, and the relationship Mg-Ca/Ca-Mg (R^2=0.576-0.653) is negative. The results can be useful to improve alfalfa fertilization system and to balance the Ca/P ratio from alfalfa plants.

Key words: nitrogen, phosphorus, calcium, magnesium, quadratic regression

Alfalfa is considered as one of the most valuable forage crops, due to high production potential, high content of protein, vitamins and minerals, high degree of digestibility, and due to the beneficial effect on soil because of the large quantities of organic matter remaining in soil with high nitrogen content.

For livestock, alfalfa is a rich source of minerals, ensuring their needs in this regard. However, the Ca content is excessive, since the Ca:P ratio in alfalfa is 5:1 or greater, as compared to the optimal ratio of 2:1 (Katić S. *et al*, 2009; Ghanizadeh N. *et al*, 2014).

Alfalfa is a large nutrients consumer plant, and a good supply of minerals to the soil (P, K, Ca, Mg, S, Co, B, Mo, Se) contributes to a normal nutrition of alfalfa plants, but also to the formation of nitrogen-fixing nodules and the their number.

According to the proportion of nutrients in the aerial part of the plant it is emphasized and highlighted its supply status. The content of alfalfa plants in minerals vary by the growth stage at harvest. This highlights the alfalfa need for nutrients differently in each phenological stage.

Regardless of the many factors which have influence on the quality of alfalfa, in the case of mineral content, time of the harvest and the anatomical plant part has a great influence (Ibriz M. *et al*, 2004; Coblentz W.K. *et al*, 2008; Stancheva I. *et al*, 2008).

The study carried seeks to show how the moment when alfalfa is harvested influence the production of dry matter and the content of N, Ca, P and Mg from the leaves, stems and alfalfa whole plants, as well as the relation of dependence between these minerals.

MATERIAL AND METHOD

The research was conducted between 2013-2014, at Ezăreni Farm, Iaşi (47°05' - 47°10' north latitude and 27°28' - 27°33' east longitude). The soil is a cambic chernozem, characterized by pH 6.73, 40.3% clay, humus 2.32%, 0.164% total nitrogen, P-Al 18 ppm, 210 ppm K-Al.

Research investigated at alfalfa (*Medicago sativa* L.), in the second, third, fourth and fifth vegetation year at the first cut, the influence of alfalfa plant phenophase at harvest on plant production of leaves, stems and whole plant (DM - dry matter) per hectare and leaves, stems and whole plant N (nitrogen), P (phosphorus), Ca (calcium) and Mg (magnesium) content.

The experiment was laid by randomized block method, with a harvested area of 10 m^2 (2 m

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x 5 m) in three replicates. Graduations of the studied factor were represented by the development phenophases: early bud (v_1), mid bud (v_2), late bud (v_3); early bloom (v_4), 10% bloom (v_5) and full bloom (v_6), phenological phases described by Kalu B.A., Fick G.W., 1981; Ball S.T., 1998; Barnes R.F., 2007; Mueller S.C., Teuber L.R., 2007.

Production was determined by weighing the yield obtained from a harvested surface of 10 m^2, which was afterwards transformed per hectare. The dry matter was determined by drying in an oven at 103ºC for 3 hours. The leaves and stems productions were calculated based on the leaves/stems ratio.

The leaves/stems ratio was determined by separating the petiole, leaflets, buds and flowers from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems).

The nitrogen content was determined with the Kjeldahl method, the magnesium and calcium content was determined with the atomic absorption spectrometry and the phosphorus content was determined with the spectrophotometric method.

The biological material used was the alfalfa variety Sandra (F 660-94), registered in 2003 at National Agricultural Research and Development Institute (NARDI) Fundulea, Bucharest (Schitea Maria, Martura T., 2004). Harvesting was carried out with 511 Bertolini mowers, at a height of 7 cm above the ground.

The data were interpreted statistically by means of variance analysis and limit differences calculation. Also, equation correlations were calculated (quadratic regression significance) between all four mineral elements (N, P, Ca and Mg).

RESULTS AND DISCUSSIONS

With the aging of plants, the whole plants and stems dry matter production has grown continuously. Leaf DM production has grown until the late bud - early bloom stages, after which decreased. The phenomenons that directly influence the production quality is represented by the etiolation, drying and leaf fall from the lower floors of the stem with the appearance of the first flowers. Thus, when the plants are harvested later than early blooming, leaf production will be lower, and also a lower forage quality (Spada Maria del Carmen, 2013; Geleti D. *et al*, 2014; SirAlkhatem M., Gabr S.A., 2014).

The analysis of growth stage at harvest on the production of leaves obtained show that differences have tended to increase until early bloomng (v_3) then decreased, but the values were higher than the control variant (statistically assured).

The stems production under the influence of growth stage at harvest has tended to increase with plants aging, the highest yield of stems, respectively 3.42 $Mg \cdot ha^{-1}$ DM, being obtained from plants harvested at full bloom (v_6). Compared to the control variant, all the differences obtained in all other harvesting growth stages were positive, distinctly significant (*table 1*).

Total production ranged from 3.20 $Mg \cdot ha^{-1}$ DM (from control variant - v_1) and 4.73 $Mg \cdot ha^{-1}$ DM (at variant harvested at full bloom - v_6).

Growth stage at harvest represents the decisive factor that influences the mineral elements content in alfalfa plants (*table 1*).

Leaves/stems ratio depends by the moment of the harvest, which directly influence the chemical composition from alfalfa forage. In this case, the leaves meant petiole, leaflets, buds and flowers separated by the stems. In the field, the percentage of leaves and stems was kept constant until the beginning blooming after which the leaves from the bottom floors of stems began to fall. Thus, at the full bloom stage there are only a few floors of leaves in the top of the stems.

The proportion of buds and flowers has increased consistently since the early bud stage to full blooming stage. So if plants are harvested at the full blooming stage production will be made from stems, flowers and a few floors leaves only (Hosseinzadeh-Moghbeli A. H. *et al*, 2013; Ghanizadeh N. *et al*, 2014; Madani H. *et al*, 2014).

With plants aging, the amount of nitrogen, phosphorus and magnesium in leaves, stems and whole plants of alfalfa has been steadily declining. The calcium amount is growing only in stems and whole plants, fact proved by studies of different authors (Dale Laura Monica *et al*, 2009; Radović J. *et al*, 2009; Katić S. *et al*, 2009). Also, with plants aging, Ca/P ratio from leaves, stems and whole plants has grown.

At a more detailed analysis of interdependencies between mineral elements studied, they have emerged some important aspects. Thus, in the leaves, there is a positive correlation, distinctly significant, between the Mg-P/P-Mg (R^2=0.821-0.869) and N-Ca/Ca-N (R^2=0.582-0.685). This result indicates the possibility of increasing P content from alfalfa leaves (is known that phosphorus, in the case of alfalfa plant is a defective element that influences the Ca/P ratio) and, also it shows that intake of nitrogen contribute to the increasing of leaves Ca content (*table 2*).

In the studied case (*table 2)*, in stems there is a positive correlation between elements N, P and Mg, but, unlike leaves, the accumulation of calcium in stems resulted in a decrease in content of other minerals studied.

Table 1

Influence of growth stage at harvest on the alfalfa DM yield and minerals content

Leaves

Experimental plot	DM yield	N	P	Ca	Mg	Ca/P
	$Mg \cdot ha^{-1}$	$g \cdot kg^{-1}$ from DM				ratio
v_1 - early bud (control)	1.21^{C}	50.22^{C}	3.82^{C}	27.23^{C}	4.77^{C}	7.13^{C}
v_2 - mid bud	1.42**	50.07	3.01^{oo}	27.27	4.34^{oo}	9.07**
v_3 - late bud	1.53**	49.24^{oo}	2.46^{oo}	27.51	4.08^{oo}	11.19**
v_4 - early bloom	1.52**	46.35^{oo}	2.28^{oo}	27.85	3.88^{oo}	12.23**
v_5 - 10% bloom	1.38**	44.53^{oo}	2.11^{oo}	27.52	3.27^{oo}	13.04**
v_6 - full bloom	1.31*	43.04^{oo}	2.02^{oo}	27.49	2.85^{oo}	13.60**
$LSD_{0.05}$	*0.08*	*0.68*	*0.09*	*0.88*	*0.11*	*0.35*
$LSD_{0.01}$	*0.12*	*0.97*	*0.13*	*1.24*	*0.15*	*0.50*

Stems

Experimental plot	DM yield	N	P	Ca	Mg	Ca/P
	$Mg \cdot ha^{-1}$	$g \cdot kg^{-1}$ from DM				ratio
v_1 - early bud (control)	1.99^{C}	23.40^{C}	2.55^{C}	7.23^{C}	2.75^{C}	2.85^{C}
v_2 - mid bud	2.55**	21.57^{oo}	2.40^{oo}	8.10**	2.12^{oo}	3.40**
v_3 - late bud	2.82**	19.83^{oo}	2.32^{oo}	9.69**	2.00^{oo}	4.20**
v_4 - early bloom	3.04**	18.24^{oo}	2.31^{oo}	11.06**	1.93^{oo}	4.82**
v_5 - 10% bloom	3.30**	16.44^{oo}	2.27^{oo}	12.53**	1.85^{oo}	5.56**
v_6 - full bloom	3.42**	15.37^{oo}	2.26^{oo}	13.73**	1.82^{oo}	6.12**
$LSD_{0.05}$	*0.17*	*0.29*	*0.04*	*0.33*	*0.07*	*0.16*
$LSD_{0.01}$	*0.24*	*0.42*	*0.06*	*0.47*	*0.10*	*0.22*

Whole plant

Experimental plot	DM yield	N	P	Ca	Mg	Ca/P
	$Mg \cdot ha^{-1}$	$g \cdot kg^{-1}$ from DM				ratio
v_1 - early bud (control)	3.20^{C}	34.88^{C}	3.07^{C}	15.27^{C}	3.57^{C}	5.02^{C}
v_2 - mid bud	3.97**	32.88^{oo}	2.63^{oo}	15.50	2.97^{oo}	5.89**
v_3 - late bud	4.36**	30.68^{oo}	2.37^{oo}	16.05*	2.75^{oo}	6.79**
v_4 - early bloom	4.56**	27.81^{oo}	2.29^{oo}	16.85**	2.60^{oo}	7.38**
v_5 - 10% bloom	4.69**	25.15^{oo}	2.22^{oo}	17.13**	2.29^{oo}	7.74**
v_6 - full bloom	4.73**	23.12^{oo}	2.19^{oo}	17.64**	2.11^{oo}	8.08**
$LSD_{0.05}$	*0.23*	*0.28*	*0.07*	*0.56*	*0.04*	*0.22*
$LSD_{0.01}$	*0.33*	*0.39*	*0.10*	*0.79*	*0.06*	*0.32*

^{C} - control; * - Significant at the 0.05 probability level; ** - Significant at the 0.01 probability level.

Higher values of the regression coefficient with statistical significance were obtained between relationships Ca-Mg/Mg-Ca (R^2=0.844-0.946) and Ca-N/N-Ca (R^2=0.529-0.532).

Leaves/stems ratio influences mineral composition of whole plants. Thus when stems will have greater weight, the value of their content in minerals or dependence of these will be more influential.

Table 2

Interdependent relationships of the main minerals in alfalfa

	Content of	+/-	Equation	R^2
Leaves				
Correlation between	N-P	+	$y = 0.009x^2 - 0.722x + 16.22$	0.496*
	N-Ca	+	$y = -0.030x^2 + 2.967x - 44.48$	0.582**
	N-Mg	+	$y = 0.004x^2 - 0.311x + 8.582$	0.331
	P-N	+	$y = -5.610x^2 + 36.89x - 8.605$	0.621**
	P-Ca	+	$y = -0.961x^2 + 5.612x + 19.76$	0.046
	P-Mg	+	$y = -0.616x^2 + 4.468x - 3.356$	0.821**
	Ca-N	+	$y = -0.656x^2 + 37.41x - 483.8$	0.685**
	Ca-P	+	$y = -0.105x^2 + 5.733x - 74.94$	0.289
	Ca-Mg	+	$y = -0.140x^2 + 7.552x - 97.28$	0.519*
	Mg-N	+	$y = -0.309x^2 + 5.860x + 29.34$	0.313
	Mg-P	+	$y = 0.626x^2 - 3.911x + 8.118$	0.869**
	Mg-Ca	+	$y = 0.101x^2 - 1.149x + 30.35$	0.028
Stems				
Correlation between	N-P	+	$y = -0.000x^2 + 0.069x + 1.368$	0.683**
	N-Ca	-	$y = 0.015x^2 - 1.057x + 24.85$	0.532**
	N-Mg	+	$y = 0.001x^2 - 0.000x + 1.496$	0.486*
	P-N	+	$y = 2.479x^2 + 8.563x - 14.75$	0.672**
	P-Ca	-	$y = -16.55x^2 + 69.12x - 60.28$	0.339
	P-Mg	+	$y = 4.500x^2 - 19.57x + 23.12$	0.668**
	Ca-N	-	$y = 0.084x^2 - 2.908x + 39.75$	0.529**
	Ca-P	-	$y = 0.010x^2 - 0.258x + 3.828$	0.395
	Ca-Mg	-	$y = 0.031x^2 - 0.767x + 6.507$	0.844**
	Mg-N	+	$y = -19.40x^2 + 97.22x - 97.08$	0.634**
	Mg-P	+	$y = -0.331x^2 + 1.858x - 0.044$	0.545**
	Mg-Ca	-	$y = 16.44x^2 - 81.64x + 107.3$	0.946**
Whole plant				
Correlation between	N-P	+	$y = 0.001x^2 - 0.034x + 2.085$	0.739**
	N-Ca	-	$y = -0.016x^2 + 0.794x + 7.440$	0.632**
	N-Mg	+	$y = 0.000x^2 + 0.044x + 0.9$	0.613**
	P-N	+	$y = -15.81x^2 + 94.89x - 107.0$	0.841**
	P-Ca	-	$y = -0.584x^2 + 1.166x + 17.13$	0.350
	P-Mg	+	$y = 0.168x^2 + 0.449x + 0.572$	0.792**
	Ca-N	-	$y = 1.214x^2 - 43.15x + 408.9$	0.571**
	Ca-P	-	$y = 0.070x^2 - 2.501x + 24.49$	0.424*
	Ca-Mg	-	$y = -0.006x^2 - 0.140x + 6.769$	0.576**
	Mg-N	+	$y = -4.517x^2 + 33.48x - 27.48$	0.661**
	Mg-P	+	$y = 0.280x^2 - 1.000x + 3.050$	0.832**
	Mg-Ca	-	$y = 1.221x^2 - 8.591x + 30.45$	0.653**

* - Significant at the 0.05 probability level; ** - Significant at the 0.01 probability level.

In the real situation, alfalfa is not harvested separately as leaves or stems. Production is represented by whole plants. Thus, in their case, it was observed that the interdependence between mineral elements is similar to stems, because they are in a higher percentage than the leaves.

Among the elements studied in whole plants, between Ca and P there is the slightest interdependent relationship. Relationships N-P/P-N (R^2=0.739-0.841) and N-Mg/Mg-N (R^2=0.613-0.661) are positive and the relationship Mg-Ca/Ca-Mg (R^2=0.576-0.653) is negative (*table 2*).

The results can be useful to improve alfalfa fertilization system and to balance the Ca/P ratio from alfalfa plants, which is 1/5-1/8 to optimum 1/2-1/3 for different animal species. Thus, a higher intake of nitrogen accumulated through an effective symbiosis, will increase P content from plants, and an optimal insurance of Ca requirements will cause a decrease in Mg content. These aspects are related to the fact that alfalfa grows well in soils rich in calcium, in which symbiotic efficiency is higher.

CONCLUSIONS

With alfalfa plants vegetation advancement, whole plant and stems dry matter production was growing.

Leaf dry matter production increased until the late bud - early bloom stages, after that period has decreased.

During growth stages succession total nitrogen content from the leaves, stems and whole plants of alfalfa has been steadily declining and influenced increasingly more by the content of stems in total nitrogen content as their proportion in production is increasingly higher, and the phosphorus content of whole plants, leaves and stems of alfalfa decreased.

During the time between the early bud and full bloom stages, the calcium content from whole plant, leaves and stems of alfalfa was growing; the alfalfa whole plants, leaves and stems Mg content was negatively correlated with growth stages succession.

Among the items studied minerals in whole plants, between Ca and P there is the slightest interdependent relationship (R^2 = 0.298-0.350), insignificant.

Relations N-P/N-P (R^2 = 0.739-0.841) and N-Mg/Mg-N (R^2 = 0.613-0.661) are positive, and the relationship Mg-Ca/Ca-Mg (R^2 = 0.576-0.653) is negative, all three they were significant and distinctly significant.

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REFERENCES

Ball S.T., 1998 - *Alfalfa Growth Stages*. New Mexico State University, Guide A-330, available on-line at: http://lubbock.tamu.edu/othercrops/pdf/alfalfa/nmsugrowthstages.pdf

Barnes R.F., 2007 - *Forages: The Science of Grassland Agriculture*, Chapter 4: Growth and Development of Forage Plants. p. 53-66.

Coblentz W.K., Brink G.E., Martin N.P., Undersander D.J., 2008 - *Harvest Timing Effects on Estimates of Rumen Degradable Protein from Alfalfa Forages*. Crop Science, 48:778-788.

Dale Laura Monica, Rotar I., Mogoş A., Florian V., Gârda Nicoleta Teodora, 2009 - *Research on protein content and total nitrogen for Medicago sativa using FT-NIR spectrometry*. Research Journal of Agricultural Science, 41(3):50-55.

Geleti D., Hailemariam M., Mengistu A., Tolera A., 2014 - *Biomass yield potential and nutritive value of selected alfalfa (Medicago sativa* L.*) cultivars grown under tepid to cool sub-moist agro-ecology of Ethiopia*. Journal of Agricultural Research and Development, 4(1):7-14.

Ghanizadeh N., Moghaddam A., Khodabandeh N., 2014 - *Comparing the yield of alfalfa cultivars in different harvests under limited irrigation condition*. International Journal of Biosciences, 4(1):131-138.

Hosseinzadeh-moghbeli A. H., Khosrowchahli M., Monirifar H., Noormohammadi G., 2013 - *Evaluation of ten alfalfa (Medicago sativa* L.*) ecotypes for salinity stress tolerance*. Technical Journal of Engineering and Applied Sciences, 3(17):2131-2133.

Ibriz M., Thami Alami I., Zenasni L., Alfaiz C., Benbella M., 2004 - *Production des luzernes des régions pré-sahariennes du Maroc en conditions salines*. Fourrages, 180:527-540.

Kalu B.A., Fick G.W., 1981 - *Quantifying Morphological Development of Alfalfa for Studies of 1 Herbage Quality*. Crop Science, 21:267-271.

Katić S., Milić D., Karagić Đ., Vasiljević S., Glamočić D. şi Jajić I., 2009 - *Variation of protein, cellulose and mineral contents of lucerne as influenced by cultivar and cut*. Biotechnology in Animal Husbandry, 25(5-6): 1189-1195.

Madani H., Stoklosa Agnieszka, Zarei J., Usefi Z., 2014 - *Alfalfa (Medicago sativa* L.*) forage yield responses to triple super phosphate, phosphate solublizing bacteria and gibberllic acid foliar application*. Scientific Papers. Series A. Agronomy, 57:246-249.

Mueller S.C., Teuber L.R., 2007 - *Alfalfa Growth and Development*, IN (**C. G. Summers** and **D. H. Putnam**, eds.), *Irrigated alfalfa management for Mediterranean and Desert zones*, Chapter 3. University of California Agriculture and Natural Resources Publication 8289.

Radović J., Sokolović D., Marković J., 2009 - *Alfalfa-most important perennial forage legume in animal husbandry*. Biotechnology in Animal Husbandry,

25(5-6):465-475.
Schitea Maria, Martura T., 2004 - *Sandra, un nou soi de lucernă creat la I.C.D.A. Fundulea*. Anual I.C.D.A. Fundulea, 71:181-190.
SirAlkhatem M., Gabr S.A., 2014 - *Effect of water stress on growth and yield of alfalfa (Medicage stavia* L.*)*. Journal of Agri-Food and Applied Sciences, 2(5):134-138.
Spada Maria del Carmen, 2013 - *Red de evaluacion de cultivares de alfalfa*. Ensayos Territoriales, 23:1-74.
Stancheva I., Geneva M., Djonova E., Kaloyanova N., Sichanova M., Boychinova M., Georgiev G., 2008 - *Response of alfalfa (Medicago sativa* L.*) growth at low accessible phosphorus source to the dual inoculation with mycorrhizal fungi and nitrogen fixing bacteria*. General and Applied Plant Physiology, 34(3-4):319-326.