オーチャードグラスの異なる器官及び番草のミネラル含有率評価に対するX線マイクロ分析の応用

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Application of X-ray Microanalysis to Evaluate Mineral Concentrations of Different Organs and Growths in Orchardgrass (*Dactylis glomerata* L.)

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Synopsis

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To assess the ability of X-ray microanalysis for evaluation of mineral concentrations of different organs and growths in orchardgrass, the relations between chemical and X-ray analyses were investigated for K, Ca Mg and P related to grass tetany and hypocalcemia. Six orchardgrass plants with high- or low-Mg concentrations were examined for three growths in 1995. The samples were separated into leaf blade, sheath, culm and head. Mg concentrations in leaf and in head were higher than in sheath and in culm. Correlations between chemical and X-ray analyses were high in leaf blade and low in culm. Among four elements, Mg showed highest correlations for all of four organs and three growths. Mg concentrations of high group selected by X-ray analysis data were significantly higher than those of low group. These high screening effects indicate high possibility to apply X-ray analysis to screen orchardgrass individuals for Mg at seedling stage.

Key words : Minerals, Mg, Orchardgrass, Screening, X-ray microanalysis.

Introduction

Several grass breeders have much interest to improve cultivars with low potential of animal disorder caused by mineral balance in forages. In the determination of mineral concentrations, wet chemical analysis is the most common method when breeders screen forage plants. However, wet chemical analysis is time-consuming procedure, and is not always suitable to screen forage plants. Then, the use of near infrared reflectance spectroscopy (NIRS) was assessed by several scientists[2,5]. They concluded that plant breeder could be used NIRS to select a large number of individuals for some elements.

The X-ray microanalysis is likely to be useful for determination of mineral concentrations for small samples of forage including seedlings, due to its efficiency and the fact that several mineral concentrations are determined simultaneously. Authors[4] tried to apply X-ray microanalysis in screening of orchardgrass for the second and third growths. As the result, high possibility was found in Mg for both growths, and in K and Ca only for the second growth. The first growth was excluded from their study because mineral concentrations were depended on the proportion of plant organs in orchardgrass. This study was to assess the ability of X-ray microanalysis in screening orchardgrass populations for the first growth which consists of four organs including head, culm, leaf blade, and leaf sheath. As these organs vary in mineral concentrations, the possibility to apply X-ray microanalysis may depends on the organs. In this study, to begin with, mineral concentrations were compared among growths, plant organs and parts by wet chemical analysis. Then, the possibility to apply X-ray microanalysis was investigated by the relations of wet chemical analysis with X-ray analyses and the tentative screening effects. The objective elements were K, Ca, Mg and P related to grass tetany and hypocalcemia.

Materials and Methods

1. Forage sample

Six orchardgrass (3 high- and 3 low-Mg concentration) plants were examined for three growths in 1995 (Table 1). These were the highest 3 and the lowest 3 plants selected for Mg concentration of the third growth in 1991 using NIRS. Basic population for selection was 499 orchardgrass plants. The reason why these 6 plant were used for materials was to deal population with wide range in Mg concentration.

For the first growth, about 5 heading stems per plant were taken at the height of 6cm (refer to Fig. 1) on the 7th day after the initial heading date. The heading date was shown in Table 1. Plant residue after sampling was cut off on July 2 when the last sample was taken. For the second and third growths, a handful of grass samples was taken on August 9 and October 10, respectively.

2. Preparation of samples

Samples for the first growth were separated into four organs : leaf blade (leaf), leaf sheath (sheath), culm and head. The second and third growths were separated into leaf blade and sheath. Furthermore, samples of the first growth were separated into upper half and lower half at the mid point of each culm for the comparison of plant parts as indicated in Fig. 1. Each sample was dried at 80℃ for 24 hours in a forced-air oven. After weighing the sample, ground to pass a 1 mm screen in a cyclone mill. These samples were used for both chemical and X-ray analyses. Mean dry weight of each organ of 6 plants was head : 11.4 g, upper leaf blade : 12.0 g, upper leaf sheath : 13.0 g, upper culm : 5.7 g, lower leaf blade : 5.3g, lower leaf sheath : 5.7 g, and lower culm : 12.1 g for the first growth. Mean dry weight of leaf blade and sheath were 39.6 g and 7.9 g for the second growth, and 8.4 g and 0.9 g for the third growth, respectively.

3. Wet chemical analyses

After samples were ashed in nitro-perchloric acid (1 : 1, v/v), K, Ca and Mg were determined using atomic absorption spectroscopy, and P by colorimetry as molybdovanado phosphoric acid.

4. X-ray microanalysis

For X-ray microanalysis, ground samples were ashed at 600℃ for two hours. The quantitative analysis of minerals was determined with the energy-dispersive X-ray (EDX) apparatus, EMAX-2770, attached with scanning electron microscopy S-2300. Efficient time of detection was 100 second. The X-ray weight density was used as a quantitative indication. The other parameters for the measurements were described in Saiga *et al.*[4].

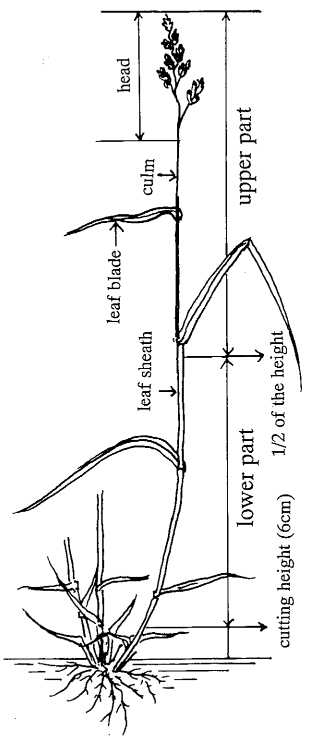

Fig. 1. Schematized orchardgrass plant of the first growth and its plant part.

5. Statistical calculation

When a sample was separated into organs and parts, the dry matter of each organ and part was weighed. Mineral concentration of each growth or each part was calculated based on the weight proportion of each organ. Statistical analysis was made by SAS statistics at the Computer Center of Iwate University.

Results

1. Comparison of mineral concentration among growths and plants

Mineral concentration was determined by wet chemical analysis in each organ of each part. To know the characteristics of materials, mineral concentrations were compared among growths, plants, organs and parts. In the comparison of mineral concentrations among these, weighted average was calculated using dry weight of each organ. Analysis of variance was done independently for growths, plants, organs and part, and replication was number of plants (n=6) or number of growths (n=3) (Table 1, Table 2).

In Table 1, mineral concentrations were compared among growths and among plants. There were significant differences among growths. Mg concentration increased as the season progressed. Phosphorous concentration was higher at the second and third growths, and K at the second growth. There was no significant difference for Ca, but the mean value was higher in the third growth.

There were significant differences among orchardgrass plants for Mg, P, and K. Especially, Mg concentration was higher in two high-Mg plants. Calcium concentrations in these two high-Mg plants tend to be higher than in the other plants, but the difference was not significant.

2. Comparison of mineral concentration among growths and parts of the first growth

Table 2 shows the comparison among organs and between parts.

Magnesium concentrations in leaf and head were higher than in sheath and in culm. Phosphorous concentration was highest in head, and lowest in sheath. Sheath and culm contained higher K than leaf and head. Calcium was contained higher in leaf than in head and culm. Potassium, Ca, Mg and P concentrations in the highest organ were 118%,

Table 1. Head emergence date and mean concentrations of minerals in the forages of three growths and 6 plants examined.

Item	n[1)]	Heading[2)]	K	Ca	Mg	P
Growth			(% DM)			
First	6	May 25	3.19[c3)]	0.276[a]	0.114[c]	0.306[b]
Second	6	—	4.58[a]	0.271[a]	0.197[b]	0.369[a]
Third	6	—	3.90[b]	0.381[a]	0.241[a]	0.370[a]
Plant[4)]						
H-1	3	May 13	3.79[abc]	0.393[a]	0.224[ab]	0.382[a]
H-2	3	May 16	3.17[c]	0.369[a]	0.247[a]	0.346[ab]
H-3	3	May 24	4.43[a]	0.248[a]	0.177[bc]	0.375[a]
L-1	3	May 24	4.18[ab]	0.307[a]	0.166[c]	0.352[ab]
L-2	3	May 22	4.10[ab]	0.294[a]	0.155[c]	0.297[b]
L-3	3	May 26	3.65[bc]	0.248[a]	0.137[c]	0.339[ab]

1) Growth : number of plant, plant : number of growths.
2) Head emergence date.
3) Means followed by the different alphabets in the same column differ at $p<0.05$.
4) H-1, 2, 3, are high Mg plants, and L-1, 2, 3 are low Mg plants.

Table 2. Comparison of mineral concentrations among organs, and parts for the first growth.

Item	n[1)]	K	Ca	Mg	P
Organ		(% DM)			
Leaf	6	2.83[c2)]	0.427[a]	0.150[a]	0.294[bc]
Sheath	6	4.43[a]	0.301[ab]	0.098[b]	0.239[c]
Culm	6	3.76[b]	0.202[b]	0.103[b]	0.336[ab]
Head	6	2.03[d]	0.215[b]	0.130[a]	0.401[a]
Part[3)]					
Upper 1	6	3.01[b]	0.267[a]	0.120[a]	0.348[a]
Upper 2	6	3.47[a]	0.306[a]	0.118[a]	0.309[b]
Lower	6	3.67[a]	0.262[a]	0.117[a]	0.294[b]

1) Number of plants.
2) Means followed by the different alphabets in the same column differ at $p<0.05$.
3) Lower : leaf+sheath+culm, Upper 1 : with head, Upper 2 : without head.

111%, 53% and 68% higher than in the lowest organ, respectively.

Mineral concentration was compared between plant parts. When head was included in upper part, there were significant differences in P and in K. Phosphorous was higher in upper part, and K was higher in lower part, while there was no significant difference in any mineral element when head was excluded from upper part.

3. Correlation between wet chemical and X-ray analyses

Correlations between wet chemical and X-ray analyses were shown in Table 3. Correlation coefficients calculated using all samples were $r=0.226$, 0.531***, 0.715***, and 0.553***, for K, Ca, Mg, and P, respectively.

When these samples were separated into each organ, correlations of leaf were significant for Ca, Mg and P. Those of head and sheath were significant only for Mg and P. In culm, any significant correlation was not obtained for all of four elements.

Correlation coefficients were calculated for each growth. For the first growth, correlations were significant for all of four elements. Especially high correlations were obtained for Mg and P. For the second growth, high correlations were obtained for Mg and Ca, and for Mg and K for the third growth. Among four elements, Mg showed the highest correlations for all of four organs and three growths.

Relations between chemical and X-ray analyses of Mg data were shown in Fig. 1. Regression equations of the first to the third growths were $y_1=0.075$

$+0.022\,x_1$, $y_2=0.067+0.058\,x_2$, $y_3=0.053+0.056\,x_3$, respectively. The b (slope) values of the second and third growths were close, but not with that of the first growth.

4. Effect of a tentative screening

As showed in Table 2, plant organs vary greatly in these mineral concentrations. The samples of the first growth can be regarded as a large population. Then the tentative screening was tried to prospect the possibility to use X-ray analysis in screening (Table 4). In this part, the effect of a tentative screening means the difference in mineral concentration between two populations separated at the mean value of the X-ray analysis data.

When all samples, pooled all growths, plants, organs and parts, were separated into two groups of high and low concentrations in each element, the statistically significant differences were obtained for Mg, Ca and P between two groups. In the population of leaf, sheath, the first growth, and the second growth, high significant differences were obtained for Mg. Other large differences ($p<0.01$) were obtained for Ca of the first growth and for P of the first growth, but any significant difference was not obtained for K. Compared with in the first growth, significance in the second and third growths was low.

There was a possibility that higher significance

Table 3. Correlation coefficient between chemical and X-ray analysis.

Group	n	K	Ca	Mg	P
All samples	60	0.226	0.531*** 1)	0.715***	0.553***
Organ					
Leaf	24 2)	0.348	0.476*	0.702***	0.587**
Sheath	18 3)	0.455	0.329	0.678**	0.513*
Culm	12 4)	0.104	0.285	0.331	−0.531
Head	6	0.570	−0.789	0.875*	0.828*
Growth					
First	42 5)	0.313*	0.509**	0.718***	0.721***
Second	12 6)	−0.022	0.712**	0.935***	0.213
Third	6	0.815*	0.293	0.850*	0.213

1) *, ** and *** Significant at $p<0.05$, 0.01, 0.001 level, respectively.
2) ((First : upper+lower)+second+third)×6 plant.
3) ((First : upper+lower)+second)×6 plants.
4) (First : upper+lower)×6 plant.
5) (Leaf+sheath+culm)×(upper+lower)×6 plants.
6) (Leaf+sheath)×6 plants.

Table 4. Mineral concentrations of two groups separated by X-ray analysis data and the significance of the difference.

Item	Organ	n 1)	K High	K Low	Ca High	Ca Low	Mg High	Mg Low	P High	P Low
			(% DM)		(% DM)		(% DM)		(% DM)	
All samples		60	4.02	3.95 ns	0.356	0.248** 2)	0.178	0.111***	0.356	0.304*
Organ										
	Leaf	24	3.65	3.35 ns	0.399	0.331 ns	0.236	0.159***	0.365	0.298*
	Sheath	18	5.56	4.57 ns	0.281	0.263 ns	0.135	0.101*	0.332	0.241*
	Culm	12	5.01	3.66 ns	0.271	0.165 ns	0.098	0.097 ns	0.348	0.297 ns
	Head	6	2.06	1.85 ns	0.320	0.110 ns	0.143	0.116 ns	0.564	0.369 ns
Growth										
	First	42	3.70	3.45 ns	0.386	0.231**	0.143	0.099***	0.352	0.264***
	Second	12	5.29	5.19 ns	0.276	0.234 ns	0.214	0.143*	0.385	0.364 ns
	Third	6	4.44	3.63 ns	0.405	0.357 ns	0.314	0.205 ns	0.377	0.365 ns
	Regrowth 3)	18	5.07	4.44 ns	0.362	0.245**	0.250	0.152***	0.378	0.370 ns

1) Refer to Table 3.
2) *, ** and *** Significant at $p<0.05$, 0.01, 0.001 level, respectively.
2) Pooled data of the second and third growths.

was not obtained because of small sample size. Samples of the second and third growths can be regarded as one population of regrowth, and then the difference of two groups were calculated in the pooled regrowth. As the results, high significant differences were obtained for Mg and Ca.

Discussion

1. Possibility to use X-ray microanalysis in screen orchardgrass plants

The technique of EDX has now been applied to biological materials[1,3]. Authors[4] investigated the possibility to use X-ray microanalysis for the second and third growths of orchardgrass 37 plants. Then, the high possibility was found in Mg for both the second and third growths, in K and Ca only for the second growth, and low possibility was found in P for both growths. In this study using 6 selected plants, correlations between chemical and X-ray analyses were high in Mg for all groups except for culm (Table 3). In addition, the results of tentative screening showed high effect for all samples, leaf, the first growth and regrowth (Table 4). The Mg concentrations of high group were higher by 0.067 unit (60.3%), 0.077 unit (48.4%), 0.044 unit (44.4%) and, 0.098 unit (64.5%) than low group, respectively. Non-significant difference observed for head could be caused by small sample size (n=6).

The X-ray microanalysis is useful for determination of mineral concentrations for small samples of forage including seedlings. The high screening effect obtained in leaf and regrowth which consists of leaf and sheath, indicates high possibility to apply X-ray analysis to screen orchardgrass individuals for Mg at seedling stage.

2. Accuracy of X-ray microanalysis

When the correlations between wet chemical analysis and X-ray analysis were calculated in each plant organ, significant correlation was not obtained for all of four elements in culm. As one of the reasons, the difference in flatness of sample surface is considered. In the preparation of samples for the X-ray analysis, we tried to brake plant particles using stainless steal spoon and crucible, and to make sample surface flat as much as possible. We knew an empirical fact that culm is more tough than the other organs even after ashed. When the sample surface is rough, X-ray will be absorbed and not contribute to the X-ray detector[6]. This is probably resulted in low accuracy of the X-ray analysis. The result indicates the importance of smooth surface of the sample in the X-ray microanalysis.

3. Growths and organs suitable for X-ray analysis

On the practical screening, it is hard and not efficient to evaluate for all growths of large population. If mineral concentrations of a certain growth were similar to that of the other growth, we can aim for the growth. Then correlations were calculated between growths using Mg data of 6 orchardgrass

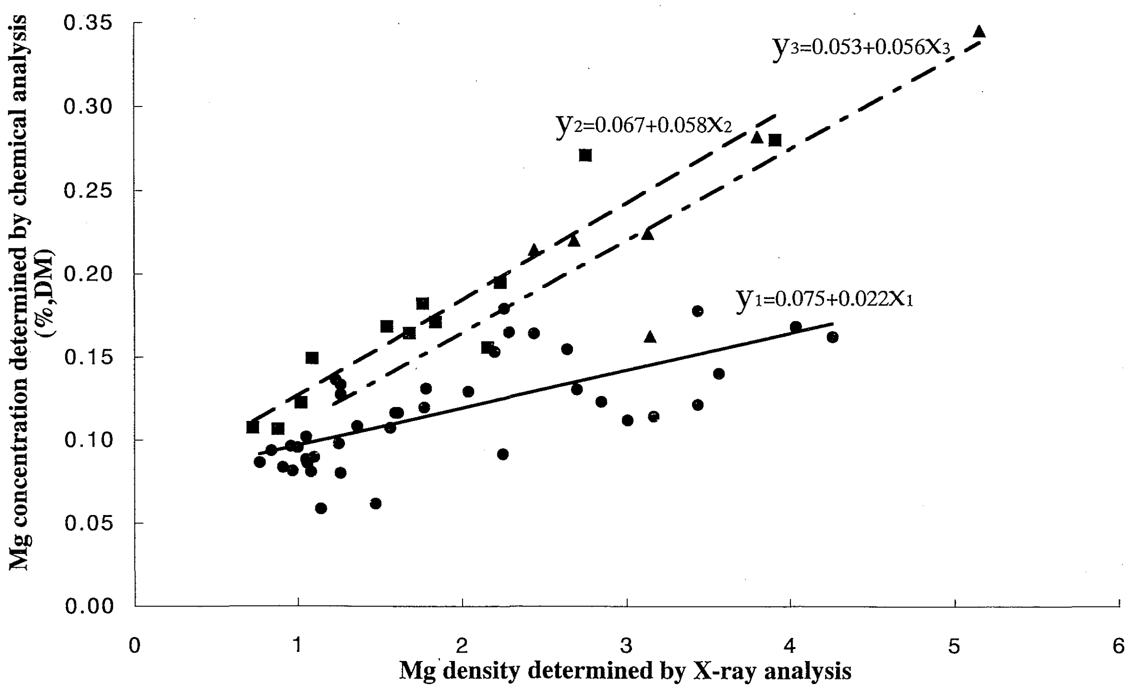

Fig. 2. Relationship between chemical and X-ray analysis for Mg concentration.
Regression functions : ●, ——— : First growth, ■, ------ : Second growth, ▲, -·-·-· : Third growth.

plants. As the result, the correlation between the second and third growths was significantly high ($r=0.950^{**}$, $n=6$), while the other combinations were not significant ($r=0.785$ and 0.617). As indicated in Fig. 2, the b value of the second and third growths were close. However, the b value of the first growth was not close with those of the other growths. Data of head affect to this results, and relations between chemical and X-ray analyses were different in head from in other organs. In another word, Mg density in head was overestimated by X-ray microanalysis. Although the correlation between chemical and X-ray analyses was high in head, data of head can not be pooled with the other organs. As indicated before, the possibility to apply X-ray analysis is low in culm. These indicate samples of the second and third growths may be more suitable for the object of screening, because any head and any culm is not contained.

References

1) McManus, W.R., R.G. Anthony, L.L. Grout, A.S. Malin, and V.N.E. Robinsn (1979) Biocrystallization of mineral material on forage plant cell walls. *Aust. J. Agric. Res.* **30**, 635-649.

2) Saiga, S., T. Sasaki, K. Nonaka, K. Takahashi, M. Watanabe, and K. Watanabe (1989) Prediction of mineral concentrations of orchardgrass (*Dactylis glomerata* L.) with near infrared reflectance spectroscopy. *J. Japan. Grassl. Sci.* **35**, 228-233.

3) Saiga, S., H. Oikawa, M. Sasaki, and H. Takusari (1993) Distribution of macrominerals in different tissues of a leaf blade of tall fescue (*Festuca arundinacea* Schreb.). *J. Japan. Grassl. Sci.* **39**, 86-92.

4) Saiga, S. and K. Izumi (1997): Comparison of X-ray microanalysis methods in screening mineral concentration for orchardgrass (*Dactylis glomerata* L.) plants. *Grassland Science* **43**, 18-23.

5) Smith, K.F., S.E. Willis and P.C. Finn (1991) Measurement of the magnesium concentration in perennial ryegrass (*Lolium perenne*) using near infrared reflectance spectroscopy. *Aust. J. Agric. Res.* **42**, 1399-1404.

6) Wood, J. (1993) X-ray mapping techniques in biology. *In* X-ray microanalysis in biology (*Eds.* D.C. Sigee, A.J. Morgan, A.T. Sumner and A. Warley) Cambridge Univ. Press, Cambridge. pp. 25-46.

要　　旨

雑賀　優・西村良恵・泉幸一郎　(1997)：オーチャードグラスの異なる器官及び番草のミネラル含有率評価に対するＸ線マイクロ分析の応用. *Grassland Science* **43**, 111-116. 岩手大学農学部 (020 盛岡市上田 3-18-8)

オーチャードグラスの異なる器官及び番草のミネラル含有率評価に，Ｘ線マイクロ分析を応用できるかの可能性を探るためにグラステタニーと起立不能症に関係した４元素について化学分析とＸ線分析結果の関係を調べた。供試材料は，オーチャードグラスの高 Mg 3 個体と低 Mg 3 個体の 6 個体で，1995 年の 1，2，3 番草で，各番草とも葉身，葉鞘，稈及び穂に分け，1 番草は更に上部と下部に分けた。それぞれの器官，部位ごとに化学分析を行った結果でミネラル含有率を比較すると，葉身と穂の Mg 含有率は葉鞘と稈のそれに比較して高く，P は穂で高く葉鞘で低かった。4 元素のうち Mg は化学分析とＸ線分析結果の相関が最も高く，模擬選抜結果でも葉身と再生草で高かったことから，Ｘ線分析をオーチャードグラスの Mg 含有率に対する幼苗選抜に使える可能性が示唆された。

キーワード：Ｘ線分析，オーチャードグラス，個体選抜，マグネシウム，ミネラル.