POSSIBILITY OF SELECTION FOR MINERAL CONCENTRATION IN ORCHARDGRASS BY X-RAY MICROANALYSIS

S. Saiga, Y. Nishimura and K. Izumi

Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka 020, Japan

ABSTRACT

To assess the ability of X-ray microanalysis for screening breeding populations of orchardgrass in mineral concentration, correlations between chemical and X-ray analysis were investigated. Six orchardgrass plants with high- and low-Mg concentrations were examined for three harvest dates in 1995. The samples were separated into leaf blade, leaf sheath, culm and head. Mg concentration in leaf and head were higher than in sheath and in culm. P concentration was highest in the head, and lowest in the sheath. Correlations between chemical and X-ray analysis were r=0.692***, 0.526***, 0.252* and 0.562*** for Mg, Ca, K and P, respectively. Correlation coefficients between chemical and X-ray analysis were highest for Mg among 4 minerals. X-ray microanalysis can be used to screen orchardgrass plants for Mg concentration at initial growth stage.

KEYWORDS

Grass tetany, minerals, Mg, orchardgrass, screening, X-ray microanalysis

INTRODUCTION

Grass breeders have been giving increasing attention to developing strains with low potential for animal disorders caused by mineral imbalances. Chemical analysis is the most common method in the determination of mineral concentrations. However, chemical analysis is a time consuming procedure, and is not always suitable for screening of forage plants. The use of near infrared reflectance spectroscopy (NIRS) has been assessed by several scientists (Saiga et al., 1989, Smith et al., 1991). They concluded that plant breeders could use NIRS to screen a large number of individuals for some mineral elements but need a small size calibration for new population.

X-ray microanalysis can easily perform elementary analysis within a small range. X-ray microanalysis is likely to be useful for determination of mineral concentrations for small forage samples including seedlings, due to its efficiency and the fact that several mineral concentrations are determined simultaneously. This study was to assess the ability of X-ray microanalysis for screening breeding populations of orchardgrass for mineral concentration. Difference in mineral concentrations among selected plants, regrowth periods, plants organs, and plant parts were compared by chemical analysis. Then, the correlations between chemical analysis and X-ray analysis were calculated.

MATERIALS AND METHODS

Forage sample: Six orchardgrass plants with high- and low-Mg concentrations were examined for three harvest dates in1995 (Table 1). For the first regrowth, a part of the plants was sampled on the 7th day after the initial heading date, then the residue was cut off on 2 July after all plants were sampled. The second and third growths were sampled on August 9 and October 10, respectively.

Samples from the first regrowth were separated into four organs: leaf blade (leaf), leaf sheath (seath), culm and head. The second and thirds regrowths were separated into leaf and sheath. Furthermore, samples of the first regrowth were separated into upper- and lower-half to compare plant parts.

All samples were dried at 80C for 24 hours in a forced-draft oven and ground to pass a 1.00 mm screen in a cyclone mill. These samples were used for both chemical and X-ray analysis. The samples for X-ray analysis were ashed at 600C for two hours.

Chemical analysis: 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.

X-ray microanalysis: 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 seconds. The other parameters for the measurements were described by Saiga et al., (1993).

RESULTS AND DISCUSSION

Comparison of mineral concentration among regrowths and plants: There were significant differences among regrowth periods for mineral concentrations. Mg concentration increased as the season progressed. Phosphorous concentrations were higher at the second and third regrowth, and K at the second regrowth. There was no significant difference for Ca, but the mean was higher at the third regrowth.

There were significant differences among orchardgrass plants for Mg, P, and K. Especially, Mg concentration was higher in two high-Mg plants. These plants were selected for high Mg concentration from the second regrowth in 1992. There were no clear relations between high- and low-Mg plants in other mineral concentrations. Differences between highest and lowest plants for Ca concentration were comparatively large, but were not statistically significant.

Differences in mineral concentration among organs and parts from the first regrowth: Table 1 shows the comparison among plant organs and between plant parts. Magnesium concentration in the leaf and in the head were higher than in the sheath and culm. Phosphorous concentration was highest in the head, and lowest in the sheath. The sheath and culm contained higher K than the leaf and the head. Calcium concentration was higher in the leaf than in the head and culm.

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

Correlations between chemical and X-ray analysis: Correlations between chemical and X-ray analysis are shown in Table 2. Correlation coefficients calculated using all data were r=0.692***, 0.526***, 0.252* and 0.562*** for Mg, P, K and P, respectively.

When these samples were separated into individual plant organs, correlations for the leaf sample were significantly high for all 4 minerals. Those of the head and sheath were significantly high only for Mg and P. In the culm, significant correlations were not obtained for all four minerals. These differences may be due to the differences in flatness of the sample surface. When the sample surface is rough, X-ray's will be absorbed and not contribute to the X-ray detector. This probably resulted in low accuracy of X-ray analysis. Even after samples were ashed at 600C, toughness of the culm would have remained.

Correlations were calculated in each regrowth period. For the first regrowth, correlations were significantly high for all four minerals. Especially high correlations were obtained for P and Mg. In the second regrowth, high correlations were observed for Mg and Ca, and for Mg and Ca in the third regrowth.

CONCLUSIONS

Correlation coefficients between chemical and X-ray analysis were highest for Mg among all 4 minerals. These correlations indicate possibility to apply X-ray microanalysis in screening large numbers of orchardgrass plants was obtained for Mg concentration. Furthermore, X-ray microanalysis, unlike normal chemical analysis, permits the identification of elements with very small samples. There is a possibility of screening at initial growth stage (seedlings).

REFERENCES

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

Saiga, S., T. Sasaki, K. Nonaka, K. Takusari, 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.

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

Table 1

Comparison of mineral concentrations among plant organs and parts for the first regrowth period in orchardgrass.

Item	n	K	Ca	Mg	P
Plant organ					
Leaf	6	2.83 c[1]	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	0.215 b	0.130 a	0.401 a
Plant part					
Upper 1[2]	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) Means followed by the different letters in the same column differ at p<0.05.
2) Lower: leaf + sheath + culm, Upper 1: with head, Upper 2: without head

Table 2

Correlation coefficient between chemical and X-ray analysis for mineral concentration in orchard grass.

Group	n	Mg	P	K	Ca
All samples	70	0.692***	0.526***	0.252*	0.562***
Plant organ					
Head	7	0.873*	0.829*	0.529	0.683
Leaf	28	0.695***	0.574**	0.374*	0.553**
Sheath	21	0.668**	0.482*	0.431	0.221
Culm	14	0.220	0.619*	0.184	0.283
Regrowth					
First	49	0.649***	0.712***	0.326*	0.523**
Second	14	0.936***	0.199	0.030	0.728**
Third	7	0.850*	0.226	0.808*	0.312

*, ** and *** Significant at p <0.05, 0.01, 0.001 level, respectively.