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SCREENING FORAGE GRASSES WITH ATOMIC ABSORPTION SPECTROMETRY, X-RAY FLUORESCENCE AND X-RAY MICROANALYSIS

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ABSTRACT

Breeding cool-season (C3) grasses with higher magnesium (Mg) content is a promising attempt for reducing grass tetany hazard in ruminants. Faster methods for plant mineral analyses could increase the number of individual plants screened for higher Mg content (High-Mg). This study evaluates the effectiveness of energy dispersive X-ray microanalysis (EDX) as well as energy reflectance X-ray spectrometry (XRF) for screening high-Mg grass genotypes. The approach was verified by using two tall fescue cultivars having known differences in magnesium (Mg) content, viz. HiMag (high-Mg cultivar) and Ky-31 (control cultivar). We assumed that cultivars with known variation in Mg concentrations could provide a test for the applicability of the new methodology in finding naturally occurring high and low Mg containing grass genotypes. Plants samples included a population of 8 plants consisting of four harvests for three years and were analyzed for Mg, calcium (Ca), and potassium (K) by EDX and ERF, and data were verified with atomic absorption spectrometry wet (AAS). While observing the frequency distribution for different nutrient concentrations, HiMag tall fescue showed higher Mg and lower K concentrations than that of Ky-31. There was positive linear relationship between AAS and EDX estimated Mg, Ca and K ($r = 0.88$, 0.62 and 0.89, respectively), indicating close agreement between AAS and EDX estimation. Also, there was a positive linear relationship between AAS and XRF, as the r values were 0.87, 0.65 and 0.88 for Mg, Ca, and K, respectively. The tetany ration was established for EDX and XRF and the results were dependable with wet chemistry.

Keywords: High-Mg C_3 grasses, Mineral, Screening, Grass Tetany Index

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INTRODUCTION

Throughout the world, grass tetany causes economic losses from death or reduced performance of livestock. Grass tetany triggered by nutritional imbalances and related with available K, Ca and Mg concentration in forages (Kemp and t' Hart, 1957). Attempts to reduce or eliminate the incidence of grass tetany have been focused on selective breeding of forages for higher levels of Mg and lower tetany potential (Asay et al., 2001). As a consequence, Magnet Italian ryegrass, Mgwell orchardgrass, and HiMag tall fescue were bred as the high-Mg cultivars of Italian ryegrass, orchardgrass and tall fescue, respectively (Hides and Thomas, 1981; Saiga et al., 2002; Mayland and Sleper, 1993). There is a continued need to breed high-Mg cultivars since the malady of grass tetany causes considerable economic loss in agriculture. Forage breeding programs for improving mineral concentrations are labor and time intensive. More efficient methods for plant analyses for minerals e.g., potassium (K), calcium (Ca) and magnesium (Mg) associated with grass tetany are necessary. Mineral element analyses by conventional methods (e.g. Titrimetric, Flame photometric, Spectrophotometric, AAS) are costly, laborious and time consuming. If faster methods of mineral analyses could be introduced, family selections by forage breeders could be speeded up and the number of individuals screened could be increased (Sleper, 1979).

Atomic absorption spectrometry is a standard and most popular technique for measuring plant nutrients. However, energy dispersive X-ray microanalyzer (EDX) analysis is a rapid method for measuring the chemical composition of plants using ashed samples. Recent research has used EDX for mineral analysis in ashed plant samples (Hodson and Sangster, 2002). Also, applicability of X-ray microanalysis (EDX) to screen orchardgrass individuals for Mg at the seedling stage has been suggested (Saiga et al., 2002). X-ray microanalysis can give the relative density of the selected mineral elements in ashed plant samples (Saiga and Izumi, 1997; Saiga et al., 1997), and thus the values could be used as general guideline for screening. Apart from this, energy reflectance X-ray spectrometry (XRF) data represent the actual mineral element concentration, but with easy sample preparation (Hutton and Norrish, 1977; Norrish and Hutton, 1977). On the other hand, AAS (wet chemistry), a widely used standard method for mineral analysis is expensive, time consuming, laborious and requires lots of attentions. This suggests that EDX and XRF should be efficient method for screening high-Mg genotype. Making comparisons of the EDX and XRF analysis data with AAS can confirm the use of these two methods for the screening of high-Mg plants. In the present study, efficacy of both the methods for mineral analyses was verified by using known high-Mg cultivar of tall fescue (*Festuca arundinacea* Schreb.), named as HiMag comparing with a control tall fescue cultivar Kentucky 31 (Ky-31). Apparently, the differences between these two tall fescue cultivars exists in their Mg content and this will emphasize the suitability of EDX and XRF for determining forage Mg content. In addition to Mg concentrations of Ca and K were also measured as those elements are responsible for tetany risk in ruminant.

MATERIALS AND METHODS

Plant materials and growth conditions

Two tall fescue (*Festuca arundinacea* Schreb.) cultivars consisting high-Mg and control cultivars namely, HiMag and Kentucky 31 (Ky-31), respectively, were used in this study. Germinated seeds of the cultivars were grown in small plastic box containing vermiculite for 70 days in a controlled environment of phytotron. The air temperature of the phytotron was maintained at 25°C day/15°C night, and the photoperiod was maintained at 14 h. Relative humidity was approximately 75% and the light intensity was 280-μmol photons m^{-2} s^{-1}. Fifty seedlings of similar growth from each cultivar were transplanted in the experimental field (Uedai: Upper grassland: 39°41'59.81"N; 141°09'0.04"E) in Morioka of Iwate University, Morioka, Japan, in a distance of 30 by 40 cm to an area of $6m^2$. The soil was characterized as Umbric Andosol, pH 6.0, with exchangeable cations: K^+, 1.22 $cmol_c$/kg; Ca^{2+} 22.10 $cmol_c$/kg; and Mg^{2+} 4.37 $cmol_c$/kg.

Plant sampling and field management

Eight plants from each cultivar were randomly selected for analysis and considered as experimental unit for four harvests. Plants were sampled (6 cm cutting height) at four sampling date of May 28 (Cut 1), June 16 (Cut 2); August 4 (Cut 3) and November 6 (Cut 4), while sampling height of the plants were in the range of 50-60 cm and the plants were in vegetative stage without any heading. After collecting sample, leftover plants in the field were harvested and thrown away. Then dolomite (one metric ton/ha), nitrogen (140 kg/ha), P_2O_5 (280 kg/ha) and K_2O (140 kg/ha) were applied to the field and left for regrowth for next sampling. Samples from different year (3yrs) and seasons (4 cuts/harvests) were collected to have much environmental influence in the mineral nutrient content of plant population.

Nutrient analyses of plant samples

Plant samples were dried at 70°C for 48 hours, and ground to pass a 1 mm screen with a cyclone mill. This sample was used for determining Mg, Ca, and K by the three methods. A subsample of dried plant (0.5g) was digested with nitric-perchloric (2:1) acid mixture (Jackson, 1973) and concentrations of magnesium (Mg), calcium (Ca), and potassium (K) in the solutions were analyzed by the Atomic Absorption Spectrophotometer (Perkin Elmer 3300). Each sample was replicated three times and the results were expressed as concentration (% DM) of each cultivar. Dried plant subsamples (1.0g) were ashed in crucibles at 600°C for 3 hours. Mineral density in the ashed shoot was determined by the energy dispersive X-ray (EDX) analyzer

(JED-2140) attached with scanning electron microscope (JSM-5800LV) as described by Saiga and Izumi (1997) to investigate the mineral density in plants. The EDX analyzer was used in the scanning mode with the following conditions: raster size 1×1 cm^2 at ×90 magnification; emission current, 79µÅ; accelerating voltage, 15 kV; specimen working distance, 10 mm; tilt angle of 35º; detector to specimen distance, 40 mm; and live time, 100s. Each sample was replicated three times by the EDX analyzer. The results obtained from EDX analyzer were converted to weight % basis.

Each plant subsample of 0.5 to 1.0 g was pressed (with a coherent disc of 2.5 cm) by using 15 tons of pressure to form a pellet with a uniform surface (Rahman and Saiga, 2007a). Experience has shown that some samples give trouble due to swelling if allowed to take up moisture from the air. The pellets should therefore be stored in a desiccator or prepared using plant powder which has been allowed to come to equilibrium with the atmosphere (Rahman and Saiga, 2007b). Magnesium, Ca, and K contents of both sides of the pellet were measured with a live time of 100s by energy reflectance X-ray fluorescence (XRF) spectrometry (JEOL Co., JSX-3200, Element Analyzer) as described by Hutton and Norrish (1977) and Norrish and Hutton (977). Each plant sample was replicated two times and the results were calculated as dry matter percentage (% DM). The results were presented with an average of four harvests.

Calculation and statistical analysis

Grass tetany (GT) index was calculated according to Kemp and ’t Hart (1957) based on average data obtained from three methods. Data for mineral element concentrations (as determined by three analytical methods) were used in developing multiple regression equations and calculating standard deviation (SD) and coefficient of variance (CV) by Microsoft Excel. The Tukey test was performed for comparison of means (SAS Institute, Cary, NC, USA). Average data for 3 years and 4 cuts with 8 plants were employed in this study.

RESULTS AND DISCUSSION

Frequency distribution of Mg, Ca and K concentrations as determined by wet chemistry of eight tall fescue samples were presented in Fig 1. We assumed that cultivars with known variation in Mg concentrations could provide a test for the applicability of the new methodology in finding naturally occurring high and low Mg containing grass genotypes. Magnesium concentrations of HiMag tall fescue ranged from 0.30 to 0.54% DM, while that of Ky-31 ranged from 0.15 to 0.30% DM. Calcium concentrations among the populations did not vary distinctly. In the case of K concentrations, the opposite trend to Mg concentrations was observed. The K concentrations of HiMag tall fescue were lower than that of Ky-31. Potassium concentrations of HiMag tall fescue ranged from 1.3 to 2.5% DM while that of Ky-31 ranged from 2.9 to 4.4%. Potassium concentrations in a large number of plants were distributed from 1.6 to 2.2% DM for HiMag while that of Ky-31 was from 3.5 to 3.8% DM. These results were in correspondence with previous findings (Wilkinson and Mayland, 1997; Sabreen et al., 2003; Sabreen and Saiga, 2004; Sabreen et al., 2004).

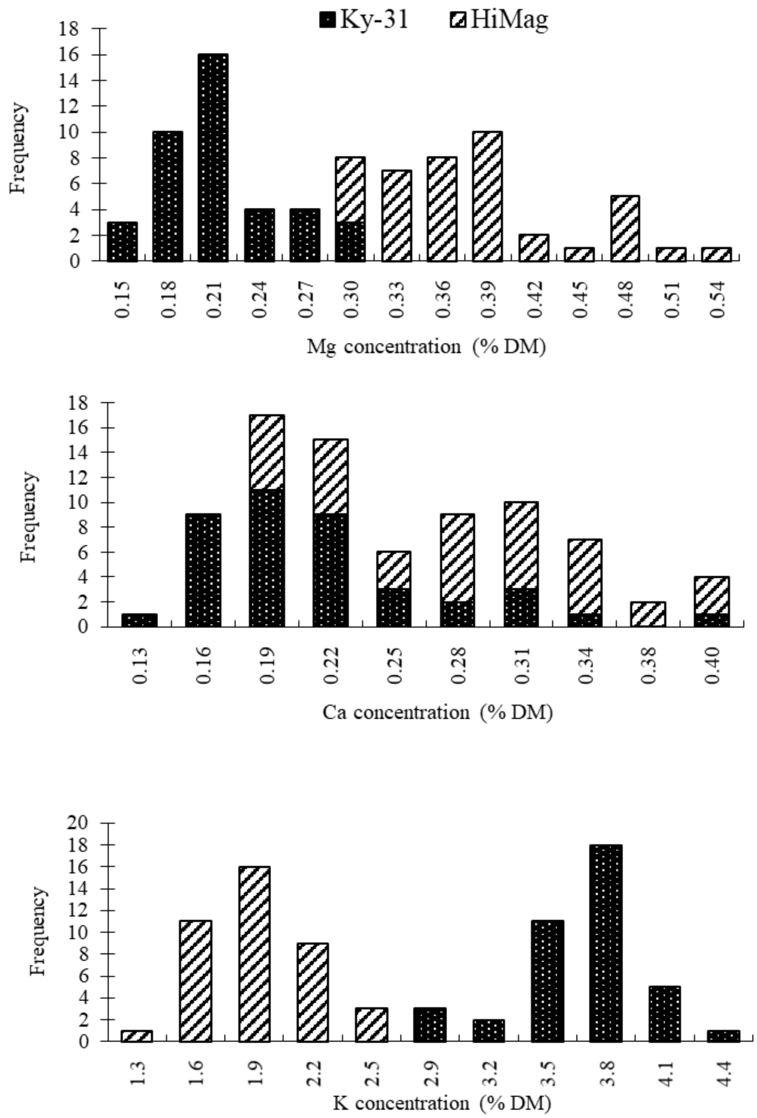

Figure 1. Frequency distribution for Mg, Ca, and K concentrations of eighty plants of two tall fescue cultivars as determined by wet chemistry.

The known high-Mg cultivar HiMag tall fescue was compared with Ky-31 to test the effectiveness of EDX and XRF analysis for screening high-Mg genotypes. Considering wet chemistry as a standard method both EDX and XRF analysis data were verified. Statistical data and equation calibration statistics for elemental concentrations determined by various analytical methods were shown in Table 1.

Table 1. Statistical data (n = 96) for Mg, Ca and K concentrations (%DM) of two tall fescue cultivars as determined by AAS, EDX and XRF.

Element	Analytical method	Forage	Range	Mean	SD[1]	CV (%)[2]
Mg	Wet chemistry	High-Mg	0.165-0.540	0.306	0.10	32.66
		Ky-31	0.211-0.641	0.341	0.11	35.56
	EDX	High-Mg	0.261-1.258	0.591	0.26	40.58
		Ky-31	0.299-1.321	0.348	0.29	41.24
	XRF	High-Mg	0.145-0.697	0.298	0.14	38.48
		Ky-31	0.154-0.769	0.435	0.15	36.01
Ca	Wet chemistry	High-Mg	0.144-0.421	0.264	0.07	26.50
		Ky-31	0.154-0.434	0.275	0.10	28.32
	EDX	High-Mg	0.703-1.335	0.929	0.16	17.23
		Ky-31	0.777-1.446	0.909	0.14	15.21
	XRF	High-Mg	0.218-0.649	0.369	0.10	25.16
		Ky-31	0.245-0.687	0.341	0.12	26.24
K	Wet chemistry	High-Mg	1.347-4.472	2.880	0.92	31.94
		Ky-31	1.457-4.997	3.714	0.99	32.24
	EDX	High-Mg	3.057-7.057	6.884	1.07	21.91
		Ky-31	3.554-7.152	6.485	1.12	22.14
	XRF	High-Mg	1.559-5.289	3.166	0.97	30.64
		Ky-31	1.614-5.352	4.655	0.98	31.21

[1]Standard deviation; [2]Coefficient of variance.

Magnesium concentrations for high Mg fodder as determined by wet chemistry ranged from 0.165 to 0.540% DM (average 0.306% DM). Corresponding values for Ca and K were 0.144 to 0.421% DM (average 0.264 %DM), and 1.347 to 4.472% DM (average 2.880% DM). The corresponding EDX data for Mg, Ca and K concentrations were 0.591, 0.929 and 6.884 weight%, respectively. On the other hand, the corresponding XRF values for average Mg, Ca and K concentrations were 0.298, 0.369 and 3.166 %DM, respectively. Means and standard deviations are listed to show the concentration level and variation among analytical methods.

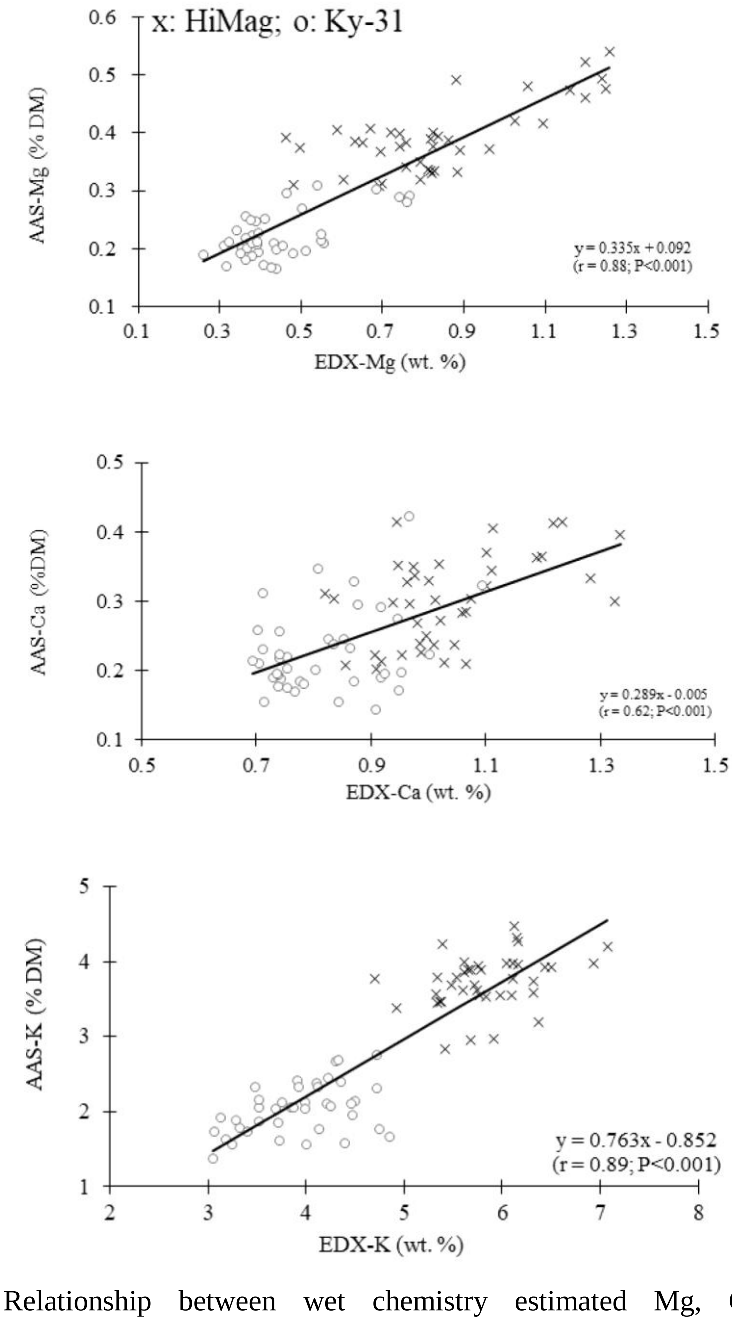

Figure 2. Relationship between wet chemistry estimated Mg, Ca and K concentrations and EDX estimated Mg, Ca and K content for eight plants of two tall fescue cultivars

X-ray microanalysis allows the use of small amounts of plant sample without the destruction of the whole plant. Efficient use of X-ray microanalysis for screening high-Mg plants can shortened the time consumed for mineral analysis for improving mineral concentrations of forage plants. The relationship between wet chemistry and EDX estimated Mg, Ca and K concentrations were shown in Fig. 2. Correlation coefficients for Mg, Ca, and K were r = 0.88, 0.62, and 0.89, respectively. This indicates the positive linear relationship between wet chemistry and EDX estimated Mg, Ca and K. Thus, close agreement between wet chemistry and EDX estimation is apparent. Apart from this, the slope for Mg and Ca was deviated from one, even though that of K was close to one. The result suggests that by using the regression equations for Mg, Ca and K effective screening for high-Mg plants can be done, as the r values were high.

The EDX estimated Mg, Ca and K is an indication of the proportion of these three mineral elements responsible for grass tetany. If the balance among these three elements is disturbed forage has increased possibility of causing grass tetany (Sleper et al., 1989). This emphasizes the importance of determining the proportion of these elements while screening high-Mg plants. The coefficient of variance inferred that even though EDX cannot estimate the exact mineral concentration this method is efficient enough to screen high-Mg genotypes if the difference within the population for Mg is large (Table 1).

The relationship between wet chemistry and XRF estimated Mg, Ca and K was shown in Fig. 3. There was a positive linear relationship between wet chemistry and ERF, and the correlation coefficient (r) values were 0.87, 0.65 and 0.88 for Mg, Ca, and K, respectively. A good calibration equation should result in a slope very close to 1.0, and this is the case for Ca (slope = 0.88) and K (slope = 0.92), while Mg had a slope of 0.60. These data indicate that the equations for Ca and K had accuracy (low bias) and precision and provided good estimates of validation samples (slope near 1.0). However, the slope for Mg was deviated from one, the intercept is near zero. This may have been due to the distinct variation for Mg concentration between high-Mg (HiMag) and control (Ky-31) cultivar of tall fescue. High correlation coefficient value for Mg is (r = 0.87) indicates the applicability of XRF analysis for screening high-Mg plants. The efficiency and dependability of X-ray fluorescence (XRF) spectroscopy to analyze macro- and micronutrients in pea (*Pisum sativum* L.) seeds were performed by Bamrah et al. (2019). They observed that the R^2 value was more than 0.85 for all studied elements except K (0.54). Based on the results they conferred that the using XRF for elements analysis is compatible with AAS method.

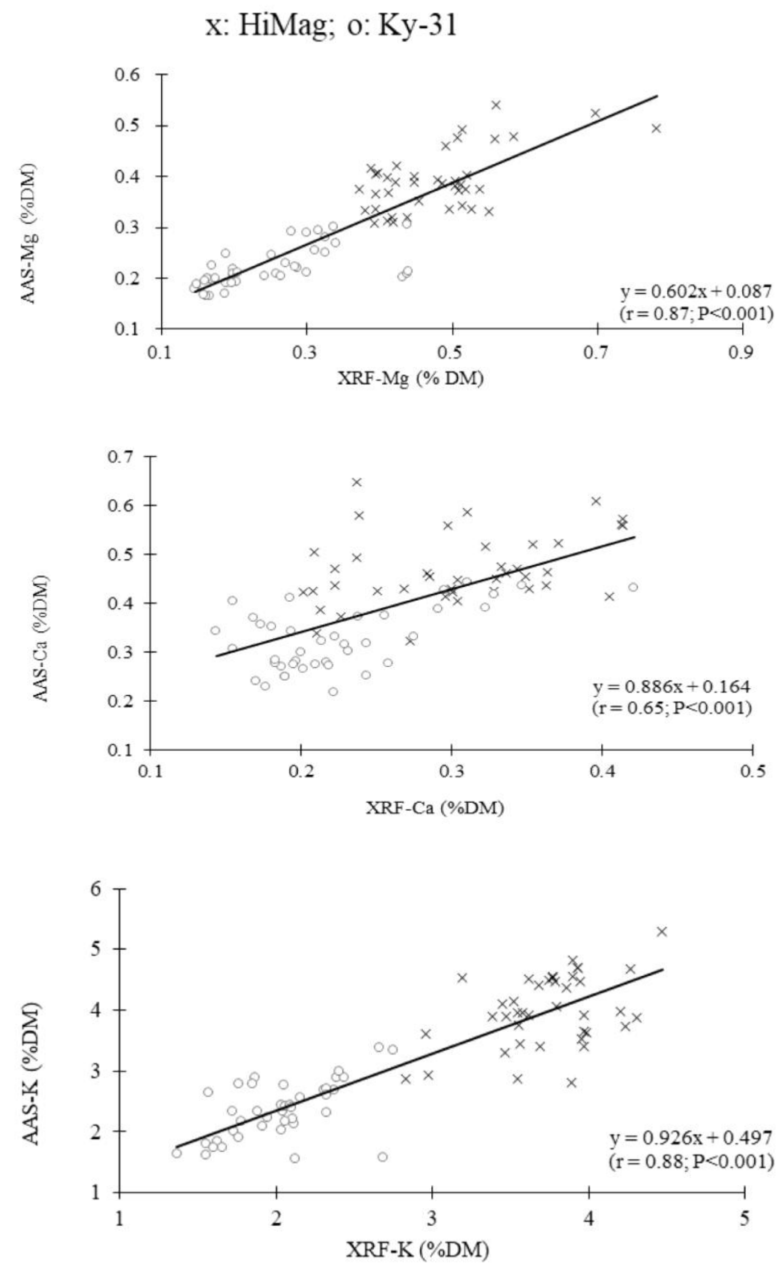

Figure 3. Relationship between wet chemistry estimated Mg, Ca and K concentrations and ERF estimated Ma, Ca and K content for eight plants for two tall fescue cultivars

The grass tetany (GT) index calculation was done as GT = K/Ca+Mg (Table 2) with average data for four cuts of eight plant for each cultivar reared for three years. The highest values for GT index were observed for AAS methods, followed by XRF and the lowest GT index were recorded for EDX. Irrespective of methods higher grass tetany index was recorded in Ky-31 compared to High-Mg cultivar.

Table 2. Grass tetany index of two tall fescue cultivars[1]

Analytical method	Forage	K/Ca+Mg	SD[2]	CV, %[3]
AAS	High-Mg	1.91	0.25	11.87
	Ky-31	2.26		
EDX	High-Mg	1.85	0.27	13.17
	Ky-31	2.23		
XRF	High-Mg	1.88	0.25	12.36
	Ky-31	2.24		
AAS	High-Mg	1.91	0.03	1.60
EDX	High-Mg	1.85		
XRF	High-Mg	1.88		
AAS	Ky-31	2.26	0.02	0.68
EDX	Ky-31	2.23		
XRF	Ky-31	2.24		

[1]Average of eight plants for 4 cuts with 3 growing seasons;

[2]Standard deviation; [3]Coefficient of variance.

The ranges of variation of values of the coefficient of variation (CV) differ among the different methods (Table 1) for nutrients and their ratios (Table 2). Irrespective of grass cultivars CV for Mg, Ca and K measured by different methods can be arranged as EDX > XRF > AAS, AAS> XRF> EDX and AAS≥ XRF> EDX, respectively (Table 1). Regardless of grass cultivars the values of CV tetany index were arranged as EDX> XRF> AAS whereas irrespective of methods the value of CV can be arranged as High-Mg> Ky-31 (Table 2). The values of CV for Mg, Ca and K were higher than their ratio, K/Ca+Mg. The coefficient of variations for nutrient ratio were 1.6% and 0.68% for High-Mg and Ky-31, respectively, for different methods (Table 2).

The coefficient of variation (CV) is close to zero suggests that there is a high precision of the sample's central tendency, i.e., the variability of the parameters measured by different methods is very low. Even though the results from EDX and/or XRF were well suited with AAS method, nevertheless, it could be recommended that the more replications are required in the nutrients analysis with EDX and/or XRF which will give reasonably concord results with AAS.

CONCLUSION

For EDX analyzer the advantage is that less skill is required to ash and analyze the samples without destroying the whole plant. Additionally, XRF has advantage of requiring less time and expertise to grind and analyze the sample. Apart from this, mineral analyses by wet chemistry can be time consuming and expensive. Also, wet chemical analysis for a large number of samples can be tedious, thus increasing error in mineral analyses data. When samples are included in the calibration equations from years and population varied in mineral concentrations, the calibration was precise enough to screen high-Mg plants of tall fescue. Therefore, this study offers the promise of a rapid and relatively inexpensive means of screening forage plants for higher Mg content by using EDX and XRF. The next step in evaluating these techniques is to use samples, which include several high-Mg cultivars from various species.

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