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USING PLANT BREEDING AND GENETICS TO OVERCOME THE INCIDENCE OF GRASS TETANY¹

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ABSTRACT

Plant breeders developing cultivars to minimize the hazards of grass tetany are concentrating largely on increasing herbage Mg concentrations in cool-season (C₃) grasses. Significant genetic variation has been found for Mg, Ca and K concentrations within C₃ grass species studied to date. For most C₃ forage grass species, heritability estimates are highest for Mg, slightly lower for Ca and lowest for K concentrations. The largest genotype × environmental interactions are found for K values, whereas small environmental effects have been observed for Mg and Ca values. No C₃ forage grass cultivar has been developed to date that would eliminate hypomagnesemia. Grass breeders need to develop more experimental C₃ plant populations that have high Mg and Ca concentrations. These experimental synthetics with genetically altered mineral concentrations need to be fed to ruminants susceptible to grass tetany to determine whether grass tetany can be eliminated or reduced. Limited feeding trials using ruminants show that improved animal performance can be expected when feeding forage grasses bred for higher Mg concentrations. (Key Words: Plant Breeding, Hypomagnesemia, Forage, Heritability, Minerals.)

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Introduction

Forage grass plant breeders increasingly have become interested in the possibility of genetically modifying cool-season forage (C₃) grasses to overcome the hazards of grass tetany in ruminants. Breeding and genetic research on tropical forage grasses (C₄) is not concerned with grass tetany; this is because the incidence of grass tetany rarely occurs in tropical, subtropical or temperate regions at the time C₄ grasses make significant growth.

Grass tetany or hypomagnesemia is a metabolic disorder of ruminants associated

with low blood serum Mg levels. Kemp (1960) showed that no cases of clinical grass tetany occurred at blood serum Mg levels above 9 µg/ml, or at an herbage Mg level above 1.9 mg/g. Kemp and t'Hart (1957) and Butler (1963) demonstrated that the incidence of grass tetany was reduced when the K/(Ca + Mg) ratio (meq basis) was less than 2.2. Grass breeders, interested in reducing the tetany potential, have directed their research efforts largely at genetic manipulation of Mg, Ca and K concentrations.

The final test to determine whether selecting for desirable mineral combinations is successful will be determined by animal evaluation trials. At the present time, few plant breeding programs have progressed to the point at which enough seed is available to establish pastures of high Mg content for ruminant experimentation. Raymond (1969) proposed that cultural practices to increase Mg concentrations in plants and animal supplementation procedures should be implemented before conducting a plant breeding program to overcome grass tetany. Unquestionably, these

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TABLE 1. CALCIUM, MAGNESIUM, AND POTASSIUM CONCENTRATIONS IN SEVERAL SPECIES OF HERBS, LEGUMES AND GRASSES^a

Species	Mg	Ca	K
	% of DM		
Herbs			
Yarrow (<i>Achillea millefolium</i> L.)	.99	1.3	3.7
Burnet (<i>Poterium sanguisorba</i> L.)	1.10	1.3	1.8
Plantain (<i>Plantago lanceolata</i> L.)	.61	1.6	2.7
Chicory (<i>Cichorium intybus</i> L.)	.64	1.4	4.6
Legumes			
Black medic (<i>Medicago Lupulina</i> L.)	.76	1.6	2.3
Alsike (<i>Trifolium hybridum</i> L.)	.62	2.0	2.1
Alfalfa (<i>Medicago sativa</i> L.)	.55	2.1	2.6
Sanfoin (<i>Onobrychis viciifolia</i> Scop.)	.79	1.0	2.5
Cool-season grasses			
Perennial ryegrass (<i>Lolium perenne</i> L.)	.21	.46	2.0
Orchardgrass (<i>Dactylis glomerata</i> L.)	.22	.42	2.3
Timothy (<i>Phleum pratense</i> L.)	.25	.41	2.2
Meadow fescue (<i>Festuca pratensis</i> Huds.)	.26	.44	2.1
Tall fescue (<i>arundinacea</i> Schreb.)	.32	.30	2.0
Red fescue (<i>F. rubra</i> L.)	.16	.40	1.8
Tropical grasses			
<i>Andropogon dummeri</i> Stapf	.16	.23	.71
<i>Bothriochloa insculpta</i> (Hochst.) A. Camus	.14	.24	.84
<i>Brachiaria decumbens</i> Stapf	.20	.39	1.32
<i>Chloris gayana</i> Kunth	.15	.29	1.17
<i>Cynodon dactylon</i> (L.) Pers.	.17	.33	1.14
<i>Digitaria scalarum</i> (Schweinf.) Chiov.	.15	.38	1.40
<i>Eragrostis tenuifolia</i> (A. Rich) Steud	.10	.21	.85
<i>Panicum maxium</i> Jacq.	.21	.56	1.51

^aAdapted from Long et al. (1969), Thomas et al. (1952) and Fleming (1973).

cultural practices can minimize the incidence of grass tetany; however, direct supplementation is expensive and adds to the labor of producing livestock. Magnesium supplements are relatively unpalatable. To improve palatability, mineral supplements often are mixed with salt or protein carriers, which also increase its cost. Although supplements have been available to the livestock producer for many years, grass tetany still occurs. Breeding C₃ grasses minimize the hazards of grass tetany is a worthy research objective.

The objective of this report is to review the literature associated with breeding C₃ grasses to prevent grass tetany.

Genetic Variation in Plant Magnesium, Calcium and Potassium

Grass tetany has occurred in ruminants grazing perennial C₃ grasses such as orchardgrass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.), tall fescue (*Festuca arundinacea* Schreb.), crested wheatgrass (*Agropyron deser-*

torum (Fisch.) Schult. and *A. cristatum* L. Gaertn) and smooth brome grass (*Bromus inermis* Leyss.) (Grunes et al., 1970). Grass tetany also has been reported in animals grazing wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), oats (*Avena sativa* L.) and barley (*Hordeum vulgare* L.). Mayland et al. (1976) estimated the tetany hazard of several cereals used as forages and found the incidence of tetany was: wheat > oats = barley > rye. Magnesium levels of wheat were the lowest, whereas those of rye were the highest. Grass tetany also has occurred in cattle grazing other annual grasses like soft chess (*B. mollis* L.) and mouse barley (*H. leporinum* Link.) (Grunes et al., 1970) and even in animals fed alfalfa hay (Bohman et al., 1977).

Plant species vary widely in concentrations of Mg, Ca and K (Table 1). Forbs and legumes generally have higher concentrations of Mg and Ca than do C₃ grasses (Thomas et al., 1952; Fleming, 1973). This may explain partially why ruminants grazing on pastures containing forbs and legumes seldom experience grass tetany. Tropical forage grasses (C₄)

have lower concentrations of Mg, Ca, K and N than the C₃ grasses do (Long et al., 1969). Why grass tetany seldom occurs in ruminants grazing C₄ grasses in the tropics is not clear. Their lower concentration of K may be important. Further, Zebu breeds are more tolerant than are English breeds to low forage Mg concentrations (Greene et al., 1989).

Heritability Estimates of Plant, Magnesium, Calcium and Potassium

The variation observed among grass species for Mg is greater than that observed by plant breeders working within the C₃ forage grass species studied to date (Sleper, 1979). Genetic variation of mineral elements, within species, associated with grass tetany have been studied in ryegrass (Cooper, 1973), tall fescue (Sleper et al., 1977; Nguyen and Sleper, 1981; Reeder et al., 1986), orchardgrass (Stratton and Sleper, 1979), reed canarygrass (*Phalaris arundinacea* L.) (Hovin et al., 1978) and crested wheatgrass (Mayland et al., 1986; Mayland and Asay, 1989). It is difficult to compare heritability estimates for Mg, Ca and K from the above reports because different experimental designs were used and the data were collected in many different environments. Nevertheless, the magnitude of the heritability estimates from the various C₃ grass species is of interest.

Magnesium consistently had the highest narrow-sense heritability (ratio of additive genetic variance to total phenotypic variance) estimate, ranging from 66 to well over 80%, for orchardgrass, tall fescue, ryegrass and reed canarygrass. In crested wheatgrass, Mayland and Asay (1989) calculated broad-sense heritability (ratio of the total genetic variance to total phenotypic variance) estimates and found that heritabilities for Ca and K were slightly higher than those for Mg. For species other than crested wheatgrass, the narrow-sense heritability estimates for Ca were slightly less than those for Mg but certainly were adequate to suggest that improvement through plant breeding would be possible. Heritability estimates for K were somewhat variable for all species studied. Potassium is influenced more highly by environment than are Mg and Ca, which suggests that selecting for lower K concentrations would be difficult.

Narrow-sense heritability estimates reported for the cation ratio K/(Ca + Mg) were 22% for reed canarygrass (Hovin et al., 1978) and 80%

for tall fescue (Sleper et al., 1977). Nguyen and Sleper (1981), using different genotypes in a later study, reported a range of 18 to 51% for the narrow-sense heritability estimate of the cation ratio. Stratton and Sleper (1979) reported that narrow-sense heritability estimates for the cation ratio in orchardgrass, over several harvests, ranged from 0 to 88%.

A broad-sense heritability estimate, averaged over years and harvests, of 78% was reported for the cation ratio in crested wheatgrass by Mayland and Asay (1989). The variable heritability estimates obtained for the cation ratio can be attributed to the greater impact of the environment on K compared with Mg and Ca.

Stability of Genotypes Under Various Conditions

The grass breeder needs information on stability of selected plant genotypes in different environments for meaningful progress to be made developing cultivars with low grass tetany potential. Sleper et al. (1980) conducted an experiment with tall fescue to determine whether temperature or interval of harvest could negate gains made in plants previously selected for differing mineral concentrations. Two tall fescue genotypes, B17-42 and B5-62, were selected for use because they were shown previously to differ in Mg content (low vs high) and in cation (K/[Ca + Mg]) ratio (high vs low), respectively, as evaluated under replicated field trials (Sleper et al., 1977). Plants were vegetatively propagated into 11- × 14-cm pots. The experiment was conducted in two growth chambers; one was programmed at 17/12°C and the other at 25/20°C (light/dark). Plants were harvested at 2-, 4-, and 6-wk intervals.

There were significant differences between genotypes at all harvest frequencies and temperatures for Mg, Ca, K and K/(Ca + Mg) (Figure 1). B5-62 always was higher than B17-42 in Mg and Ca concentration and lower than B17-42 in K concentration and the cation ratio K/(Ca + Mg). The harvest interval × genotype interaction and temperature × genotype interaction were never significant. This suggested that genotypes were behaving similarly throughout the duration of the experiment.

Genotype B5-62 had the highest concentration of Mg throughout the experiment. Consistently above 1.9 mg/g, it was considered by Kemp (1960) to be safe from grass tetany at all

Figure 1. Concentration of mineral elements and the cation ratio for two contrasting genotypes of tall fescue grown under different temperature and harvest frequencies (from Sleper et al., 1980).

treatment combinations. In addition, B5-62 had the lowest K/(Ca + Mg) value over all treatments. All cation values for B5-62 were considerably below the 2.2 critical level established by Kemp and t'Hart (1957). One certainly would want to avoid B17-42 and its progeny in breeding for high concentrations of Mg in tall fescue because heritabilities have been reported to be high. The B5-62 genotype is one of the parents contributing to the newly released cultivar of 'Martin' tall fescue. 'Martin' (tested as experimental LMR) has shown favorable levels of Mg on both amended and nonamended minesoils (Hanson et al., 1982).

Another experiment using the same two tall fescue genotypes was conducted by Brown and Sleper (1980) to determine whether the inherited ability of B5-62 and B17-42 to accumulate Mg and Ca could be offset by increasing the K made available to the plants. This experiment was conducted in the greenhouse with genotypes grown in soils uniformly fertilized with an initial treatment of 100 ppm N and with 50, 150 and 250 ppm K and harvested at three different dates.

Although added K depressed Mg levels in both selections at all harvest dates, the concentration of Mg in B5-62 always was

greater than that in B17-42 (Figure 2). Grunes et al. (1968) found similar trends in perennial ryegrass. Adding increasing amounts of K increased the K/(Ca + Mg) ratio in both genotypes (Figure 3). The cation ratio for B5-62 always was significantly lower than the ratio for B17-42. The cation ratio for genotype B5-62 always was below 2.2, whereas that for B17-42 occasionally was above the 2.2 critical ratio. These experiments provided additional evidence that plants selected for high Mg may maintain their relative rankings with respect to Mg concentration and the cation ratio under widely divergent environmental conditions.

Another environmental variable that influences Mg accumulation in cool-season forage grasses is level of soil O₂. Soil O₂ levels often are low during the grass tetany season because soils often are saturated with water. Elkins and Hoveland (1977) showed that low soil O₂ levels caused a decrease in Mg concentration of annual ryegrass (*Lolium multiflorum* L.). Haaland et al. (1978), in a greenhouse study, examined the effects of low soil O₂ levels on Mg concentration in tall fescue and found similar results. But Haaland et al. (1978) showed that a tall fescue genotype, selected for high concentrations of Mg, remained high in both high and low soil O₂ levels.

The above studies relating to the environmental influence on concentrations of mineral elements important in grass tetany were done in the laboratory. To further assess the

Figure 2. The effect of applied K on the Mg concentration for two contrasting genotypes of tall fescue (from Brown and Sleper, 1980).

Figure 3. The effect of applied K on the K/(Ca + Mg) ratio for two contrasting genotypes of tall fescue (from Brown and Sleper, 1980).

importance of genotype $\times$ environmental interactions on Mg, Ca and K contents, it is necessary to examine experiments conducted in the field. Field studies conducted by Nguyen and Sleper (1981) showed significant genotype $\times$ environmental interactions in tall fescue for Mg, Ca, K and K/(Ca + Mg) levels. The genotype $\times$ environmental interactions for K were between 20 and 30 times greater than for Mg and Ca. This high genotype $\times$ environment interaction for K also led to extremely high genotype $\times$ environment interactions for the cation ratio K/(Ca + Mg). Mayland et al. (1986) found significant genotype $\times$ environment interactions for K and K/(Ca + Mg) but not for Mg and Ca. The significant genotype $\times$ environment interaction for the cation ratio was due to a large effect of environment on K concentration in crested wheatgrass.

Before initiating a selection experiment to genetically modify minerals in cool-season grasses, the plant breeder needs information on the magnitude of genetic variation and heritability of the trait (i.e., the relative importance of the genetic variance to the total phenotypic variance). The magnitude of the genotype $\times$ environmental interaction compared with that of the genetic variance also is of major concern in grass breeding. It can be concluded from the studies examined here that it is possible to select for increased levels of Mg and Ca. Selection for lower concentrations of K is

likely to be more difficult because of the high environmental influence.

Selection

Cool-season perennial grass cultivars identified as causing grass tetany have a broad genetic base and most contain from 6 to over 30 parents. Generally, they are cross-pollinated, which makes them highly heterozygous and heterogeneous. Released cultivars of these species are referred to as synthetics (Sleper, 1987). A synthetic cultivar is one in which the parents have been selected for good general combining ability and mated in all possible combinations. The cultivar is maintained through open pollination.

Because heritability estimates generally are high for Mg, plant breeders should have success in improving the Mg concentration by using either mass selection or by using a between- and within-family breeding procedure. Sleper and Mayland (unpublished data) have completed two cycles of selection for high Mg concentration in tall fescue using mass selection. One thousand plants were evaluated for each cycle of selection. Sixty-five plants were chosen from the base population to serve as parents to produce seed of the first cycle of selection that had Mg and Ca concentrations generally greater than 5 g/kg and K concentrations less than 340 g/kg. Similar selection criteria were used for selected parents that produced seed of the second cycle of selection. Sleper and Mayland (unpublished data) presently are increasing seed of all cycles of selection for the purpose of evaluating the progress through plant breeding and to obtain enough seed of the second cycle of selection to establish small plot management trials useful in evaluating animal response to the high Mg genotypes.

Hides and Thomas (1981) used mass selection to increase the Mg concentration in annual ryegrass. Three cycles of selection were completed for high and low Mg concentrations and for improved agronomic performance, which included improved herbage yield, persistence, winter hardiness and disease resistance. After three cycles of selection, the high Mg population showed an increase of 35% in Mg concentration, whereas the low Mg population showed a 24% decline in Mg concentration compared with the control cultivar. There was no relationship between herbage yield and

Mg concentration in the source population. In the second cycle of selection, there was a decline in herbage yield compared with the control, whereas no significant decline in herbage yield was observed for the third cycle of selection. Nguyen and Sleper (1981) found that correlations between herbage yield and Mg, Ca and K concentrations were low in tall fescue. Similar results were reported in reed canarygrass by Hovin et al. (1978).

The mineral elements Mg, Ca and K are not necessarily inherited independently of each other. Nguyen and Sleper (1981) found that correlations between Mg and Ca concentrations in a broad-based population of tall fescue ranged from $r = .16$ to $.67$ over three springs and ranged from $r = .58$ to $.74$ over three falls. Correlations between Mg and K and between Ca and K in the same study generally were low. Sleper and Mayland (unpublished data) found that the correlation between Mg and Ca concentration in the first cycle, selected for high Mg concentration in tall fescue, was $.44$. Correlations between Mg and K and between Ca and K, in the same first-cycle tall fescue population, were close to 0. Mayland et al. (1986) found similar correlation estimates between the same three minerals in crested wheatgrass. These studies suggested that selecting for higher Mg concentrations could lead to higher Ca concentrations with little effect on K concentrations.

The final step before releasing a cultivar with reduced grass tetany potential is animal evaluation. Merely having an experimental cultivar with high Mg concentration may not necessarily result in reducing the incidence of grass tetany.

Mosely and Griffiths (1984) have conducted animal trials with the annual ryegrass selections with high and low Mg content developed by Hides and Thomas (1981). The two experimental selections of annual ryegrass were established as swards, harvested fresh daily and fed to sheep. The dry matter digestibility was similar for both experimental grasses, but the mean dry matter intake by the sheep was 25% greater for the high than for the low Mg selection. The mineral balance data showed that the intake of Mg and Ca was significantly greater for the animals consuming the high Mg selection. Magnesium apparent availability and retention also were significantly greater with the high than with the low Mg selection. This is the only study in which

ruminants were fed experimental selections having high Mg contents developed by plant breeding. This study does not prove that selecting cool-season forage grasses for high Mg content can reduce or eliminate grass tetany, but it does suggest that development of herbage containing high Mg can have an effect on animal performance. More ruminant studies, using experimental C_3 grasses selected for greater Mg concentrations, are needed.

Conclusions

There is significant genetic variation for Mg and Ca contents in all C_3 forage grasses evaluated to date. The largest portion of this genetic variation is additive, and plant breeders should be able to increase the Mg and Ca concentrations by using either mass selection or by using between- and within-family breeding procedures. Selection has occurred for high Mg content in two C_3 forage grass species, tall fescue and annual ryegrass. There also is genetic variability for K concentration in most cool-season forage grasses, but K is more responsive to the environment than are Mg and Ca, so the results for K concentration are not so consistent as they are for Mg and Ca. Selection likely will improve the cation ratio $K/(Ca + Mg)$, because Mg and Ca values are negatively correlated with the ratio. Selection for low K values likely will not be an effective means of reducing this ratio by plant breeding because of the large genotype $\times$ environmental effect on K. Information is needed on how environmental conditions and different forage management systems affect mineral element concentrations of herbage, particularly improved experimental synthetics developed by plant breeders.

Cool-season forage grass breeders need to develop more experimental populations that differ in Mg, Ca and K concentrations and feed these to ruminant livestock. Will genetically altered plant populations for mineral contents have an affect on animal performance? Based on the limited work in this area, results look encouraging.

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