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Genetic variability of Mg, Ca, and K in crested wheatgrass

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Abstract

Increasing available Mg in crested wheatgrass (*Agropyron* spp.) could reduce the incidence of grass tetany (hypomagnesemia) in ruminants grazing this forage. Raising the Mg levels might be done through genetic processes if enough variation in ion concentration existed in the *Agropyrons*. The purpose of this study was to determine the genetic variation in Mg, Ca, and K concentrations in 2 crested wheatgrass populations. Parent plants were vegetatively propagated to provide 6 replicates each of 12 clones of crested wheatgrass *(A. desertorum)* and 16 F_3 clones of colchicine-induced tetraploid *A. cristatum* × natural tetraploid *A. desertorum*. Each plant was selected on a basis of seedling and mature plant vigor, forage, and seed yield, leafiness, resistance to pests, and response to environmental stress. The 2 populations were grown in separate, space-planted nurseries at Logan, Utah. Herbage was harvested at the pre-boot and early flowering stage in each of 2 years. Magnesium and Ca were determined by atomic absorption and K by flame emission. A reduced tetany potential (RTP) index for each clone was calculated as the sum of normalized Mg and (Ca+Mg)/K values. Significant ($P<0.01$) differences for all traits were detected among clones in each population. All traits, except K and RTP, were closely correlated. Broad-sense heritability values for most traits ranged from 0.61 to 0.84. Enough genotypic variation existed in both populations to warrant breeding lines with higher concentrations of Mg and larger RTP values. Such changes could reduce the incidence of grass tetany in livestock grazing crested wheatgrass.

Key Words: *Agropyron desertorum*, grass tetany, hypomagnesemia, broad sense heritability, forage quality

Cool-season (C_3) grasses are responsible for most cases of grass tetany (hypomagnesemia) in ruminants. The risk of tetany can be reduced by supplying more available Mg to the animals. The quantity of available Mg may increase with increases in Mg concentration in forage (Moseley and Griffiths 1984). However, increasing the concentration of K relative to Mg and Ca reduces the amount of available Mg and increases the risk of tetany (Mayland 1988). This risk is presumed to increase exponentially when the forage K/(Ca + Mg) value, expressed on an equivalent basis, exceeds 2.2 (Kemp and 't Hart 1957). Other factors may also reduce available Mg, but these are likely to be less important (Mayland 1988).

There is increasing evidence that ion uptake by plants is under genetic control (Clark 1983, Saric 1983, and Vose 1963). Such control has already been shown for Ca, Mg, and K in perennial ryegrass (*Lolium perenne*, Cooper 1973), Italian ryegrass (*Lolium multiflorum*, Hides and Thomas 1981), *Lolium-Festuca* hybrids (Buckner et al. 1981), reed canarygrass (*Phalaris arundinacea*, Hovin et al. 1978), orchardgrass (*Dactylis glomerata*, Mika et al. 1988 and Stratton and Sleper 1979), and tall fescue (*Festuca arundinacea*, Nguyen and Sleper 1981). Sleper et al. (1977) noted that K/(Ca + Mg) was highly heritable in *Dactylis* and *Festuca* spp.

Plant breeding programs to increase available Mg have been started on Italian ryegrass (Hides and Thomas 1981) and tall fescue (Sleper et al. In Press). Crested wheatgrass (*Agropyrons* spp.) may have enough genetic variation in Mg, Ca, and K values to justify a breeding program to increase available Mg. This is particularly important because crested wheatgrass may account for 30% of the grass tetany cases in the United States (Mayland 1986). To test this potential, we determined the magnitude of genetic variability and broad-sense heritability in Mg, Ca, and K uptake by crested wheatgrass.

Materials and Procedures

Forage samples were obtained from 2 *Agropyron* populations grown in separate nurseries near Logan, Utah. Parent plants were vegetatively propagated to provide 6 replicates of each clone. The first nursery contained 12 clones of standard crested wheatgrass *(Agropyron desertorum)*. Each plant was selected on a basis of seedling and mature plant vigor, forage and seed yield, leafiness, resistance to pests, and response to environmental stress. The second nursery included 16 of the original 18 parents of a synthetic strain, later released as the cultivar 'Hycrest' (Asay et al. 1986). These parents were the F_3 plants of colchicine-induced tetraploids of *A. cristatum* × natural tetraploid of *A. desertorum*. The 18 parents had been selected from among 8,000 plants, and selection criteria were the same as for the other population. The 2 populations will be referred to as Agdes and Hycrest, respectively.

Nursery plants were spaced 1 m apart in a randomized, complete block design having 6 replications. The areas were fertilized with nitrogen and phosphorus and sprinkler irrigated as needed. The soil at the Hycrest site was a Nibley silty clay loam series, a fine mixed mesic aquic Argiustoll. The Agdes study was conducted on a Timpanogos silt loam series, a fine loamy mixed mesic calcic Argixeroll.

One-half of each plant was clipped to a standard 5-cm stubble height at the early boot stage (8 May 1985 and 15 May 1986), and the remaining half was clipped at flowering (13 June 1985 and 15 June 1986) in each of 2 years. This harvested leaf and stem material was initially dried in the greenhouse and, later, in forced draft ovens at 60° C for 24 hours. Samples were ground to pass a 40-mesh Wiley screen. Subsamples were digested in 3:1 nitric:-perchloric acid and diluted with 1 g La/liter as $LaCl_3$. Magnesium and Ca were analyzed by atomic absorption and K by flame emission.

Analytical precision was typified by an in-house alfalfa sample which was analyzed with the wheatgrass series. This sample was analyzed to contain 3.4 ± 0.3 mg Mg/g, 14.5 ± 0.8 mg Ca/g and 23.5 ± 1.0 mg K/g. All elemental data were expressed on a dry matter basis. The ratio of K/(Ca + Mg) was calculated on an equivalent basis.

An index for reduced tetany potential (RTP) was calculated as follows:

$$RTP_u = \frac{Mg_i - Mg_p}{s\,Mg_p} + \frac{\left[\frac{Ca + Mg}{K}\right]_i - \left[\frac{Ca + Mg}{K}\right]_p}{s\left[\frac{Ca + Mg}{K}\right]_p}$$

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where the subscript 'i' is the value for the individual plant. The subscript 'p' is the mean value for the population, and the 's' is the square root of the error mean square in the F test for the appropriate trait at a given harvest date. The RTP_u (as shown above) is a normalized function having a mean value of zero for a given population. Values of RTP in this paper are shown as $RTP_u + 10$ so all values are positive.

Analysis of variance was calculated with replications, clones, and years considered as random variables and harvests as a fixed variable. Broad-sense heritabilities (H_b) were computed on a mean basis from variance components (Burton and DeVane 1953). The H_b was the ratio σ^2C/σ^2_{ph}, where σ^2C was the variance component due to differences among clones, and σ^2_{ph} was the variance of the mean for each clone.

Results and Discussion

Significant differences ($P<0.01$) were found among clones for all traits measured in the Agdes and Hycrest populations (Table 1). Differences between harvests and years were also significant, corroborating the year and seasonal variation in the occurrence of grass tetany. Harvest and year mean squares for RTP were zero because values were normalized within each harvest.

The mean square error terms for clones were highly significant for all traits measured in each of the Agdes harvests (combined data shown in Table 1). Similarly, all main effects were significant ($P<0.05$) for Hycrest, except Ca in harvest 2 in 1985, K/(Ca + Mg) ratio in harvest 2 and the 1986 combined analysis, and the RTP index in harvest 2 of 1986. It is concluded from these results that elemental uptake is under genetic control in both populations and that genetic variability is available for selection purposes.

Clone × harvest and clone × year interactions were often significant because relative differences among clones were not consistent across harvests and years. Some clones had consistently high or low values for the measured characteristics.

The RTP index should be a good measure of "available Mg". It includes information on the interactions of Mg, Ca, and K (Kemp and 't Hart 1957, Mayland and Grunes 1979) while also giving additional weight or consideration to the Mg concentration in the forage. Using the RTP index would allow an evaluation of progress in selection programs.

There were wide ranges among the clones for all traits studied in Agdes (Table 2) and Hycrest (Table 3). Dividing the range (max − min) by the mean provided a normalized measure of dispersion. Relative ranges among means of Agdes clones, when computed over years and harvests, made up 31, 36, 26, 27, and 38% of the mean Ca, Mg, K, (K/(Ca + Mg), and RTP index, respectively. Corresponding values for Hycrest were 40, 37, 24, 17, and 27%. These ranges provide additional evidence of the opportunities for increasing Mg concentrations in crested wheatgrass through breeding and selection and reducing the incidence of grass tetany in livestock.

Calcium, Mg, and K concentrations were lower in the second harvest than in the first in both populations (Tables 2 and 3). A reduction was expected as the plants accumulated more photosynthate thereby diluting the elemental concentrations. Values for Ca and Mg decreased more than K when evaluating the changes associated with increased maturation. This resulted in K/(Ca + Mg) ratios which were 50% higher in the second harvest of Agdes and 12% higher in second harvest of Hycrest. The ratio means exceeded the 2.2 critical level at the second harvest for both populations. Means of elemental traits for Hycrest and Agdes could not be statistically compared because the 2 populations were grown on different soils.

The Agdes plants contained higher concentrations of Ca, Mg, and K in 1986 than in 1985. Hycrest clones also had higher Ca concentrations in 1986 but lower levels of Mg and especially K than did these same plants in 1985. This may reflect small differences in phenology at the time of sampling each year or perhaps differences in soil moisture, temperature, and nutrition over sites and years. The 1985 K/(Ca + Mg) values were slightly higher than in 1986 for the Agdes population (Table 2) but much higher for the Hycrest populations (Table 3).

Table 1. Analysis of variance of elemental traits combined over harvests and years for *Agropyron desertorum* and Hycrest clones.

Source		d.f.	Mean squares Ca	Mg	K	K/(Ca + Mg)	RTP index
Agropyron desertorum							
BLOCKS	(B)	5	0.6	0.2	8.9	24.5	1392**
CLONES	(C)	11	2.3**	0.4**	47.0**	91.2**	2238**
ERROR	a	55	0.3	0.0	3.4	7.6	304
HARVEST	(H)	1	286. **	40.0**	1283. **	5150. **	0
C X H		11	0.3	0.1	22.2**	48.2**	1472**
YEARS	(Y)	1	20.2**	2.0**	18.6**	380. **	0
C X Y		11	0.8**	0.3**	6.9**	19.8**	1409**
H X Y		1	2.1**	1.2**	38.4**	70.1**	0
C X H X Y		11	0.3	0.1	2.2	9.1	300*
Residual		180	0.3	0.1	2.2	7.7	372
Hycrest							
BLOCKS	(B)	5	0.8	0.2	35.9	12.8	1090**
CLONES	(C)	15	2.2**	0.3**	32.5**	37.8**	1640**
ERROR	a	75	0.2	0.0	5.4	8.2	320
HARVEST	(H)	1	146. **	31.4**	5010. **	599. **	0
C X H		15	0.6**	0.1*	8.	13.5	496**
YEARS	(Y)	1	47.6**	0.6**	4710. **	8940. **	0
C X Y		15	0.6**	0.1*	5.2	16.5	681*
H X Y		1	2.5**	1.1**	2160. **	152. **	0
C X H X Y		15	0.2	0.1*	3.1	7.4	360**
RESIDUAL		240	0.2	0.0	5.1	9.8	384

*,**Significant at $P<0.05$ and $P<0.01$, respectively.

Table 2. Ranges, means, standard errors of the difference (SEd), and broad-sense heritabilities (H) of elemental traits in 12 *Agropyron desertorum* clones grown at Logan, Utah, in 1985 and 1986.

	Years Combined		Harvests Combined		Yrs-Harv Comb.
Traits	Cut 1	Cut 2	1985	1986	
Calcium[1]					
Range	3.53–4.69	1.58–2.43	2.18–3.33	2.80–3.97	2.56–3.50
Mean	4.04**	2.05**	2.78**	3.31**	3.04**
SEd	0.12	0.12	0.19	0.22	0.15
H	0.52	0.81	0.88	0.77	0.64
Magnesium[1]					
Range	1.23–1.89	0.73–.93	0.94–1.37	0.98–1.58	0.98–1.41
Mean	1.57**	0.82**	1.11**	1.28**	1.19**
SEd	0.13	0.04	0.09	0.11	0.07
H	0.23	0.38	0.80	0.81	0.27
Potassium[1]					
Range	15.5–22.6	14.0–18.2	15.9–19.8	14.8–21.0	15.4–20.0
Mean	20.4**	16.2**	18.0**	18.5**	18.3**
SEd	0.80	0.52	0.25	0.74	0.53
H	0.88	0.78	0.85	0.91	0.83
K/(Ca + Mg)					
Range	1.27–1.94	1.99–2.83	1.82–2.50	1.62–2.45	1.82–2.37
Mean	1.64**	2.48**	2.18**	1.95**	2.06**
SEd	0.096	0.104	0.127	0.100	0.080
H	0.72	0.91	0.87	0.82	0.78
RTP index					
Range	8.54–11.5	7.78–12.5	8.14–12.1	8.01–12.6	8.16–12.0
Mean	10.0**	10.0**	10.0**	10.0**	10.0**
SEd	0.72	0.61	0.76	0.76	0.50
H	0.43	0.75	0.82	0.80	0.40

[1]Traits expressed in mg/g, except H.
*,**Mean squares for genotypes are significantly different at $P<0.05$ or 0.01 respectively.

Table 3. Ranges, means, standard errors of the difference (SEd), and broad-sense heritabilities (H) of elemental traits in 16 Hycrest clones grown at Logan, Utah, in 1985 and 1986.

	Years Combined		Harvests Combined		Yrs-Harv Comb.
Traits	Cut 1	Cut 2	1985	1986	
Calcium[1]					
Range	2.73–4.56	1.88–2.78	2.23–3.14	2.39–3.85	2.31–3.49
Mean	3.59**	2.36**	2.62**	3.33**	2.97**
SEd	0.18	0.16	0.14	0.20	0.12
H	0.80	0.50	0.83	0.89	0.71
Magnesium[1]					
Range	1.13–1.80	0.73–1.02	1.02–1.36	0.84–1.49	0.93–1.35
Mean	1.48**	0.91**	1.24**	1.16**	1.20**
SEd	0.10	0.05	0.07	0.10	0.06
H	0.63	0.78	0.72	0.76	0.61
Potassium[1]					
Range	19.4–25.6	14.7–18.7	21.3–25.8	12.8–18.3	17.0–22.1
Mean	24.3**	17.1**	24.2**	17.2**	20.7**
SEd	0.9	0.7	0.7	0.8	0.7
H	0.93	0.69	0.78	0.82	0.83
K/(Ca+Mg)					
Range	1.78–2.34	2.07–2.58	2.39–2.94	1.54–1.94	2.04–2.43
Mean	2.11**	2.36**	2.72**	1.75*	2.23**
SEd	0.09	0.12	0.12	0.12	0.08
H	0.81	0.56	0.78	0.50	0.61
RTP index					
Range	8.38–11.4	8.36–11.6	8.26–11.9	8.48–11.9	8.37–11.1
Mean	10.0**	10.0**	10.0**	10.0**	10.0**
SEd	0.72	0.70	0.84	0.73	0.52
H	0.51	0.76	0.72	0.60	0.63

[1]Traits expressed in mg/g, except H.
*,**Mean squares for genotypes are significantly different at $P<0.05$ or 0.01 respectively.

Table 4. Correlations among Ca, Mg, K, K/(Ca + Mg) and index values for *Agropyron desertorum* (upper right) and Hycrest (lower left) populations combined over years.[1]

	Harvest	Ca	Mg	K	K/(Ca+MG)	RTP index
Ca	1		0.77**	0.14	-0.77**	0.75**
	2		0.43**	0.24**	-0.69**	0.60**
	Combined		0.89**	0.63**	-0.88**	0.38**
Mg	1	0.41**		0.32**	-0.64**	0.83**
	2	0.51**		0.37**	-0.38**	0.73**
	Combined	0.72**		0.66**	-0.78**	0.46**
K	1	-0.23**	0.43**		0.41**	-0.06
	2	-0.15*	0.29**		0.43**	-0.15
	Combined	0.29**	0.66**		-0.29**	-0.07
K/(Ca+Mg)	1	-0.63**	0.05	0.87**		-0.77**
	2	-0.85**	-0.43**	0.56**		-0.82**
	Combined	-0.66**	-0.22**	0.47**		-0.54**
RTP index	1	0.64**	0.79**	-0.04	-0.43**	
	2	0.50**	0.90**	0.08	-0.53**	
	Combined	0.42**	0.52**	0.01	-0.46**	

[1] n = 144 for individual and 288 for combined harvest of *A. desertorum*, n = 192 for individual and 384 for combined harvest of Hycrest.
*,**Significant at $P<0.05$ and $P<0.01$, respectively.

In general, more than 50% of the phenotypic variance among clones in the 2 populations was due to genetic effects, even when considered over years and harvests. Broad-sense heritabilities (H) ranged from 0.27 to 0.83 in Agdes and 0.61 to 0.83 in Hycrest for the analyses combined over years and harvests (Table 2, 3). Heritabilities of Mg were much lower for Agdes in the analyses combined over years within each harvest than comparable values obtained from the analyses for each year combined over harvests (Table 2). This can be explained, at least partially, because years were considered as random variables while harvests were treated as fixed in the computation of the genetic variance among clones. Heritabilities are big enough that selection for RTP would be effective in both populations, especially Hycrest.

Correlations among the 5 traits were computed from data combined over the 2 years (Table 4). Magnesium was significantly ($P<0.01$) and positively correlated with Ca in both the Agdes (r = 0.89) and Hycrest (r = 0.72) populations. Magnesium was more closely associated with K (r = 0.66 and 0.66) than was Ca (r = 0.63 and 0.23) in the 2 respective populations. The K/(Ca + Mg) ratio was negatively related to Mg and Ca and positively to the K values. The correlations of K with the ratio in each of the Agdes harvests were positive (r = 0.41 and 0.43). However, K levels were only slightly less in the second than the first harvest whereas Ca and Mg levels were much less. This resulted in a small negative correlation (r = -0.29) between the ratio and K levels. Similar shifts in regression slopes occurred between Ca and K in Hycrest.

The RTP index was significantly correlated with Mg, Ca, and the K/(Ca + Mg) ratio but not with K (Table 4). Simple and stepwise multiple regression of the RTP index against the other elements illustrated that Mg accounted for 21 and 27% of the variation in the RTP of Agdes and Hycrest, respectively. Potassium was the second most important parameter which, including Mg, accounted for 33 and 49% of the variation in RTP in the 2 populations, respectively. Regressing the RTP index against Mg, Ca, K, and the K/(Ca + Mg) data accounted for 42 and 51% of the variability in the RTP index in the Agdes and Hycrest populations, respectively. Variation in the Mg:K, Ca:K, and Mg:Ca values plus the biological and analytical errors were responsible for the remaining variability in the RTP values. Thus, the RTP index seems most sensitive to variability in Mg and, to a lesser extent, to that in K concentrations in these 2 populations. Examining the correlations among elemental parameters in other populations would be useful in determining the value of the RTP index.

Conclusions

A wide range in genetic variability and values for heritability were measured for ion uptake in these *Agropyrons*. Developing germplasm with higher Mg concentrations is a realistic goal to reduce the grass tetany potential of this genus. However, mineral elements are not inherited independently of each other. Genetic increases in Mg will likely be accompanied by increases in Ca, a positive response, and by slight increases in K, a negative response. The present studies were not designed to derive estimates of narrow-sense heritabilities or the proportion of the genetic variance that is conditioned by additive effects. Research is underway to determine these values and to test genetic gains in progenies of selected plants. The relationships between the RTP index and traits such as seedling establishment, drought tolerance, winter hardiness, insect and disease tolerance, forage yield, and forage quality must also be assessed.

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