Title page:

Revisiting variation in leaf magnesium concentrations in forage grasses for improved animal health

Running title: Variation in leaf magnesium (Mg) concentrations in forage grasses

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Abstract

Aims

An Italian ryegrass cultivar (*Lolium multiflorum* Lam. cv. Bb2067), selected and bred for increased leaf magnesium (Mg) concentration in the 1970s, reduced the incidence of hypomagnesaemia in sheep under experimental grazings. Here, we report evidence from archival experiments showing that cv. Bb2067 had consistently greater Mg concentrations at multiple sites. We also aimed to quantify variation in leaf Mg concentration among modern perennial ryegrass (*Lolium perenne* L.), hybrid ryegrass (*Lolium perenne* × *multiflorum*), and tall fescue (*Festuca arundinacea* Shreb.) cultivars.

Methods

Data are reported from unpublished 1980s field-plot experiments for cv. Bb2067 and contemporaneous reference cultivars, sown at two UK locations in 1983 and 1984, and from 397 cultivars of modern forage grass in 13 UK-based breeding experiments sampled in spring 2013.

Results

Across sites, years and cuts, cv. Bb2067 had a consistently greater leaf Mg concentration and lower potassium (K) concentration and forage tetany index (FTI) than reference cultivars in the 1980s experiments. Seasonal variation in leaf Mg and K concentrations and FTI were observed in the 1980s experiments, with K concentrations being generally greatest and Mg concentrations smallest in spring. Among modern forage grasses, there was large variation in leaf Mg concentration (up to 6.3-fold) and FTI (up to 2.1-fold), both within and between species, which varied independently of yield. Among a subset of hybrid ryegrass cultivars, there is evidence that the high Mg trait is already present in some modern breeding lines, albeit previously unreported.

Conclusions

The variation in leaf Mg concentration and FTI among old and new cultivars shows there is considerable potential to breed forages with improved mineral quality to improve livestock health, potentially without compromising yield or other nutritional traits.

Keywords: biofortification, forages, grass staggers, hypomagnesaemia, ryegrass, tetany

Introduction

Magnesium (Mg) is an essential element for ruminant nutrition, required for normal nerve and muscle function, bone formation and as a cofactor for enzymatic reactions in metabolic pathways (Schonewille et al. 2013). Animals are unable to mobilise large amounts of Mg from the bone or soft tissues, and therefore maintaining adequate plasma Mg concentrations relies almost entirely on Mg intake and absorption (Martens and Stumpff 2019). Thus, inadequate dietary Mg intake from forage by ruminants can lead to hypomagnesaemia-related conditions such as grass staggers or grass tetany (Underwood and Suttle 1999). Additionally, large rumen potassium (K) concentrations caused by high intake of K from forage inhibits uptake of Mg in the rumen, causing effective Mg deficiency and grass tetany symptoms (Leonhard-Marek and Martens 1996). The inhibitory effect of K on Mg absorption is greater at low Mg concentrations (Martens and Stumpff 2019). Reduced calcium in the cerebrospinal fluid also increases the effect of Mg deficiency on the brain (Allsop and Pauli 1975).

A 'tetany risk ratio' or forage tetany index (FTI; Equation 1), which accounts for the relationship between K, Mg and Ca concentrations is used to identify forages that are at risk of causing grass tetany (Kemp and t'Hart 1957).

$$\text{Equation 1} \quad \text{Forage tetany index (FTI)} = \frac{\frac{K}{39.1}}{\left(\frac{Ca}{40.1} + \frac{Mg}{24.3}\right) * 2}$$

where, the elemental leaf concentration (g kg⁻¹ dry weight) is divided by the atomic weight and multiplied by the valence of the respective elements. If the FTI is above 2.2, then a pasture or forage is considered at risk of causing grass tetany. This FTI level is consistent with evidence from both modelling (Martens and Stumpff 2019) and experimental studies (Ram et al. 1998).

The Mg nutrition of intensive ruminant systems in the UK is usually controlled by supplements in the form of blocks, licks, drenches and boluses (Kumssa et al. 2019a). Increasing the ability of forages to supply adequate Mg through crop breeding or use of Mg fertilisers has been a relatively neglected area of research (Kumssa et al. 2019b). There are reports of several grass cultivars that have been selectively bred to have increased Mg concentrations. These include cultivars of tall fescue (*Festuca arundinacea* Shreb.; cultivar=HiMag; Mayland and Slepser 1993; Wilkinson and Mayland 1997) and cocksfoot/orchardgrass (*Dactylis glomerata* L.; cultivar=Mgwell; Sabreen et al. 2003) in the USA and perennial ryegrass (*Lolium perenne* L.; cultivar=Ramore) in Ireland (Binnie et al. 1996). Variation in Mg concentrations has also been investigated in other forage grass species, such as Russian wildrye (*Psathyrostachys juncea* L.; Asay and Mayland 1990) and crested wheatgrass (*Agropyron* spp.; Mayland and Asay 1989). However, it is not clear if any of these cultivars have since been adopted in terms of being commercialised or used in further breeding programmes.

A cultivar of Italian ryegrass (*Lolium multiflorum* Lam. var. Bb2067; 'Magnet') was developed in the 1970s and 1980s at the Welsh Plant Breeding Station (first reported in the Report of the Welsh Plant Breeding Station, 1973, p. 82). In a sheep grazing experiment, cv. Bb2067 had a 48% greater forage Mg concentration and a lower FTI than in a contemporaneous commercial cultivar, cv. RvP. On pasture sown with cv. Bb2067, the 2.5% incidence of clinical hypomagnesaemia in sheep was far lower than the 21% incidence reported for sheep grazing on pasture sown with cv. RvP (Moseley and Baker 1991). Sheep that were transferred to grazing cv. RvP a 35% decline in serum Mg concentration was observed, whereas sheep grazing cv. Bb2067 did not show any changes in serum Mg concentration. Despite having

increased concentrations of Mg and reports of efficacy in controlling hypomagnesaemia, cv. Bb2067 was never commercialised, due to minor yield penalties in subsequent National List trials. Results of multi-site, multi-year field experiments conducted using Bb2067 and related and contemporaneous cultivars were not published at the time.

New cultivars of Italian ryegrass, perennial ryegrass (*Lolium perenne*), tall fescue and hybrid ryegrass (*Lolium multiflorum* × *Lolium perenne*) have since been bred for other nutritive value traits (e.g. high sugar, adequate fibre, protein, and dry matter digestibility) combined with high yield. Variation in leaf Mg concentration or FTI among these more modern cultivars of forage grasses has not yet been explored and it may therefore be possible to exploit this variation to find cultivars with favourable yield and quality traits as well as elevated leaf Mg concentrations and lower FTI.

The primary aims of this study were: (1) to assess the leaf Mg concentration of cv. Bb2067 and contemporaneous Italian ryegrass cultivars, grown at multiple sites and years in the 1980s by extracting information from unpublished hardcopy laboratory notebooks; (2) to quantify variation in leaf Mg concentration of more modern forage grass cultivars of Italian ryegrass, perennial ryegrass, hybrid ryegrass and tall fescue which were being grown in pre-breeding plot trials, for their wider agronomic performance and other quality traits.

Materials and Methods

1980s Italian ryegrass experiments

Three experiments were sown in the 1980s; one at a site near to Aberystwyth in 1983, and two in 1984, at sites near to Aberystwyth and Edinburgh, to evaluate the performance of cultivars bred to have high and low leaf Mg concentrations and to compare against leading commercial cultivars of the time. These data have not previously been reported.

Experimental design and management

The 1983 experiment was sown near Aberystwyth at IGER (Institute of Grassland and Environmental Research, now the Gogerddan campus of Aberystwyth University). This consisted of five Italian ryegrass cultivars; one high Mg cultivar (Bb2067_1), one low Mg cultivar (Bb1276), which is an ecotype from the Po valley in Italy and was found to have lower leaf Mg concentrations, and three commercial cultivars acting as controls (RvP, Optima and S22; Table 1). This experiment was cut six times in both 1984 and 1985 (Table 1).

The two experiments in 1984 were sown in Aberystwyth and in Edinburgh at Science and Advice for Scottish Agriculture (SASA). These consisted of ten Italian ryegrass cultivars; three cultivars bred for greater leaf Mg concentrations (Bb2067_1 and Bb2067_2, which were sibling families, and Bb1843 which was an earlier generation of the Bb2067 lines), three cultivars segregating for lower leaf Mg concentrations during the selection of Bb2067 (Bb2068_2, and Bb1842, which are previous generations of the Bb2068 breeding lines, and Bb1267), and four commercial cultivars acting as controls (RvP, Optima, S22 and Exalta; Table 1). These experiments were cut 5-7 times in each of 1985 and 1986 at both sites (Table 1).

The soil type at Aberystwyth is well drained loam over gravel in the Eutric Endoskeleti-Eutric Cambisols (IUSS Working Group WRB 2007) Rheidol series (Cranfield University 2019). The site at Edinburgh has a coarse textured soil (sandy-silt-loam or sandy-clay-loam) in the Macmerry series (The James Hutton Institute 2019).

Sample collection and analysis

At each cut, above-ground biomass was collected from across the full plot. A dried subsample of each plot was then milled, and analysed for leaf Mg, Ca and K concentrations using atomic absorption spectrophotometry after dry ashing.

Modern forage grass experiments

A total of 397 cultivars representing four forage grass species (Italian ryegrass, perennial ryegrass, hybrid ryegrass and tall fescue) were collected in spring 2013, from 1349 plots from 13 established breeding experiments located close to Aberystwyth, UK (Table 2). The 13 experiments each consisted of 40-416 plots (1.00-4.68 m² area; Table 2). All plots were sown between 2010 and 2012 as part of a wider grass breeding programme. Plots sown in 2010 were located in Field 1 (52°25'40.1"N 4°01'25.5"W); plots sown in 2011 were located in Field 2 (52°26'01.1"N 4°00'42.1"W); plots sown in 2012 in Field 3 (52°26'03.6"N 4°00'27.5"W).

Soil collection and analysis

Soil (0-15 cm) was collected from the modern established breeding experiments in July 2013 from five points spanning each experimental area, in a 'W' shape.

Soil pH was measured using the method as the Countryside Survey (Emmett et al. 2010). The soil pH was acidic across experiments (4.83-6.88; Table 2). For Mg, K and Ca extractions, soil was ground in a ball mill using agate bowls and balls. A subsample of ~2 g was placed in centrifuge tubes and 20 mL of 1 M ammonium nitrate was added to each tube. Tubes were then agitated using an end-over-end shaker for ~12 hours. The tubes were then centrifuged at 2500 rpm for 10 minutes and 10 mL of the supernatant was passed through a 22 µm syringe-driven filter into a universal tube. A 1 mL subsample of the filtered supernatant was then transferred via pipette into ICP tubes and diluted with 9 mL of 2% nitric acid, and analysed using inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo-Fisher Scientific X-Series II instrument (Thermo Fisher Scientific Inc., Waltham, MA, USA; Penrose et al. 2017).

Plant sample collection and preparation

The plots from the modern established breeding experiments were cut to approximately 5 cm height using a custom Haldrup F-55 grass harvester (Haldrup GmbH, Ilshofen, Germany), all the cuttings were collected in the harvester and weighed to calculate fresh weight. A subsample was collected and dried at ~80 °C to calculate the percentage of dry matter (DM), and this was used to convert the fresh yield to DM yield. The spring 2013 cuts were the first cut of the year for experiments 14 and 16, the third cut for experiment 10 and the second cut for all other experiments. Unfortunately, yield was not recorded for Experiments 1 and 4.

A subsample of sward (200-300 g fresh weight) was also taken by hand from cuttings ejected from the harvester. Samples were oven dried (~80°C; Peverill, 1993) for 2-5 d, depending on the moisture content of the samples and then milled using a cutting mill, with ~10 g dry mass (DM) retained for further analyses. A subsample of 0.3 g DM of ground grass leaves were digested using microwave digester (Multiwave 3000 with 48-vessel 48MF50 rotor; Anton Paar GmbH, Graz, Austria) and diluted in preparation for analysis using inductively-coupled plasma mass spectrometry (ICP-MS; Penrose et al, 2017).

Plant mineral analysis

Multi-element analysis of diluted solutions was undertaken by ICP-MS. Samples from the spring (May-June) cut 2013 of experiments 1, 4, 7, 11, 13, 14 and 16 were analysed using a

Thermo-Fisher Scientific X-Series II instrument (Thermo Fisher Scientific Inc., Waltham, MA, USA). All other samples were analysed using a Thermo-Fisher Scientific iCAP-Q instrument (Thermo Fisher Scientific, Bremen, Germany), with appropriate cross-calibration between instruments.

Data analysis

The statistical significance of the differences between the leaf Mg concentrations of the cultivars grown in the 1980s experiments in Aberystwyth and Edinburgh was tested using ANOVA, with cultivar, experiment, sowing year, harvest year and the interactions between cultivar and experiment, cultivar and sowing year and cultivar and harvest year as factors. The model was then tested using Tukey's post-hoc test. Means were considered significantly different to one another at $\alpha=0.05$.

As the FTI was found to be non-normally distributed, the significance of the differences between the leaf FTI of the cultivars grown in the 1980s experiments in Aberystwyth and Edinburgh was tested using a Kruskal-Wallis test, with cultivar as the factor. The model was then tested using a pairwise-Wilcoxon post-hoc test. Means were considered significantly different to one another at $\alpha=0.05$.

Variation among cultivars in terms of leaf Mg, K and Ca concentration were calculated by dividing the mean concentration of the cultivar with the highest mean leaf concentration of that element by the mean concentration of the cultivar with the lowest mean leaf concentration of that element. Variation among cultivars in terms of FTI was calculated in the same way. All data analyses were conducted using R (R Core Team, 2017).

Results

Differences in leaf Mg concentrations and tetany indexes between 1980s cultivars

Bb2067_1 and the other cultivars bred for greater leaf Mg concentrations (Bb1843 and Bb2067_2) had consistently greater Mg concentrations (~20-50 % greater) than the cultivars bred for low Mg (Bb1276, Bb1842 and Bb2068_2) and also the reference cultivars (S22, RvP, Exalta and Optima in both Aberystwyth and Edinburgh (Fig. 1). The FTI of the cultivars bred for greater leaf Mg concentrations was consistently lower than the reference cultivars (by ~10-20 %), but this was not statistically significant at $\alpha=0.05$. Cultivar, experiment and harvest year statistically significantly affected leaf Mg concentrations, whereas the interactions between cultivar and experiment or the year of harvest were not statistically significant (Supplementary Table 1).

Seasonal variation in leaf Mg, Ca, K concentrations and forage tetany indexes in 1980s experiments

Leaf K and Mg concentrations varied between cuts in both the 1983 sown grasses (Aberystwyth, Figs 2a, 2b, 2e and 2f) and 1984 sown grasses (Aberystwyth and Edinburgh, Figs 3a-h). However, the seasonal variation was different in each site and year, with no consistent seasonal trends (Figs 2a, 2b, 2e, 2f and Figs 3a-h). However, generally K concentrations were higher in spring (Cut 1) and autumn cuts (Cuts 5-7) than in summer cuts (Cuts 2-4), and leaf Mg concentrations were generally lower in spring and early summer, rising through the summer and peaking in autumn (Figs 2a, 2b, 2e, 2f and Figs 3a-h). Due to generally greater K and lower Mg leaf concentrations in the spring, the FTI was greater in spring cuts

than in later cuts, except in the 1986 Aberystwyth cuts, where the FTI was fairly consistent across all the cuts (Figs 2g and 2h and Figs 3i-l).

Differences in leaf Mg concentrations and tetany indexes between 1980s cultivars and modern cultivars

Modern Italian ryegrass cultivars had highly variable leaf Mg concentrations (cultivar means=976–1872 mg kg⁻¹) which were similar to the Mg concentrations of the 1980s Italian ryegrass low-Mg cultivars (cultivar means=1498–1564 mg kg⁻¹) and control cultivars (cultivar means=1722–1903 mg kg⁻¹) and smaller than the high-Mg cultivars (cultivar means=2095–2279 mg kg⁻¹). The maximum FTI was greater for the modern Italian ryegrasses (1.53–2.65) than in any of the 1980s cultivars (low-Mg cultivars= 1.88–1.92; control cultivars=1.64–1.85; high-Mg cultivars=1.48–1.62). Data for both the 1980s and modern experiments are given in Online Resources (Supplementary Tables 2-3).

Variation in leaf Mg concentration and forage tetany index between modern grass cultivars

The variation in leaf Mg concentration within species (i.e. between cultivars) was largest in hybrid ryegrass (6.3-fold; 800–5014 mg kg⁻¹) and smallest in tall fescue (1.4-fold; 1161–1584 mg kg⁻¹; Fig. 4a). Variation in leaf K and Ca concentrations within species was smaller than variation in leaf Mg concentrations (Fig. 4). The largest variation in leaf K and Ca concentrations was between perennial ryegrass cultivars (K=3.0-fold, 10402–31474 mg kg⁻¹; Ca=3.5-fold, 2029–6189 mg kg⁻¹; Fig. 4b) and the smallest in tall fescue (K=1.1-fold; 27133–30025 mg kg⁻¹; Ca=1.3-fold, 3043–3945 mg kg⁻¹ Fig. 4b). FTI varied less than either leaf Mg or K concentrations (Fig. 4c); the variation was 2.1-fold in hybrid ryegrass, 2.0-fold in perennial ryegrass, 1.7-fold in Italian ryegrass and 1.2-fold in tall fescue.

Variation in leaf Mg concentrations and forage tetany indexes between modern grass species

Tall fescue had the greatest median leaf Mg concentration (1481±151 mg kg⁻¹; median ± standard deviation); ~40% greater than the median leaf Mg concentration of perennial ryegrass (1007±227 mg kg⁻¹), which had the lowest median leaf Mg concentration (Fig. 4a). Hybrid ryegrass had the greatest median leaf K concentration (28860±6112 mg kg⁻¹), followed by tall fescue (28472±1920 mg kg⁻¹), Italian ryegrass (25303±4271 mg kg⁻¹) and perennial ryegrass (24325±5362 mg kg⁻¹; Fig. 4b). Italian ryegrass had the greatest median leaf Ca concentration (4695±829 mg kg⁻¹), and tall fescue had the lowest median leaf Ca concentration (3551±375 mg kg⁻¹; Fig. 4c). Tall fescue had the greatest median FTI (2.46±0.18), and all tall fescue samples had a FTI of >2.2 (Fig. 4d), whereas hybrid, Italian and perennial ryegrasses all had median FTI <2.2, however, all of these species had some cultivars with a FTI of >2.2 (Fig. 4d).

Relationship between yield and leaf Mg concentrations

A weak negative correlation between leaf Mg concentration and yield can be observed for perennial ryegrass (Fig. 5), suggesting a minor dilution effect on leaf Mg concentrations, although it is unlikely to have a large effect in nutritional terms. It was also evident that there are a subset of hybrid ryegrass cultivars that have a much greater mean leaf Mg concentrations (mean leaf Mg concentrations for each cultivar of ~2500 to 4000 mg kg⁻¹) and which segregate from the the main cluster of cultivars (leaf Mg concentrations of ~500 to 1500 mg kg⁻¹; Fig. 5).

This observation indicates that the high Mg trait might already be present in some modern breeding lines of hybrid ryegrass, which could have a large nutritional effect.

Discussion

1980s grass experiments

In the designed field experiments conducted in the 1980s at two different sites, those cultivars which had been selectively bred to have a greater leaf Mg concentration (i.e. Bb2067_1-Magnet, Bb2067_2 and Bb1843) had consistently greater Mg concentrations than both the reference cultivar (RvP), and the Bb2068 and related cultivars which segregated with lower Mg concentrations. This effect was independent of site, cut time or year. In general, Bb2067 and its related high Mg cultivars also had lower FTI than the other cultivars. To our knowledge, Bb2067 was the first Italian ryegrass cultivar to be selected and bred specifically for increased leaf Mg concentration. Cultivars of other species have also been bred using selective breeding to have increased leaf Mg concentrations (perennial ryegrass, Binnie et al. 1996; tall fescue, Wilkinson and Mayland 1997 and cocksfoot, Sabreen et al. 2003) have also been reported, supporting the idea that the heritability of Mg plant tissue concentrations is sufficiently high to be a selectable trait. Narrow-sense heritability has been reported as 0.66-0.80 for the forage grasses tall fescue, ryegrass and reed canarygrass (*Phalaris arundinacea*); Sleper et al. 1989). Strong heritability was also suggested in our study, where the interactions between cultivar and experiment or harvest year were found not to significantly affect leaf Mg concentration.

Results from the 1980s experiments show that there is a strong seasonal effect on leaf Mg and K concentrations, and consequently FTI, with generally higher FTI in spring. This is consistent with Jones and Tracey (2015) who observed greater K concentration and lower Mg concentrations in spring than at other times. High K concentrations in spring are associated with rapid spring growth of the grass (White and Brown 2010), which unfortunately coincides with a higher requirement of Mg for lactation for ruminants (Grunes and Welch 1989). Although there remains a risk of grass tetany even with the cultivars that were bred for greater leaf Mg concentrations (such as Bb2067), this risk is reduced due to their greater leaf Mg concentrations and lower FTI when compared to the control cultivars.

The 1980s control Italian ryegrass cultivars had generally similar leaf Mg concentrations to modern cultivars, though there were some modern Italian ryegrass cultivars with greater leaf Mg concentrations. This may be due to the greater number of cultivars in the modern experiments in comparison to the number of control cultivars in the 1980s experiments; as the number of cultivars increases, the likelihood of finding greater variation in elemental concentrations increases (Penrose et al. 2017). As modern techniques allow us to screen many cultivars relatively cheaply and easily for Mg and other minerals, screening large numbers of cultivars is recommended to find cultivars with greater leaf Mg concentrations. Furthermore, leaf Mg concentrations were still greater for the 1980s cultivars bred for high Mg than for the modern Italian ryegrass cultivars. This suggests that the 1980s high-Mg cultivars would be useful breeding material to use to create modern high-Mg Italian or hybrid ryegrass varieties, combining their superior leaf Mg concentrations with the high yield and favourable nutritive value traits possessed by modern Italian ryegrass varieties.

Modern grass experiments

The data from the modern grass cultivars suggest that there is large variation in leaf Mg concentrations within species, particularly hybrid ryegrass, which has not yet been considered in breeding programmes focused on Mg concentration or FTI. Although we found a weak negative correlation between yield and leaf Mg concentration across the perennial ryegrass cultivars, which suggests a minor dilution effect, a distinct cluster of hybrid ryegrass cultivars segregated clearly with greater leaf Mg concentrations independent of yield (Fig. 5). This observation indicates that the high Mg trait might already be present in some modern breeding lines of hybrid ryegrass, although we are not aware that this will have been selected intentionally. These cultivars would be good candidates to include in systematic experiments across contrasting environments and crop management scenarios, such as under different fertiliser regimes (Kumssa et al., 2019b) to investigate the relationship between leaf Mg concentrations and other agronomically important traits such as nutritional value and heading date.

Potential genes and genetic loci controlling Mg uptake and root to shoot transport have been identified in *Arabidopsis thaliana* (e.g. Guo et al. 2010), *Brassica* spp. (e.g. Broadley et al. 2008; Graham et al. 2014; Alcock et al. 2017), and rice (*Oryza sativa*; e.g. Horie et al. 2011). Identification of genetic markers associated with Mg uptake and transport in these forage grasses could be a useful next step to accelerate selection of cultivars with enhanced Mg concentrations. The 1980s grass Bb2067 and its related high-Mg cultivars as well as these modern hybrid ryegrass cultivars with augmented leaf Mg concentrations could be used for initial screening to potentially identify markers responsible for consistently greater Mg uptake or translocation in ryegrasses.

In previous studies on modern grass cultivars, using the same four species as in this current study, large genetic sources of variation in the leaf concentration of the non-essential elements, strontium (Sr) and caesium (Cs), were reported between cultivars which may have radiological significance (Penrose et al. 2017). There are chemical similarities between Cs and K, and between Sr, Ca, and Mg. However, there was much less variation between cultivars in the essential minerals (maximum inter-variety variation for K=3.0-fold; Ca=3.5-fold; Mg=6.3-fold) than for Sr and Cs, which are unlikely to be under the same regulatory control. We also found large variation in leaf Mg, K and Ca concentrations and FTI between the four different grass species, which again is similar to our findings for Sr and Cs (Penrose et al. 2017). For example, farmers concerned about Mg deficiency in their livestock could use hybrid ryegrass which has, on average, greater leaf Mg concentrations and lower FTI than perennial ryegrass. However, moving from perennial ryegrass pastures to hybrid ryegrass pastures is not a simple change. Despite the fact yields are generally greater in hybrid ryegrasses in comparison to perennial ryegrasses, hybrids are less persistent, meaning the producer would have to re-sow earlier and more frequently than for perennial ryegrass. Additionally, different grass species are suited to different environmental conditions and management practices. Therefore, providing producers with options to increase forage Mg concentrations in their chosen grass species, either agronomically or by providing a cultivar with a greater leaf Mg concentration, is more likely to lead to successful adoption.

Potassium concentrations had a larger influence on FTI than Mg concentrations in our study, as shown by the greater inter-variety variation in FTI than in Mg concentrations. This supports the findings of Nguyen and Sleper (1981), who suggested that K concentration drives FTI to a greater extent than Mg concentration. It is therefore important when selecting for cultivars with greater leaf Mg concentrations to ensure that moderate to low K concentrations are maintained so that the benefits of increased Mg (and Ca) can be realised. However, leaf K concentrations

have been found to have lower narrow-sense heritabilities than Mg (Nguyen and Sleper 1981), which may make it more difficult to breed for consistently lower leaf K concentrations as well as greater leaf Mg concentrations, and so management of soil K will become a critical factor.

Recent studies have shown that application of Mg fertiliser can increase leaf Mg concentration and reduce leaf K concentrations in Italian ryegrass, including Bb_2067, therefore the use of breeding and agronomy can both be considered to increase leaf Mg concentration and reduce FTI (Kumssa et al, 2019b). Among four Italian ryegrass varieties, the response to Mg fertilisers was consistent at two sites and in two seasons, however, the consistency of the response needs to be explored in more environments and with more varieties and forage species. The effect of K fertilisation and liming on leaf Mg concentrations of different varieties and forage species should also be explored in future research.

Conclusions

Evidence from the breeding trials in the 1980s confirm that leaf Mg concentration and FTI are selectable traits for increasing the nutritional quality of Italian ryegrass. The large variation in leaf Mg concentration between modern cultivars, and between species overall, show that genetic variation could potentially be exploited to create high yielding cultivars of hybrid ryegrass, perennial ryegrass, Italian ryegrass and tall fescue that have greater leaf Mg concentrations alongside other important nutritional traits. Among a subset of hybrid ryegrass cultivars, there is also evidence that the high Mg trait is already present in some modern breeding lines, albeit previously unreported. Future work is required to understand the genetic basis of these high Mg traits and to identify markers, to assist future breeding of cultivars with greater Mg concentrations and lower FTI, which could help to reduce the incidence of hypomagnesaemic tetany in ruminant livestock.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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