Journal of Horticulture
ISSN: 2376-0354
OPEN ACCESS Freely available online
Research Article

Gamete Selection for Macro-Nutrient Selection of Ca, Mg and K in Tall fescue

Bryan Kindiger*

Forage and Livestock Production Research Unit, Grazinglands Research Laboratory-USDA-ARS, USA

ABSTRACT

Innovations in plant breeding can achieve advances toward plant improvement by simplifying selection procedures and allowing rapid consolidation of advantageous gene systems. A gamete selection, dihaploid induction approach is being investigated for tall fescue (*Festuca arundinacea* Schreb. (syn=*Lolium arundinaceum* (Schreb.) Darbysh)) that can provide for rapid nutritional improvements in the forage profile of the species. Grass tetany or hypomagnesemia causes severe losses from death or reduced performance in livestock throughout the world. Dietary Mg supplementation adds to the annual cost of labour and production of grazing livestock and does not always ensure that all animals receive adequate amounts of Mg. The research presented here reviews the outcome of the implementation of a new dihaploid breeding and selection approach that can stabilize the complex genetic mineral characteristics of Ca, Mg and K of tall fescue into a homozygous, dihaploid state. Results of the study suggest the application of a dihaploid selection approach will be an efficient and advantageous methodology for future tall fescue cultivar development and reducing livestock losses due to hypomagnesemia.

Keywords: Nutrient; Species; Genome

INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb. syn=*Lolium arundinaceum* (Schreb) Darbysh; *Schedonorus arundinaceus* (*Schreb. Dumort.*)), 2n=6x=42, represents the predominant, introduced perennial cool season grass forage in the USA. Its wide adaptation, excellent spring, summer and fall production, deep root system, tolerance to heat and persistence over summer conditions make this a highly desirable species for hay, pasture and turf. It responds well to fertilizer, but can maintain itself under limited fertility conditions and is adapted to moderately acid and wet soils [1]. Tall fescue is an outbreeding allohexaploid that represents a species complex that consists of three major types (Continental, Mediterranean and rhizomatous) filling a niche as a highly important agricultural species [2]. Tall fescue, being an allopolyploid species and characteristically being an outcrossing species with self-incompatibility, creates unique breeding inconveniences related to gene, agronomic and nutritional selection. A major breeding inconvenience in allopolyploid tall fescue is due to the presence of multiple alleles associated with a single locus or trait, which during selection, is complicated by its allopolyploid nature. A tall fescue hexaploid will possess six alleles per locus and the difficulty in fixating or selectively pyramiding the appropriate gametic and filial frequencies for such a trait can be daunting. Self-incompatibility complicates these difficulties [3]. The diversity of tall fescue genotypes reflects varied degrees of species morphology, agronomic attributes, persistence, drought and other agronomic attributes which have and are the focus of various breeding or selection schemes within the Lolium genus and in particular, tall fescue [4-6].

Gamete selection as originally defined by [7] is based on the principle that selection exerted at the gametophytic level can increase desirable allelic frequencies detectable at the sporophytic level. If superior gametes can be recognized with certainty through a selection cycle, then such a system would be theoretically more efficient than one based on zygotic selection [8]. In practice, gamete selection ordinarily involves two steps:

(1) Selection on the basis of outcross performance testing of individual plants of a variety or population

(2) A similar controlled selection for outstanding individuals exhibiting desirable agronomic attributes.

Following the identification of superior genotypes, such individuals would undergo continued selfing, followed by phenotypic selection, to generate a homozygous line fixed for the desired ag-

Correspondence to: Bryan Kindiger, Forage and Livestock Production Research Unit, Grazinglands Research Laboratory-USDA-ARS, USA; Tel: 405-262-5291; Email: bryan.kindiger@ars.usda.gov

Received: 01-Mar-2022, Manuscript No. Horticulture-22-22035; **Editor assigned:** 03-Mar-2022, PreQC No. Horticulture-22-22305 (PQ); **Reviewed:** 17-Mar-2022, QC No. Horticulture-22-22305; **Revised:** 24-Mar-2022, Manuscript No. Horticulture-22-14870 (R); **Published:** 31-Mar-2022, DOI: 10.35248/2376-0354.22.9.296

Citation: Bryan Kindiger (2022) Gamete Selection for Macro-Nutrient Selection of Ca, Mg and K in Tall fescue. J Hortic. 9: 296

ronomic characteristics. In instances where haploids can be generated through microspore culture, followed by genome doubling, or utilizing an alternative approach to induce homozygosity, homozygous or dihaploid lines will result.

The clinical diagnosed disease of hypomagnesaemia tetany is known by many common names such as grass tetany, grass staggers, winter tetany, etc. Hypomagnesaemia occurs when the levels of magnesium in a cultivar are too low to meet a livestock's nutritional requirements. Hypomagnesaemia can arise in ruminant livestock such as beef cattle, dairy cattle and sheep, usually after grazing on pastures of rapidly growing grass forages such as ryegrass, wheat, rye and other cool season perennial and annual forages in late winter and early spring. Other environmental factors can elevate the incidence of hypomagnesaemia in pastures when there is freezing in early spring pastures or sudden growth in cool season pastures after rainfall following drought [9]. Hypomagnesaemia commonly arises when the forage or feed intake contains less than 0.20% magnesium on a dry matter basis. Hypomagnesaemia may also occur with the application of potash fertilizers, where the potassium disrupts the absorption of magnesium within the rumen [10]. This commonly occurs in older, lactating beef cows, four to eight weeks after calving but can occur in non-lactating animals as well. These rapid growing, cool season forages are often high in potassium and nitrogen and low in magnesium and sodium, each contributing to decreased absorption of magnesium through the rumen wall [10]. Though reviews of the disease are plentiful, it is difficult to find information to assess the financial costs to livestock producers that can be attributed to hypomagnesaemia. The statistics are few as ranchers have difficulty in distinguishing the disease and rarely the reporting of such losses is recorded. However, some older information pertaining to actual financial losses is available. In France, the financial losses attributed to hypomagnesaemia exceeds £ 700,000 or 817,414 € a year [11]. Additional country statistics and incidence in the EU and US are generally equally impressive [11]. The utilization of magnesium based supplements as a preventative measure complementing livestock rations is the typical recommendation for combatting hypomagnesemia worldwide [10]. The application of dolomitic lime to pasture forages with a surfactant has also been suggested to be an appropriate treatment. Previous research has indicated that the application of magnesium compounds to increase the Mg concentration in grasses was economically unfeasible [12] and as a consequence, plant breeders have attempted to respond to the problem by traditional breeding approaches [13]. One traditional selection approach resulted in the USDA release of the 'HiMag' tall fescue cultivar [14]. The successful development of a high magnesium cultivar indicated that selection for mineral composition through genotype manipulation, utilizing a clonal and diallele selection approach can successfully produce a tall fescue cultivar exhibiting balanced Ca, Mg and K levels that can reduce the incidence of grass tetany [15-17]. Recently, two *Lolium multiflorum* Lam. subsp. *multiflorum* lines were released (IL1, IL2) that allow low levels of dihaploid generation in hybrids when using the IL lines as the maternal parent in crosses that utilize the tall fescue parent as the paternal, pollen parent [18,19]. The present research describes a novel, gametic selection strategy for the development of tall fescue dihaploids possessing enhanced Ca, Mg and K mineral compositions.

Traditionally, an advantage of a dihaploid generation and selection strategy resides with generating a dihaploid possessing a homozygous condition for the alleles of a particular trait that is accomplished in a single generation. Multiple generations of selfing a line to reach an inbred, fixed genotypic condition are omitted. In the case of allopolyploid, self-incompatible tall fescue, generations of selfing to generate homozygous lines will be difficult and time consuming. The application of the two new dihaploid induction lines IL1 and IL2, in a breeding or selection program for tall fescue, can overcome some of these significant breeding obstructions [20]. In this research, a dihaploid gamete selection approach is examined for the fixation of alleles conferring an elevated and advantageous balance of Ca, Mg and K mineral composition in tall fescue.

MATERIALS AND METHODS

The USDA-ARS has recently released two annual ryegrass (*Lolium multiflorum* Lam. subsp. *multiflorum*) (2n=2x=14) genetic stocks, identified as IL1 and IL2 [18,19]. Each is characterized by a genome loss phenomenon following hybridization with tall fescue (*Festuca arundinacea* Schreb. (syn=*Lolium arundinaceum* (Schreb.) Darbysh.)) (2n=6x=42), which is then followed by a low level of parthenogenic development of an embryo in the inflorescence. The IL1 and IL2 genetic stocks exhibit few advantageous agronomic characteristics and are notable primarily for their ability to induce genome loss following hybridization. Each IL line is free of the fungal endophyte *Epichloë* sp. or *Neotyphodium* sp. [21-23].

Pollinations between the IL lines and tall fescue samples were generated by hand using the IL lines as the maternal parent and applying a bulk of randomly obtained tall fescue pollen from several commercial and public cultivars in the greenhouse. Hybrid seed generated from the hybridizations were collected and in the greenhouse, germinated in small trays containing a light potting soil mixture. The resultant F1 hybrids were then transferred to eight inch plastic pots containing a light potting soil mix. The F1 were allowed to grow to maturity in a pollen free environment. The F1 hybrids in the pollen isolated greenhouse were grown to maturity and, as is typical of such hybrids, were observed to be pollen sterile. However, it is noted that some level of pollen fertility can occasionally occur [24,25]. If any F1 appeared to indicate some level of pollen fertility by exerting its anthers, it was placed in isolation in an adjoining greenhouse bay. When mature, the inflorescences were harvested and threshed by hand or machine to remove any stems. The cleaned seed heads were then placed in trays containing a light potting soil mix for germination and the eventual identification and selection for recovered ryegrass or tall fescue seedlings. It is important to note that embryo culture or embryo rescue methods were not employed for DH generation. Following two to three weeks of germination, seedlings were allowed to grow to appropriate size to allow for phenotypic identification and eventual transplanting to pots. The germinating seedlings generally represent a mixture of ryegrass DH recoveries possessing a chromosome number of 2n=2x=14 or tall fescue DH recoveries possessing a chromosome number of 2n=6x=42. Discrimination between the ryegrass and tall fescue DH recoveries was performed by visual examination of phenotypic characters such as leaf width and growth habit and, as required and verified by root tip chromosome counts. Chromosome counting was performed by published methods [26]. The 42 chromosome tall fescue DH seedlings were transferred to 8 inch pots containing a light potting soil mixture for additional growth.

In September 2011, 25 two years old F1's and one each of their

respective one year old DH recoveries were transplanted to a non-replicated space planted nursery at the of Kansas State University, Southeast Agricultural Research Center, Parsons, KS. The F1 and their respective DH offspring were transplanted adjacent to each other at a distance of 50 cm, plant center to plant center. These closely adjacent plantings were performed to minimize soil differences across the field that would affect the forage quality determinations. There was no intent in this experiment to evaluate forage quality across genotypes as the non-replicated nursery negated the ability to compare different genotypes in differing areas of the nursery due to anticipated field differences. Instead, the expression of a set of forage quality components in the F1 and their expression to their recovered DH offspring were determined. The nursery was fertilized in October with 40 lbs/ac granular nitrogen and weeding was performed by hand as was necessary.

On April, 23, 2012, 25 leaf samples were obtained from the F1 and their 25 DH offspring. At least 300 grams of fresh leaf material per individual were obtained, sealed in Ziploc® bags and posted on a next day delivery schedule to ANALab, Fulton, IL for estimations of crude protein (CP), soluble protein (SP), magnesium (Mg), calcium (Ca) and potassium (K). The results of the analysis are provided in Table 1. Research has shown that when the tetany ratio of K to the sum of Ca and Mg was less than 2.2, there is a low incidence of tetany. The tetany ratios are expressed as K/(Ca+Mg) in milliequivalents (mEq) per kg of dry matter and are used as a measure for predicting tetany likelihood [16,27]. Milliequivalents take into account both molecular weight and valence (i.e. charge) because acid base balance is affected by electrical charge rather than mass. Table 1 includes the conversion factors [28,29] for the estimation of milliequivalent determinations and the calculations for mEq/kg from the ANALabs estimated dietary percentages of K, Ca and Mg.

Table 1: Crude Protein, Soluble Protein, Presence of Endophyte, Percentage Ca, Mg, and K concentrations of twenty, dihaploid tall fescue recoveries. The tetany ratio of K/(Ca+Mg) is also provided to identify low likelihood tetany inducing tall fescue germplasm.

Sample	Crude Prot	Soluble Prot	E+	Ca%	mEq Ca% x 499.0	Mg%	mEQ Mg% x 822.64	K%	mEq K% x 282.06	mEq K/(Ca+Mg) Ratio
DH150B (37)	15.47	37.01	no	0.33	164.67	0.14	115.1696	2.66	750.2796	2.681105891
DH117B (1)	15.77	43.93	no	0.34	169.66	0.13	106.9432	1.74	490.7844	1.774326544
DH122B (2)	17.8	39.64	no	0.48	239.52	0.18	148.0752	2.59	730.5354	1.884789595
DH120B (4)	20.68	37.47	no	0.42	209.58	0.27	222.1128	2.07	583.8642	1.352499277
DH113B (5)	19.89	35.56	no	0.34	169.66	0.19	156.3016	2.39	674.1234	2.068106795
DH141B (21)	19.8	42.23	no	0.31	154.69	0.29	238.5656	3.08	868.7448	2.2091098
DH138B (22)	17.85	33.84	no	0.37	184.63	0.21	172.7544	1.82	513.3492	1.43640629
DH133B (23)	19.84	38.21	no	0.40	199.6	0.14	115.1696	2.41	679.7646	2.159562423
DH140B (25)	18.63	38.81	no	0.43	214.57	0.3	246.792	2.95	832.077	1.803523047
DH134B (26)	20.65	37.51	no	0.37	184.63	0.16	131.6224	3.1	874.386	2.764835935
DH146B (30)	18.73	29.38	no	0.41	204.59	0.22	180.9808	2.72	767.2032	1.989785534
DH142B (33)	20.8	34.38	no	0.44	219.56	0.29	238.5656	2.87	809.5122	1.767009309
DH154B (34)	18.45	33.82	no	0.33	164.67	0.25	205.66	2.16	609.2496	1.645153242
DH153B (35)	18.88	32.74	no	0.42	209.58	0.19	156.3016	2.43	685.4058	1.87329945
DH152B (36)	16.29	32.56	no	0.55	274.45	0.26	213.8864	2.55	719.253	1.472863788
DH149B (38)	15.83	37.95	no	0.36	179.64	0.16	131.6224	2.59	730.5354	2.347008183
DH160B (41)	19.52	34.96	no	0.26	129.74	0.17	139.8488	2.66	750.2796	2.783051818

DH159B (42)	20.32	33.53	no	0.54	269.46	0.21	172.7544	1.97	555.6582	1.256535744
DH156B (45)	20.08	37.82	no	0.39	194.61	0.16	131.6224	2.84	801.0504	2.45545936
DH155B (46)	22.04	36.29	no	0.41	204.59	0.19	156.3016	2.98	840.5388	2.329061691

RESULTS AND DISCUSSION

Research has shown that when the tetany ratio of K/(Ca+Mg) in milliequivalents (mEq) per kg of dry matter K to the sum of Ca and Mg was less than 2.2, there is a low incidence of tetany (Wilkinson and Mayland, 1997; Elliott, 2008). A review of the twenty dihaploid tall fescue recoveries listed in Table 1 indicates that most of the DH lines fall within this level. This is not surprising as the parental F1 were also expressed and were selected for a <2.2 tetany ratio. From a breeding, selection or gene expression level, this value is fixed in a homozygous condition in these dihaploid recoveries. There is no opportunity for gene segregation due to the homozygous genetic condition of these lines. The expression of these lower tetany ratios is a product of a single tall fescue gamete possessing an appropriate genotype conferring the advantageous K, Ca and Mg levels. Gamete selection as originally defined by [7] is based on the principal that selection exerted at the gametophytic level can increase desirable allelic frequencies detectable at the sporophytic level. If superior gametes can be recognized with certainty through a selection cycle, then such a system would be theoretically more efficient than one based on zygotic selection [8,19] and provide materials particularly useful in genetic analysis and breeding systems when properly exploited [30].

A list of dihaploid individuals exhibiting a tetany ratio less than 2.2 is provided in Table 1. The combining of a few or several of these homozygous lines could be used to generate endophyte free, F1 hybrid tall fescue having a superior balance of Ca, Mg and K, allowing a lower incidence of hypomagnesemia in grazing livestock. These same lines could be pooled to form a low incidence hypomagnesemia population whereby more traditional tall fescue selection methods could be utilized to generate a new cultivar having a low hypomagnesemia ratio similar to the earlier release of the HiMag cultivar [14]. Though forages containing adequate levels of magnesium (0.2 per cent Mg) can be generated, rapid, spring growing, cool season forages are often high in potassium and nitrogen and low in sodium, even with a superior hypomagnesemia ratio, such germplasm will require proper management in such pastures if the Mg levels are to remain biologically available to the grazing livestock.

CONCLUSION

This breeding and selection technique has clear advantages in fixing advantageous gene combinations for other mineral and other forage quality traits. In this study, the dihaploid, gamete selection approach has indicated its value in the rapid and efficient generation of several tall fescue genotypes exhibiting balanced levels of K, Ca and Mg and a low hypomagnesemia ratio. It is anticipated that this approach can be efficiently applied toward the selection of other important quantitative or qualitative traits.

REFERENCES

1. Jennings JA, Beck P, West C. Tall fescue for forage. University of Arkansas Extension Bulletin, Dept. of Agricultural and Natural Resources. 2008; FSA 2133.
2. Hand ML, Cogan NO, Stewrt AV, Forster JW. Evolutionary history of tall fescue morphotypes inferred from molecular phylogenetics of the Lolium-Festuca species complex. BMC Evol Biol. 2010; 10: 303.
3. Comai L. The Advantages and Disadvantages of being polyploidy. Nature. 2005; 6: 836-846.
4. Kasperbauer MJ, Buckner RC, Springer WD. Haploid plants by anther-panicle culture of tall fescue. Crop Sci. 1980; 20:103-107.
5. Humphreys MW, Canter PJ, Thomas HM. Advances in introgression technologies for precision breeding within the Lolium-Festuca complex. Ann Appl Biol. 2003; 143:1-10.
6. Volaire F, Norton MR. Summer dormancy in perennial temperate grasses. Annals of Botany. 2006; 98: 927-933.
7. Stadler LJ. Gamete selection in corn breeding. J Amer Soc Agron. 1944; 36: 988-989.
8. Richey FD. Corn breeding: Gamete selection, the Oenothera method and related miscellany. J Amer Soc Agron. 1947; 39: 403-411.
9. Thomas HS. Cows Need Salt to Avoid Grass Tetany. Angus Media publ. 2016.
10. Arnold M, Lehmkuhler J. Forage-related cattle disorders: hypomagnesemic tetany of "grass tetany". Univ. of Kentucky. 2014.
11. Voison A, Grass Tetany. The symptoms of grass tetany and the losses it incurs. Published by Crosby, Lockwood and Son Limited, London. 1963; 262.
12. Grunes DL, Stout PR, Brownell JR. Grass tetany of ruminants. Adv Agron. 1970; 22: 331-374.
13. Sleper DA, Garner GB, Asay KH, Boland R, Pickett EE. Breeding for Mg, Ca, K, and P Content in Tall fescue. Crop Sci. 1977; 17: 433-438.
14. Sleper DA, Mayland HF, Crawford Jr RJ, Shewmaker GE, Massie MD. Registration of HiMag tall fescue germplasm. Crop Sci. 2002; 42(1): 318-319.
15. Nguyen HT, Sleper DA. Genetic variability of mineral concentrations in *festuca arundinacea* schreb. TAG. 1981; 59: 57-63.
16. Wilkinson SR, Mayland HF. Yield and mineral concentration of HiMag compared to other tall fescue cultivars grown in the Southern Piedmont. J Plt Nutr. 1997; 20: 1317-1331.

17. Crawford RJ Jr, Massie MD, Sleper DA, Mayland HF. Use of experimental high-magnesium tall fescue to reduce grass tetany in cattle. J Prod Agric. 1998; 11: 409-496.

18. Kindiger B, Singh D. Registration of annual ryegrass genetic stock IL2. J of Plant Reg. 2011; 5:254-256.

19. Kindiger B. Sampling the genetic diversity of tall fescue utilizing gamete selection. Gene Diversity Plt. 2012; 271-284.

20. Kindiger B. Generation of paternal dihaploids in tall fescue. Grassl Sci. 2016; 62: 243-247.

21. Carroll GC. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology. 1988; 692-699.

22. Moon CD, Tapper BA, Scott B. Identification of Epichloe Endophytes in planta by a microsatellite-based PCR fingerprinting assay with automated analysis. Appl. Environ. Micro. 1994; 65:1268-1279.

23. Pedersen JF, Sleper DA. Considerations in breeding endophyte-free tall fescue forage cultivars. J Prod Agric. 1994; 1: 127-132.

24. Buckner RC, Fergus EN. Improvement of tall fescue for palatability by selection within inbred lines. Agron. J. 1960; 52: 173–176.

25. Buckner RC Hill, HD, Burrus PB. Some characteristics of perennial and annual ryegrass x tall fescue hybrids and of the amphidiploid progenies of annual ryegrass x tall fescue. Crop Sci. 1961; 1: 75-80.

26. Kindiger B. A technique for the preparation of somatic chromosomes of maize. In: M. Freeling and V. Walbot (Eds), "The Maize Handbook". Springer. 1994; 481-483.

27. Elliott M. Grass tetany in cattle predicting its likelihood. PrimeFacts. 2008; 785.

28. Oetzel G. Use of anionic salts for prevention of milk fever in dairy cattle. Compendium on continuing education for practicing veterinarians. 1993; 15: 1138.

29. Karen Dupchak. A Review of potassium and tetany ratios. Nutrition update. MAFRI. 2004; 15(2).

30. Dunwell JW. Haploids in flowering plants: Origins and exploitation. Plt Biotech J. 2010; 8: 377-424. .