GENETIC ANALYSIS AND EVALUATION OF TALL FESCUE FOR LOW

MAINTENANCE APPLICATIONS

by

YUANSHUO QU 曲远硕

A dissertation submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Plant Biology

Written under the direction of

William A. Meyer

And approved by

_____

_____

_____

_____

_____

New Brunswick, New Jersey

October, 2020

ABSTRACT OF THE DISSERTATION

GENETIC ANALYSIS AND EVALUATION OF TALL FESCUE FOR LOW

MAINTENANCE APPLICATIONS

by

YUANSHUO QU 曲远硕

Dissertation Director:

William A. Meyer

Tall fescue [*Festuca arundinacea* (Schreb.)] is a cool-season turfgrass species that

has shown great potential for low-maintenance turfgrass applications. This dissertation

sought to explore and demonstrate the applications of statistical models in the genetic

analysis and cultivar development of tall fescue. Specific attention was placed on two

low-maintenance traits that the Rutgers turfgrass breeding program has been focusing on,

improvement of drought tolerance using the rainout shelter and resistance to red thread

disease caused by *Laetisaria fuciformis* (Berk.) Burds.

Rainout shelters have been widely used in the breeding of tall fescue for improved

drought tolerance. Persistence of green coloration of leaves during drought is one of the

crucial traits with noticeable variations for selection. In this project, we studied two

consecutive generations of tall fescue evaluated in rainout shelter trials with different experimental designs. Bayesian mixed linear models were applied to collected datasets. Variance components, narrow-sense heritability (h^2), and prediction accuracy of estimated breeding value (EBV) were estimated. The theoretical foundations of genetic analysis and application to the breeding of tall fescue were also discussed. We first reported h^2 for green persistence of tall fescue in rainout shelter selection. Mean heritability from parental generation was 0.18 with a 95% highest density interval (HDI) of (0.04,0.51), while that from progeny generation was 0.08 with a 95% HDI of (0.01,0.23). Though significantly greater than zero, both estimates were low, indicating a large proportion of non-genetic variance. Given the heritability estimate and experimental design in the progeny generation, the prediction accuracy for EBV with different selection methods was calculated. Selection methods ranked by mean prediction accuracy from the highest to the lowest are parental selection > family selection > mass selection. Given the heritability estimations, the theoretical prediction accuracy for these selection methods was also calculated. Extra attention was paid to stratified mass selection. Under the stratified mass selection method, our work demonstrated the application of best linear unbiased prediction(BLUP), A-BLUP, and G-BLUP in the breeding tall fescue, and illustrated how prediction accuracy could be further improved by increasing the number of blocks or/and the implementation of A-BLUP and G-BLUP.

Data analysis in the studies has so far focused on continuous data. However, it is not uncommon to see discrete data in the breeding of tall fescue. The last chapter of this dissertation provided an example of an analysis of binary disease incidence data. The study investigated binary red thread disease incidence in tall fescue populations evaluated

in two locations over multiple years, highlighted the importance of specific selection effort against red thread disease in tall fescue, and estimated heritability of disease incidence with two different experimental designs. Narrow-sense heritability (0.52,0.74 and 0.48) estimated from different experimental designs are all in the range of moderate to high, supporting the idea that additive genetic variance accounts for large phenotypic variance in red thread incidence in tall fescue populations. Hence, disease incidence of red thread in tall fescue can be effectively reduced through selection and breeding. This is also the first report documenting the efficacy of family selection in reducing red thread incidence in tall fescue.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1. Brief history of turf-type tall fescue in the United States

Tall fescue [*Festuca arundinacea* (Schreb.)] is an allohexaploid (2n=6x = 42) with the genomic constitution of $\mathbf{PPG_1G_1G_2G_2}$. The $\mathbf{P}$ genome appears to have come from meadow fescue (2n=2x=14, *Festuca pratensis* [Huds.] Darbysh.), while the two $\mathbf{G}$ genomes come from the tetraploid fescue (*Festuca arundinacea* var. *glaucescens* Boss)(Sleper & West, 1996). It is widely adopted as a turfgrass in many areas of the world, including many parts of North America, South America, Europe, and the cooler parts of Asia, Africa, Australia, and New Zealand(Meyer & Watkins, 2003). Tall fescue was introduced from Europe to the United States before the 1850s by early settlers. Since the 1940s, it has been used for lawns, roadsides, pasture and soil conservation purposes.

The rapid and extensive acceptance of tall fescue was primarily due to its valuable qualities as a pasture grass. It is better adapted to disease, insects, heat, and drought, compared with other cool-season grasses. However, most of these pasture type tall fescue cultivars have a coarse texture, lighter color, and clumping growth habits, which are not desirable for turf situations. Before 1979, only cultivars like Kentucky 31 and ALTA were considered for turf situations (Cowan, 1956). Thanks to a low seed price, they are still in demand nowadays. Over 50 million kg of Kentucky 31 seeds are produced each year, mainly in Missouri (Meyer & Watkins, 2003). The era of turf-type tall fescue was initiated by the release of Rebel tall fescue in 1979 by Dr. C. Reed Funk at the New Jersey Agricultural Experiment Station, and for the first time, a tall fescue cultivar could produce turf which was heat and drought tolerant, attractive in color and texture, and persistent without the clumping growth habit (Funk, Engel, Dickson, & Hurley, 1981).

Almost complete self-incompatibility of tall fescue results in cross-pollination. Current breeding efforts of tall fescue generally focus on creating superior synthetic cultivars with improved turf quality and low maintenance requirements, such as better drought tolerance and enhanced disease resistance.

1.2. Selection of tall fescue for drought tolerance

Global climate change presents a significant challenge for turfgrass research. The predicted rise in global temperature is expected to intensify both the frequency and severity of drought (Jacob et al., 2014; Min, Zhang, Zwiers, & Hegerl, 2011). Facing this challenge, the development of turfgrass cultivars that can thrive under drought stress remains one of the most important research objectives. Previous studies have shown that tall fescue responds to drought stress with various mechanisms, including escape, avoidance, and tolerance (Huang & Gao, 1999; Pirnajmedin, Majidi, & Gheysari, 2015). Drought escape refers to the rapid phenological development of plants to complete life cycle, followed by dormancy during severe drought stress(Wang, Bughrara, & Nelson, 2008), while the latter two mechanisms serve to improve water uptake, minimize water loss and avoid dormancy(Pirnajmedin et al., 2015). Although escaping drought stress by dormancy may aid in the long-term survival of tall fescue, most turfgrass managers prefer to maintain a dense green surface for aesthetics, playability, and safety (Cross, Bonos, Huang, & Meyer, 2013; Karcher, Richardson, Hignight, & Rush, 2008).

Recent years have seen increasing applications of rainout shelter (ROS) in the selection and evaluation of turfgrass and forage cultivars (Hatier et al.,2014; Lootens et al., 2016; Schwartz et al., 2018; Steinke et al., 2010; Zhou et al., 2009). ROS allows precise control and alteration of water availability on outdoor field research plots,

creating relative uniform selection environments (Fay, Carlisle, Knapp, Blair, & Collins, 2000). There are many selection methods in the breeding of tall fescue, among which mass selection, parental selection, family selection, and stratified mass selection are most commonly used. In this study, we sought to compare these methods in ROS selection of tall fescue populations.

Green persistence of leaves during drought is one of the crucial traits with noticeable phenotypic variations upon which to base selection. With the increasing demand in high-throughput phenotyping technologies, normalized difference vegetation index (NDVI) was adopted to capture and document plant greenness under drought stress in this study. NDVI is an effective indicator of vegetation response to drought stress, with high potential applications for determining vegetation health and density in a high-throughput manner in plant breeding (Condorelli et al., 2018; Karnieli et al., 2010; Khosravi, Haydari, Shekoohizadegan, & Zareie, 2017; Zhang et al., 2019). It is calculated by standardizing the difference between the reflection of radiation in the near-infrared and that in the red spectral region. The underlying principle of this calculation is that radiation from the red region is poorly reflected due to the absorption by chlorophyll in green plants, while radiation from the near-infrared region is strongly reflected by the spongy mesophyll structure (Tucker, 1979).

The traditional approach to analyze phenotypic data is the partitioning of variances using the one-way analysis of variance (ANOVA). Given a half-sib design, ANOVA provides a logical connection from observed variance components estimated from the data to the underlying genetic components, allowing the estimation of additive genetic variance hence narrow-sense heritability (h^2). These parameters of interest are essential

for the prediction of response to selection and prediction accuracy of estimated breeding value (EBV), contributing to a better understanding of the underlying genetic mechanism (Nguyen & Sleper, 1983). ANOVA was initially designed for analysis of balanced data, which is often approachable in laboratory or greenhouse studies. However, field research often challenges investigators with highly unbalanced datasets. Alternative approaches using mixed models either under frequentist or Bayesian framework have been developed for estimation of variance components, and quickly gained popularity in the field of quantitative genetics.

In this study, two consecutive generations of tall fescue half-sib populations were evaluated in multiple ROS trials, with green persistence data documented using NDVI. Bayesian mixed linear models were then applied to collected NDVI data for estimation breeding values, variance components, and heritability. Given the heritability estimation and the specific experimental design in the progeny generation, the prediction accuracy for different selection methods was estimated. The theoretical foundation of genetic analysis and its application to the breeding of tall fescue was also reviewed and discussed. Through genetic analysis, we sought to gain a better understanding of the inheritance of green persistence of tall fescue under drought stress and to optimize the breeding process.

1.3. Selection of tall fescue for improved red thread disease resistance

Another low-maintenance trait studied was the resistance to red thread disease, caused by *Laetisaria fuciformis* (Berk.) Burds. It is a common disease on many cool-season and some warm-season grasses throughout the world (Bonos, Wilson, Meyer, & Funk, 2005; Raikes, Lepp, & Canaway, 1996; Zhang et al., 2015). Symptoms of red

thread disease on turf stands are characterized by circular or irregularly shaped, small to large patches ranging from 5 to 60 cm, with the presence of red, thread-like sclerotia, hence the name. In addition to the "red thread" outgrowth, small cream, or pink-colored mycelial mats and flocks of arthroconidia are often observed (Smiley, Dernoeden, & Clarke, 2005). The sclerotia can survive adverse environmental conditions and stay viable for some time. When conditions permit, the sclerotia germinate and start a new cycle of infection. Sclerotia and arthroconidia can be disseminated by water, mowing equipment, or foot traffic to initiate secondary inoculation (Stalpers & Loerakker, 1982). Outbreaks of red thread disease usually occur during cloudy and rainy conditions in low maintenance turf stands such as residential lawns, parks, golf course rough areas, and low budget athletic fields. Damage may be more severe on infrequently moved areas, or when low temperature or fertility slows the growth of turfgrasses. Applications of nitrogen fertilizer not only encourage faster recovery but also reduce disease incidence. Other control methods include enhanced light intensity or light penetration (when in shade), increased ventilation, clipping removal during major infection, and use of resistant cultivars (Raikes et al., 1996; Smiley et al., 2005; Tani & Beard, 1997). Traditionally, red thread has been a disease on perennial ryegrass (*Lolium perenne* L.), especially when it is under fertility stress (Qu et al., 2005). Red thread epidemics in tall fescue have increased as the use of tall fescue gained popularity. Selection of turfgrass species for resistance to red thread has not progressed as much, compared to other major diseases of turfgrasses, such as dollar spot caused by *Clarireedia jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch (Bonos, 2006), or gray leaf spot caused by *Pyricularia oryzae* Cavara (Bonos, Kubik, Clarke, & Meyer, 2004; Han, Bonos, Clarke, Meyer, 2006). Additionally,

genetic studies have not been conducted for red thread resistance in any turfgrass species; this is primarily due to the low reproducibility of experiments carried out under natural infection and variable success with artificial inoculations (Berestetski, Ehrig, & Kastirr 2002).

This study was initiated by investigating red thread disease incidence in a collection of tall fescue maternal half-sib populations and commercial synthetics in two disease outbreaks in 2016 and 2017 induced by artificial inoculation. We then proceed to estimate disease incidence rate in different populations and the narrow-sense heritability of red thread incidence in tall fescue using a maternal half-sib design. Based on estimated heritability and disease incidence, family selection was applied to identify resistant and susceptible genotypes. Diallelic crosses were subsequently conducted to evaluate the efficacy of family selection and to confirm the repeatability of estimated genetic parameters.

2. GENETIC ANALYSIS OF TALL FESCUE HALF-SIB POPULATIONS IN RAINOUT SHELTER SELECTION

Abstract

Rainout shelters have been widely used as a tool in the breeding of tall fescue for improved drought tolerance. Green persistence of leaves during drought is one of the crucial traits with noticeable quantitative variations for selection. In this study, we studied two consecutive generations of tall fescue evaluated in rainout shelter trials with half-sib designs. Bayesian mixed linear models were applied to collected datasets. Variance components, narrow-sense heritability (h^2), and prediction accuracy of estimated breeding value (EBV) were estimated. The theoretical foundations of genetic analysis using half-sib designs and application to the breeding of tall fescue were also discussed. We first reported h^2 for green persistence of tall fescue in rainout shelter selection. Mean heritability from parental generation was 0.18 with a 95% highest density interval (HDI) of (0.04,0.51), while that from progeny generation was 0.08 with a 95% HDI of (0.01,0.23). Though significantly greater than zero, both estimates were low, indicating a large proportion of non-genetic variance. Given the heritability estimate and experimental design in the progeny generation, the prediction accuracy for EBV with different selection methods was calculated. Selection methods ranked by mean prediction accuracy from the highest to the lowest are parental selection > family selection > mass selection.

Keywords: Mixed linear models, variance components, heritability, estimated breeding value, prediction accuracy.

2.1. Introduction

The scarcity of water is one of the most significant environmental constraints to the survival and growth of plants in agriculture worldwide (Cattivelli et al., 2008; Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009). Selection of turfgrass species and cultivars with improved drought tolerance has become a focus of breeding to provide more options for climates and locations that most commonly encounter warter scarcity. Tall fescue is a cool-season turfgrass species that has shown significant potential for drought tolerance because of its deep root system as well as other physiological characteristics (Cross et al., 2013; Fu, Fry, & Huang, 2007; Jiang & Huang, 2001). Tall fescue is a cross-pollinated, self-incompatible, sexual perennial grass, and the general breeding aim has so far been to develop improved synthetic populations (Meyer & Watkins, 2003; Nguyen & Sleper, 1983). Allowing a relatively large number of superior genotypes to inter-cross creates a diverse genetic combination in the progeny. These progenies are then maintained as half-sib populations for further evaluation, as well as base populations from which superior genotypes for production of next generation are selected. For efficient identification of superior genotypes, experimental approaches to creating uniform drought conditions are often desired. The application of rainout shelter (ROS) allows precise control and alteration of water availability on outdoor research plots (Fay et al., 2000).

Turfgrass breeders use different selection methods depending on the mode of reproduction of a species. When evaluating half-sib populations of tall fescue, the most widely used methods include mass selection, family selection, and parental selection. The differences among these methods lie in the predictors for estimated breeding value (EBV), choice of recombination units, and length of selection cycle. In mass selection,

EBVs are predicted based on the phenotypic performance of evaluated clones, while in

parental selection, the selection is performed on shared parental clones based on the mean

phenotypic performance of evaluated progenies. Family selection combines parental

selection and mass selection, wherein elite families are first identified using the mean

phenotypic performance of evaluated progenies, followed by a mass selection of elite

genotype(s) within identified families. The length of selection cycles is another difference

among these methods. Mass selection does not require the production and evaluation of

progenies but will suffer low prediction accuracy for EBV on traits with low heritability.

Parental selection and family selection yield better prediction accuracy at the cost of the

additional season and effort for production and evaluation of progenies. In this study, we

sought to compare these methods in ROS selection of tall fescue populations.

Green persistence of leaves during drought is one of the crucial traits with

noticeable phenotypic variations upon which to base selection. With the increasing

demand in high-throughput phenotyping technologies, normalized difference vegetation

index (NDVI) was adopted to capture and document plant greenness under drought stress

in this study. NDVI is an effective indicator of vegetation response to drought stress, with

high potential applications for determining vegetation health and density in a high-

throughput manner in plant breeding (Condorelli et al., 2018; Karnieli et al., 2010;

Khosravi et al., 2017; Zhang et al., 2019). It is calculated by standardizing the difference

between the reflection of radiation in the near-infrared and that in the red spectral region.

The underlying principle of this calculation is that radiation from the red region is poorly

reflected due to the absorption by chlorophyll in green plants, while radiation from the

near-infrared region is strongly reflected by the spongy mesophyll structure (Tucker, 1979).

The traditional approach to analyze collected phenotypic data is partitioning of variances using the one-way analysis of variance (ANOVA). Given a half-sib design, ANOVA provides a logical connection from observed variance components estimated from the data to the underlying genetic components, allowing the estimation of additive genetic variance hence narrow-sense heritability (h^2). These parameters of interest are essential for the prediction of response to selection and prediction accuracy of EBV, contributing to a better understanding of the underlying genetic mechanism (Nguyen & Sleper, 1983). ANOVA was initially designed for analysis of balanced data (i.e., equal numbers of progenies in different half-sib populations), which is often approachable in laboratory or greenhouse studies. However, field researches often challenge the investigators with highly unbalanced datasets. Alternative approaches using mixed models either under frequentist or Bayesian framework have been developed for estimation of variance components, and quickly gained popularity in the field of quantitative genetics.

In this study, two consecutive generations of tall fescue half-sib populations were evaluated in multiple ROS trials, with green persistence data documented using NDVI. Bayesian mixed linear models were then applied to collected NDVI data for estimation breeding values, variance components, and heritability. Given the heritability estimation and the specific experimental design in the progeny generation, the prediction accuracy for three different selection methods, namely, mass selection, family selection, and parental selection, was estimated. The theoretical foundation of genetic analysis using

half-sib designs and its application to the breeding of tall fescue was also reviewed and discussed. Using a thorough genetic analysis, we sought to gain a better understanding of the inheritance of green persistence of tall fescue under drought stress and to optimize this breeding process.

2.2. Material and methods

2.2.1. Experiment design and data collection

The experiments were conducted during the summer of 2017 and 2019 in two Rain-out Shelters (ROS) at the Rutgers Plant Biology Research and Extension Farm, Freehold, NJ. ROS1 is an automated double-layered canopy that is signaled to protect the trial area from precipitations by air moisture sensors, while ROS2 is a stationary greenhouse that covers the trial area during the entire period of drought treatment. The soil in both shelters is categorized as sandy loam, with a slight acidity. Mechanical analysis indicates soil from ROS1 consists of 56% sand, 26% silt, and 18% clay, while that from ROS2 consists of 70% sand, 15% silt, and 16% clay. During the summer of 2017, 620 clones randomly selected from 20 maternal populations in the parental generation were evaluated for green persistence under drought stress for 62 days. Ninety-six clones were subsequently selected from all 20 maternal populations by visual inspection. After matching the maturity in the spring of 2018, the progeny generation was created by poly-crossing 38 out of the 96 selected clones. Maternal membership of these 38 clones was shown in Table 2.1 Maternal effects of selected tall fescue clones and corresponding maternal plots evaluated in rainout shelter trials at Freehold, NJ.. A random selection of 380 clones from 38 maternal populations in the progeny generation and 360 clones from the 20 maternal populations in the parental generation were

evaluated in the summer of 2019 in ROS1 and replicated in ROS2(Maternal populations

were replicated, and the clones are not. Detailed entry information was presented in

Table 2.2 Entry information experimental maternal half-sibs evaluated in rainout

shelter trials at Freehold, NJ.). All trials were planted in the fall of prior years and

organized as spaced plant with 30.5cm spacing between each clone. Once established,

clones were mowed weekly at 7.62cm during growing seasons. NDVI was measured for

each clone with a FieldScout TCM 500 NDVI Turf Color Meter (Item#:2975NDVI,

Spectrum Technologies, 3600 Thayer Court, Aurora, IL) every week from initiation to

termination of a drought treatment. Segregation of parental populations in different trials

was monitored using the standard deviation of all clones. For the two trials in 2019,

drought treatment was terminated once the evaluated populations segregated to the same

level as in the 2017-ROS1 trial (

Figure 2.1 Standard deviation of tested populations in the rainout shelter trials.).

The last datasets collected before termination of trials were scaled, analyzed, and

presented in the following sections.

2.2.2. Statistical model and theoretical foundation

The following model was fitted separately to the parental and progeny generation.

In each generation, let Y_{ijk} denote the scaled NDVI value of the kth progeny of dam i in

environment j, and the model is then

$$Y_{ijk} = \beta_j + u_i + e_{ijk} \qquad \text{Equation 2.1}$$

In matrix notation, let **y** denote the vector of all scaled NDVI values. For $\boldsymbol{\beta} = \{\beta_j\}_{j=1}^{J}$,

$\mathbf{u} = \{u_i\}_{i=1}^{I}$ and $\mathbf{e} = \{\{\{e_{ijk}\}_{i=1}^{I}\}_{j=1}^{J}\}_{k=1}^{K}$, the model can also be expressed by the

following equation,

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e} \qquad\qquad \text{Equation 2.2}$$

where $\boldsymbol{\beta}$, $\mathbf{u}$, and $\mathbf{e}$ denote the environmental effect, maternal effect, and unexplained

residual effect respectively, $\mathbf{X}$ and $\mathbf{Z}$ are the corresponding design matrices for

environmental and maternal effect. Under the Bayesian framework, all effects are

assumed to be random. We further assumed that they all followed independent

normal distributions. Specifically,

$$
\begin{aligned}
\boldsymbol{\beta} \quad &\sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_\beta^2) \\
\mathbf{u} \quad &\sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_u^2) \\
\mathbf{e} \quad &\sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_e^2)
\end{aligned}
\qquad \text{Equation 2.3}
$$

where $\mathbf{I}$ is the identity matrix; σ_β^2, σ_u^2, and σ_e^2 are the variance components ascribed to

environmental effect, maternal effect, and unexplained residual effect respectively. The

model also assumes that all variance components follow an inverse-gamma distribution

with shape and scale parameter values of 0.001.

Assuming that epistasis is of minor importance, the variance of maternal effect

provides an approximation of one-quarter of the additive genetic variance in half-sib

designs, i.e. $\sigma_u^2 = \frac{1}{4}\sigma_A^2$ (Lynch & Walsh, 1998). Therefore, the additive genetic variance

can be estimated by $4\sigma_u^2$. The total phenotypic variance, σ_P^2, can be expressed by the

following equation.

$$\sigma_P^2 = \sigma_\beta^2 + \sigma_u^2 + \sigma_e^2 \qquad\qquad \text{Equation 2.4}$$

Hence the narrow-sense heritability is

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} = \frac{4\sigma_u^2}{\sigma_\beta^2 + \sigma_u^2 + \sigma_e^2} \qquad\qquad \text{Equation 2.5}$$

Given the same level of selection intensity and a defined population, the expected

response is determined by the accuracy of predicting breeding value, ρ, which measures

the correlation between true breeding values (TBVs) and EBVs. In mass selection, ρ equals the square root of the narrow-sense heritability estimate, h. In parental selection with half-sibs, the prediction accuracy is given by

$$\rho = \sqrt{\frac{nh^2}{4+(n-1)h^2}}$$
Equation 2.6

in which n is the number of half-sibs (Walsh & Lynch, 2018). In family selection with half-sib, wherein measured half-sibs from each family are used to create the next generation, the prediction accuracy takes the correlation between half-sibs into account,

$$\rho = \frac{n+3}{2}\sqrt{\frac{h^2}{4n+n(n-1)h^2}}$$
Equation 2.7

as described by Lush (1947). Similarly, n is the number of half-sibs.

The infinitesimal model is robust and straightforward for the inheritance of quantitative traits, in which total phenotypic variance is partitioned into a genetic and a non-genetic(environmental) components, and the genetic component of progeny traits follows a normal distribution around the mean of the parents; variance of this distribution is independent of the parental trait values and decreases due to selection and inbreeding(Barton, Etheridge, & Véber, 2017; Fisher, 1918). Under half-sib designs, the variance component was partitioned into environmental variance, maternal variance, and unexplained residual variance. The genetic component, specifically additive genetic component, can be subsequently estimated from the maternal variance. The genotypic covariance of maternal half-sibs plays a pivotal role in this estimation process. On the one hand, the genotype of kth progeny of ith dam in $Y_{ijk}=\beta_j + u_i +$

e_{ijk} Equation 2.1 can be expressed as follows.

$$G_{ik} = Y_{ijk} - \beta_j = u_i + e_{ijk} \qquad \text{Equation 2.8}$$

Given the genotypic values of two maternal half-sibs, G_{ik} and G_{il}, the genotypic

covariance of the two equals the variance of maternal effect, that is,

$$
\begin{aligned}
Cov(G_{ik}, G_{il}) &= Cov\big[(u_i + e_{ijk}), (u_i + e_{ijl})\big] \\
&= Cov(u_i, u_i) + Cov(u_i, e_{ijl}) + Cov(e_{ijk}, u_i) + Cov(e_{ijk}, e_{ijl}) \\
&= \sigma_u^2
\end{aligned}
$$

$$\text{Equation 2.9}$$

On the other hand, for two genetically related diploid clones, U and V, their genotypic

covariance can be partitioned into additive genetic variance and dominance genetic

variance, assuming no epistasis. We have that,

$$\text{Cov}(G_U, G_V) = \gamma\sigma_A^2 + \theta\sigma_D^2 \qquad \text{Equation 2.10}$$

where G_U and G_V are the genotype values of U and V, γ is the probability alleles drawn

randomly from U and V are identical-by-descent (IBD), θ is the probability that U and V

share two alleles IBD, σ_A^2 and σ_D^2 are additive genetic variance and dominance genetic

effect, respectively(Jacquard, 1974; Wright, 1966). With the previous example of the

genotypic values of two maternal half-sibs, G_{ik} and G_{il} , γ and θ take on values $\frac{1}{4}$ and 0

respectively, it follows,

$$\text{Cov}(G_{ik}, G_{il}) = \frac{1}{4}\sigma_A^2 \qquad \text{Equation 2.11}$$

which provides a logical connection between observed variance components that have

been estimated from the data and the underlying genetic components.

$$4\sigma_u^2 = 4\text{Cov}(G_{ik}, G_{il}) = \sigma_A^2 \qquad \text{Equation 2.12}$$

When it comes to the application to polyploid species like tall fescue, dominance genetic variance must be considered. Under similar assumptions, for two autohexaploid half-sibs, again U and V, it can be shown mathematically that

$$\text{Cov}(G_U, G_V) = \frac{1}{4}\sigma_A^2 + \frac{1}{25}\Delta_{UV}^{(2)}\sigma_{D_2}^2 + \frac{1}{400}\Delta_{UV}^{(3)}\sigma_{D_3}^2 \qquad \text{Equation 2.13}$$

where $\Delta_{UV}^{(t)}$ is the probability that all members of random t-allelic sets from U and V are IBD and $\sigma_{D_t}^2$ is the dominance genetic variance resulting from t-allelic interaction. Hence,

$$4\sigma_u^2 = 4\sigma_u^2\text{Cov}(G_U, G_V) = \sigma_A^2 + \frac{4}{25}\Delta_{UV}^{(2)}\sigma_{D_2}^2 + \frac{1}{100}\Delta_{UV}^{(3)}\sigma_{D_3}^2 \qquad \text{Equation 2.14}$$

The tall fescue studied in this project is allohexaploid exhibited disomic inheritance, whose genome is designated by $\mathbf{PPG_1G_1G_2G_2}$ (Meyer and Watkins, 2003; Pedersen & Sleper 1993). The value of $4\sigma_u^2$ most likely provided an estimate between the two extreme situations expressed in $4\sigma u2 = 4\text{Cov}(G_{ik}, G_{il}) = \sigma_A^2$ $\qquad$ Equation 2.12 and

$4\sigma u2 = 4\sigma u2\text{Cov}(G_U, G_V) = \sigma_A^2 + \frac{4}{25}\Delta_{UV}^{(2)}\sigma_{D_2}^2 + \frac{1}{100}\Delta_{UV}^{(3)}\sigma_{D_3}^2$ $\qquad$ Equation 2.14. When using $4\sigma_u^2$ as an estimate for the additive genetic effect of tall fescue population, it inevitably took a proportion of dominance genetic variance into account, leading to inflated results (Lynch & Walsh, 1998). Unfortunately, there has been no remedy to this problem yet to our knowledge.

2.2.3. Model implementation

Posterior distributions of parameters in the model were sampled in R 3.5.3(R Core Team, 2019) environment with package 'R2jags' (Su & Yajima, 2015). Codes of model implementation were presented as supporting information. The Gibbs sampler was

implemented with 5 Markov chain Monte Carlo chains, each with 50,000 iterations. The

first 10,000 iterations were discarded to minimize the effect of initial values, and the rest

iterations were thinned by taking every 10th to reduce sample autocorrelation. The

convergence of chains was confirmed via visual inspection. Analysis of results was

visualized using package 'tidyverse' (Wickham et al., 2019).

For comparison of parameter estimates from two generations, distributions of the

differences were simulated by randomly drawing 4000 samples (2,000 for each

generation) from the posterior samples with replacement.

2.3. Results

The posterior estimates of variance components (σ_u^2, σ_A^2, σ_e^2 and σ_β^2) from both
generations were presented in
Figure 2.2 Boxplot for posterior estimates of variance components from parental
and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials
in the summer of 2017 and 2019 at Freehold, NJ.. Variance components from two
generations showed a similar pattern. The largest variance component is environmental
variance, accounting for 59.41% of total phenotypic variance in the parental generation
and 92.33% of that in the progeny generation. The unexplained residual variance is the
second-largest component, constituting 20.75% of the total phenotypic variance in the
parental generation and 7.22% of that in the progeny generation, suggesting a better
model fitting to data collected from progeny generation. The smallest variance
component is the variance of maternal effect, which leads to low estimates of additive
genetic variance. Reduction in mean additive genetic variance and phenotypic variance
were observed from parental to progeny generation (
Figure 2.2 Boxplot for posterior estimates of variance components from parental

and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials

in the summer of 2017 and 2019 at Freehold, NJ.). Given an α level of 0.05, maternal

variance, additive genetic variance, and residual variance estimated from paternal

generation were significantly higher than those estimated from the progeny population.

The posterior distribution for heritability estimate from parental generation has a mean of

0.18, a mode of 0.09, and a 95% HDI of (0.04,0.51), while that from progeny generation

has a mean of 0.08, a mode of 0.02, and a 95% HDI of (0.01,0.23). Though significantly

greater than zero, both estimates were quite low, indicating most of the phenotypic

variance is not genetic. The difference between the mean heritability estimates was

estimated to be 0.11 with a 95% HDI of (-0.11, 0.45), suggesting no significant

differences between heritability estimates between the two generations. The difference in

population means of the two generations in trial 2019-ROS1 is 0.03 with a 95% HDI of (-

0.15, 0.18), and that in trial 2019-ROS2 is 0.00 with a 95% HDI of (-0.19, 0.18), showing

no significant differences in either trial (Figure 2.3 Population distributions of parental

and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials

in the summer of 2019 at Freehold, NJ.).

The

To

2.4. Discussion

Development of tall fescue cultivars with improved drought tolerance is hindered

by poor understanding of mechanisms of drought responding and by inadequate selection

techniques. A large volume of studies have been devoted to the physiological strategies

of tall fescue under drought stress. These strategies include escape, avoidance, and

tolerance to overcome drought stress (Huang & Gao, 1999; Pirnajmedin, Majidi, &

Gheysari, 2016; Tuberosa, 2012; Wang, Bughrara, & Nelson 2008). However,

information on the underlying genetics is still limited. In this project, we sought to

investigate the selection process using ROS by estimating genetic variance components

and their functions given that drought tolerance is characterized as a quantitative trait in

grass species (Jiang et al., 2017; Merewitz, Belanger, Warnke, Huang, & Bonos, 2014).

Turfgrass breeders have paid particular attention to additive genetic variance, as the most commonly used cool-season turfgrasses are cross-pollinated (Bonos, Clarke, & Meyer, 2006; Chen, Wang, Waltz, & Raymer, 2009), and only additive genetic effects can stably pass on from parent to offspring in long term selection (Nyquist & Baker, 1991). It is a common practice in cool-season turfgrass breeding to maintain maternal populations for selection. The half-sib design is, therefore, a convenient way to estimate the maternal breeding value and to conduct genetic experiments (Aastveit & Aastveit, 1990). Although it allows the evaluation of a broad spectrum of germplasms, half-sib design provides no estimation for dominance variance. The situation is more complicated given that tall fescue is an allohexaploid species, as pointed out in $4\sigma u2 = 4\sigma u2 \text{Cov}(G_U, G_V) = \sigma_A^2 + \frac{4}{25}\Delta_{UV}^{(2)}\sigma_{D_2}^2 + \frac{1}{100}\Delta_{UV}^{(3)}\sigma_{D_3}^2$ Equation 2.14. Other experimental designs, e.g., nested full-sib design, diallelic design, might provide estimations for dominance variance and additive genetic variance so long as estimated variance components can be translated into covariances of relatives. It is also worth noting that $\text{Cov}(G_U, G_V) = \frac{1}{4}\sigma_A^2 + \frac{1}{25}\Delta_{UV}^{(2)}\sigma_{D_2}^2 + \frac{1}{400}\Delta_{UV}^{(3)}\sigma_{D_3}^2$ Equation 2.13 points out an interesting consequence of polyploidy that the covariance of half-sibs can be influenced by dominance effect because parents pass more than one allele on to the progeny. In short term selection, breeders can take advantage of such dominance effect for higher response to selection. However, in long term recurrent selection breeding programs, dominance effect may vanish due to the segregation of alleles.

The posterior mean for h^2 estimates in this study were 0.18 for parental and 0.08 for progeny generation, suggesting a small proportion of genetic component in total phenotypic variance. In summary, environmental effects have significant impacts on the

performance of tall fescue populations during selection process using ROS. Despite being low, the heritability estimates were still significantly greater than zero, leading to a significantly positive correlation in EBVs of the two generations (Figure 2.6 Correlation of maternal effects estimated from parental and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials in the summer of 2017 and 2019 at Freehold, NJ.). Under Fisher's (1918) infinitesimal model, in the absence of any epistatic variance or genotype by environment interaction, directional truncation selection generates the same amount of negative disequilibrium contribution to additive genetic variance and phenotypic variance (Walsh & Lynch, 2018). That is to subtract the same amount of disequilibrium contribution from both the nominator and denominator in $h^2 =$

$$\frac{\sigma_A^2}{\sigma_P^2} = \frac{4\sigma_u^2}{\sigma_\beta^2 + \sigma_u^2 + \sigma_e^2}$$
Equation 2.5, leading to a smaller heritability

estimate in the progeny generation. We did not see a significant reduction in heritability or phenotypic variance from the parental generation to progeny generation, which is presumably due to large variances of parameters. However, additive genetic variance estimated from progeny generation was significantly lower than that estimated from parental generation, following Fisher's infinitesimal model. This study is the first to document the low heritability of green persistence of tall fescue in directional selection using ROS. Heritability estimates for tall fescue or any other turfgrass species for green persistence under drought stress have not been reported. However, decreased heritability of agronomic traits under drought stress has been documented for other crops. In maize (*Zea mays*), selection under drought conditions has often been considered less efficient comparing with non-stressed conditions due to a reduction in heritability for grain yield under stress (Bolaños & Edmeades, 1996; Rosielle & Hamblin, 1981). Similar

phenomena were also found in the breeding of wheat. Mathew et al. (2018) evaluated 99

genotypes of wheat (*Triticum aestivum* L.) and one triticale (X *Triticosecale* Wittmack)

under drought-stressed and non-stressed conditions in both field and greenhouse

environments. Drought stress decreased broad-sense heritability of root to shoot ratio

from 0.47 to 0.28 and grain yield from 0.55 to 0.17. Caution must be exercised for such

comparisons, as trait heritability varies greatly depending on the genetic makeup of the

materials studied, environmental conditions under which materials are evaluated, the

accuracy of phenotypic data, and even model specification. It is essential to state clearly

the model structure and formula for heritability estimation. In linear mixed models under

the frequentist framework, it is common that spatial and temporal factors (e.g., year

effect, location effect) are treated as fixed effects and genetic factors as random effects

(Smith, Cullis, & Thompson, 2005). In such a setting, the total phenotypic variance is

estimated only by the variances of random effects and unexplained residuals. While

under the Bayesian framework, all factors are random variables, environmental variances

must be included in the estimation of total phenotypic variance. The concepts of

heritability and variance components are features that describe the properties of

populations. They may not be transferable from one population or environment to

another.

　　　　Heritability measures the efficiency of response to selection. In mass selection,

low heritability leads to poor prediction accuracy. In the selection of tall fescue under

ROS, the phenotypic value of a tall fescue clone is not a good predictor of its breeding

value due to the low heritability estimation. The significance of this finding is that

minimal differences were reported between parental and progeny generation (Figure 2.3

Population distributions of parental and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials in the summer of 2019 at Freehold, NJ.). Selection in stress-managed environments, like ROS, assumes the site is not affected from additional factors, such as weeds, diseases, salinity, etc. In the breeding of turfgrasses, it is not uncommon to evaluate materials selected from ROS to additional screening improved overall turf quality. Breeders may adopt selection criteria for traits that may not be compatible with what is necessary for improvement of drought tolerance. Several previous studies have reported that Ky-31, with poor turf qualities, exhibits enhanced drought tolerance as compared to some of the advanced generation, dwarf-type tall fescue cultivars (Carrow & Duncan, 2003; Fu & Huang, 2004; Huang & Gao, 1999). Our study may offer some insight into this relationship from a genetic perspective.

In plant breeding, traits of low heritability can still be selected for successfully as long as appropriate selection methods are adopted. This study demonstrated that family selection and parental selection could both increase the prediction accuracy of EBV, compared with mass selection. It should be noted that, though similar, Figure 2.6 Correlation of maternal effects estimated from parental and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials in the summer of 2017 and 2019 at Freehold, NJ.was not a parent-offspring regression. Instead, it demonstrated the low correlation of EBVs estimated from two consecutive generations, which is also a manifestation of the low heritability for green persistence under drought stress. This correlation provides the theoretical foundation for the efficiency of family selection, which has played an important role in the selection of cool-season turfgrasses. Due to the low correlation, the mean prediction accuracy with family selection was only slightly

higher than that of mass selection. Parental selection yielded the highest prediction accuracy; however, it came with the price that fewer genotypes were evaluated. Compared with mass selection, both parental selection and family selection also require the production and evaluation of progenies, leading to longer selection cycles.

In summary, we have reviewed the theoretical foundations of genetic analysis in half-sib design under Fisher's infinitesimal model. Under this hypothesis, Bayesian linear mixed models were applied to the analysis of two consecutive generations of tall fescue populations tested in the ROS, and variance components and genetic parameters were estimated for this process. We first reported the heritability estimate for green persistence measured by NDVI of tall fescue in ROS selection. Mean heritability estimate from parental generation was 0.18, with a 95% credit interval of (0.04,0.51), while that from progeny generation was 0.08 with a 95% credit interval of (0.01,0.23). Given the heritability estimates and experimental design in the progeny generation, we then estimated the prediction accuracy with different selection methods. Selection methods ranked by mean prediction accuracy from the highest to the lowest are parental selection, family selection, and mass selection.

Table 2.1 Maternal effects of selected tall fescue clones and corresponding maternal plots evaluated in rainout shelter trials at Freehold, NJ.

Selected clones	Maternal plots	Maternal effect of selected clones		Maternal effect of maternal plots	
		mean	95% HDI[a]	mean	95% HDI[a]
ROS16-68	A15-509	0.09	(-0.11,0.29)	0.38	(0.15,0.62)
ROS16-69	A15-509	-0.07	(-0.27,0.12)		
ROS16-91	A15-513	0.14	(-0.05,0.35)	0.37	(0.13,0.62)
ROS16-92	A15-513	0.11	(-0.09,0.32)		
ROS16-80	A15-505	-0.07	(-0.27,0.13)	0.29	(0.04,0.53)
ROS16-34	A15-436	-0.04	(-0.25,0.17)	0.20	(0.01,0.39)
ROS16-36	A15-436	0.04	(-0.16,0.25)		

ROS16-37	A15-436	-0.02	(-0.22,0.18)		
ROS16-39	A15-436	0.08	(-0.11,0.28)		
ROS16-02	A15-311	0.16	(-0.04,0.39)	0.17	(-0.03,0.37)
ROS16-03	A15-311	0.21	(0.00,0.44)		
ROS16-26	A15-341	0.03	(-0.17,0.23)		
ROS16-30	A15-341	0.06	(-0.14,0.26)	0.16	(-0.04,0.36)
ROS16-32	A15-341	0.17	(-0.03,0.38)		
ROS16-85	A15-725	-0.10	(-0.32,0.10)	0.16	(-0.09,0.40)
ROS16-86	A15-725	0.03	(-0.16,0.23)		
ROS16-89	A15-675	-0.14	(-0.36,0.06)	0.15	(-0.09,0.39)
ROS16-72	A15-677	0.07	(-0.12,0.27)	0.13	(-0.10,0.37)
ROS16-40	A15-463	-0.02	(-0.23,0.17)	0.10	(-0.10,0.29)
ROS16-94	A15-463	0.00	(-0.20,0.19)		
ROS16-20	A15-316	-0.02	(-0.22,0.18)	0.08	(-0.11,0.28)
ROS16-21	A15-316	0.10	(-0.09,0.32)		
ROS16-54	A15-451	0.03	(-0.17,0.23)		
ROS16-55	A15-451	-0.20	(-0.43,0.00)	-0.03	(-0.22,0.17)
ROS16-56	A15-451	-0.09	(-0.30,0.11)		
ROS16-45	A15-708	-0.07	(-0.27,0.12)		
ROS16-46	A15-708	0.06	(-0.13,0.26)	-0.06	(-0.26,0.14)
ROS16-48	A15-708	-0.09	(-0.30,0.11)		
ROS16-78	A15-489	-0.05	(-0.25,0.15)	-0.07	(-0.31,0.17)
ROS16-13	A15-668	-0.02	(-0.22,0.17)	-0.12	(-0.32,0.07)
ROS16-08	A15-511	-0.18	(-0.40,0.02)	-0.15	(-0.34,0.05)
ROS16-10	A15-511	0.07	(-0.12,0.28)		
ROS16-04	A15-682	-0.17	(-0.39,0.02)		
ROS16-50	A15-711	-0.10	(-0.31,0.10)	-0.39	(-0.58, -0.19)
ROS16-51	A15-711	0.14	(-0.06,0.36)		
ROS16-53	A15-021	0.04	(-0.15,0.24)	-0.45	(-0.65, -0.25)
ROS16-14	A15-021	0.04	(-0.16,0.25)		
ROS16-16	A15-515	-0.20	(-0.43,0.00)	-0.47	(-0.67, -0.28)

[a] Highest density interval

Table 2.2 Entry information experimental maternal half-sibs evaluated in rainout shelter trials at Freehold, NJ.

Maternal population	Year-Location	Date of Data Collection	Days of Drought	Generation	Number of clones
A15-021	2019-ROS1	2019-08-07	63	Parental	40
A15-021	2019-ROS2	2019-07-17	42	Parental	40
A15-311	2017-ROS1	2017-08-01	62	Parental	40
A15-311	2019-ROS1	2019-08-07	63	Parental	22
A15-311	2019-ROS2	2019-07-17	42	Parental	22
A15-316	2017-ROS1	2017-08-01	62	Parental	40
A15-316	2019-ROS1	2019-08-07	63	Parental	22
A15-316	2019-ROS2	2019-07-17	42	Parental	22
A15-341	2017-ROS1	2017-08-01	62	Parental	40

A15-341	2019-ROS1	2019-08-07	63	Parental	22
A15-341	2019-ROS2	2019-07-17	42	Parental	22
A15-436	2017-ROS1	2017-08-01	62	Parental	40
A15-436	2019-ROS1	2019-08-07	63	Parental	22
A15-436	2019-ROS2	2019-07-17	42	Parental	22
A15-451	2017-ROS1	2017-08-01	62	Parental	40
A15-451	2019-ROS1	2019-08-07	63	Parental	22
A15-451	2019-ROS2	2019-07-17	42	Parental	22
A15-463	2017-ROS1	2017-08-01	62	Parental	40
A15-463	2019-ROS1	2019-08-07	63	Parental	20
A15-463	2019-ROS2	2019-07-17	42	Parental	20
A15-489	2017-ROS1	2017-08-01	62	Parental	20
A15-489	2019-ROS1	2019-08-07	63	Parental	10
A15-489	2019-ROS2	2019-07-17	42	Parental	10
A15-505	2017-ROS1	2017-08-01	62	Parental	20
A15-505	2019-ROS1	2019-08-07	63	Parental	10
A15-505	2019-ROS2	2019-07-17	42	Parental	10
A15-509	2017-ROS1	2017-08-01	62	Parental	20
A15-509	2019-ROS1	2019-08-07	63	Parental	10
A15-509	2019-ROS2	2019-07-17	42	Parental	10
A15-511	2017-ROS1	2017-08-01	62	Parental	40
A15-511	2019-ROS1	2019-08-07	63	Parental	20
A15-511	2019-ROS2	2019-07-17	42	Parental	20
A15-513	2017-ROS1	2017-08-01	62	Parental	20
A15-513	2019-ROS1	2019-08-07	63	Parental	10
A15-513	2019-ROS2	2019-07-17	42	Parental	10
A15-515	2017-ROS1	2017-08-01	62	Parental	40
A15-515	2019-ROS1	2019-08-07	63	Parental	20
A15-515	2019-ROS2	2019-07-17	42	Parental	20
A15-668	2017-ROS1	2017-08-01	62	Parental	40
A15-668	2019-ROS1	2019-08-07	63	Parental	20
A15-668	2019-ROS2	2019-07-17	42	Parental	20
A15-675	2017-ROS1	2017-08-01	62	Parental	20
A15-675	2019-ROS1	2019-08-07	63	Parental	10
A15-675	2019-ROS2	2019-07-17	42	Parental	10
A15-677	2017-ROS1	2017-08-01	62	Parental	20

A15-677	2019-ROS1	2019-08-07	63	Parental	10
A15-677	2019-ROS2	2019-07-17	42	Parental	10
A15-682	2017-ROS1	2017-08-01	62	Parental	40
A15-682	2019-ROS1	2019-08-07	63	Parental	20
A15-682	2019-ROS2	2019-07-17	42	Parental	20
A15-708	2017-ROS1	2017-08-01	62	Parental	40
A15-708	2019-ROS1	2019-08-07	63	Parental	20
A15-708	2019-ROS2	2019-07-17	42	Parental	20
A15-711	2017-ROS1	2017-08-01	62	Parental	40
A15-711	2019-ROS1	2019-08-07	63	Parental	20
A15-711	2019-ROS2	2019-07-17	42	Parental	20
A15-725	2017-ROS1	2017-08-01	62	Parental	20
A15-725	2019-ROS1	2019-08-07	63	Parental	10
A15-725	2019-ROS2	2019-07-17	42	Parental	10
ROS16-02	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-02	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-03	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-03	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-04	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-04	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-08	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-08	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-10	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-10	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-13	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-13	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-14	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-14	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-16	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-16	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-20	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-20	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-21	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-21	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-26	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-26	2019-ROS2	2019-07-17	42	Progeny	10

ROS16-30	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-30	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-32	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-32	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-34	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-34	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-36	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-36	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-37	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-37	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-39	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-39	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-40	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-40	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-45	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-45	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-46	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-46	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-48	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-48	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-50	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-50	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-51	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-51	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-53	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-53	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-54	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-54	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-55	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-55	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-56	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-56	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-68	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-68	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-69	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-69	2019-ROS2	2019-07-17	42	Progeny	10

ROS16-72	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-72	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-78	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-78	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-80	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-80	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-85	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-85	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-86	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-86	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-89	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-89	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-91	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-91	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-92	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-92	2019-ROS2	2019-07-17	42	Progeny	10
ROS16-94	2019-ROS1	2019-08-10	66	Progeny	10
ROS16-94	2019-ROS2	2019-07-17	42	Progeny	10

Figure 2.1 Standard deviation of tested populations in the rainout shelter trials.

Note:

σ_u^2 : variance of maternal effect

σ_A^2 : additive genetic variance

σ_e^2 : unexplained residual variance

σ_β^2 : variance of year effect

Figure 2.2 Boxplot for posterior estimates of variance components from parental and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials in the summer of 2017 and 2019 at Freehold, NJ.

Figure 2.3 Population distributions of parental and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials in the summer of 2019 at Freehold, NJ.

Note: Plots with positive mean maternal effects were depicted in red, while those with negative mean maternal effects in green.

Figure 2.4 Boxplot for posterior estimates of maternal effects of tall fescue parental generation evaluated in rainout shelter trials in the summer of 2017 and 2019 at Freehold, NJ

Note: Clones with positive mean maternal effects were depicted in red, while those with negative mean maternal effects in green.

Figure 2.5 Boxplot for posterior estimates of maternal effects of tall fescue progeny generation evaluated in rainout shelter trials in the summer of 2019 at Freehold, NJ.

Figure 2.6 Correlation of maternal effects estimated from parental and progeny generation of tall fescue maternal half-sibs evaluated in rainout shelter trials in the summer of 2017 and 2019 at Freehold, NJ.

3. ANALYSIS AND OPTIMIZATION OF STRATIFIED MASS SELECTION METHOD IN RAINOUT SHELTER SELECTION OF TALL FESCUE

Abstract

The choice of selection method has significant implications for selection gain in a tall fescue breeding program. In this study, 96 selected tall fescue genotypes were evaluated in a series of outdoor rain-out shelter trials carried out in the summer of 2017, 2018, and 2019 using a ten-block stratified mass selection design. Data on the green persistence under drought was fitted using mixed linear models under the Bayesian framework for the partition of total phenotypic variance and prediction of breeding value. The study first estimated the heritability of green persistence of tall fescue under drought. The mean heritability estimation was 0.07.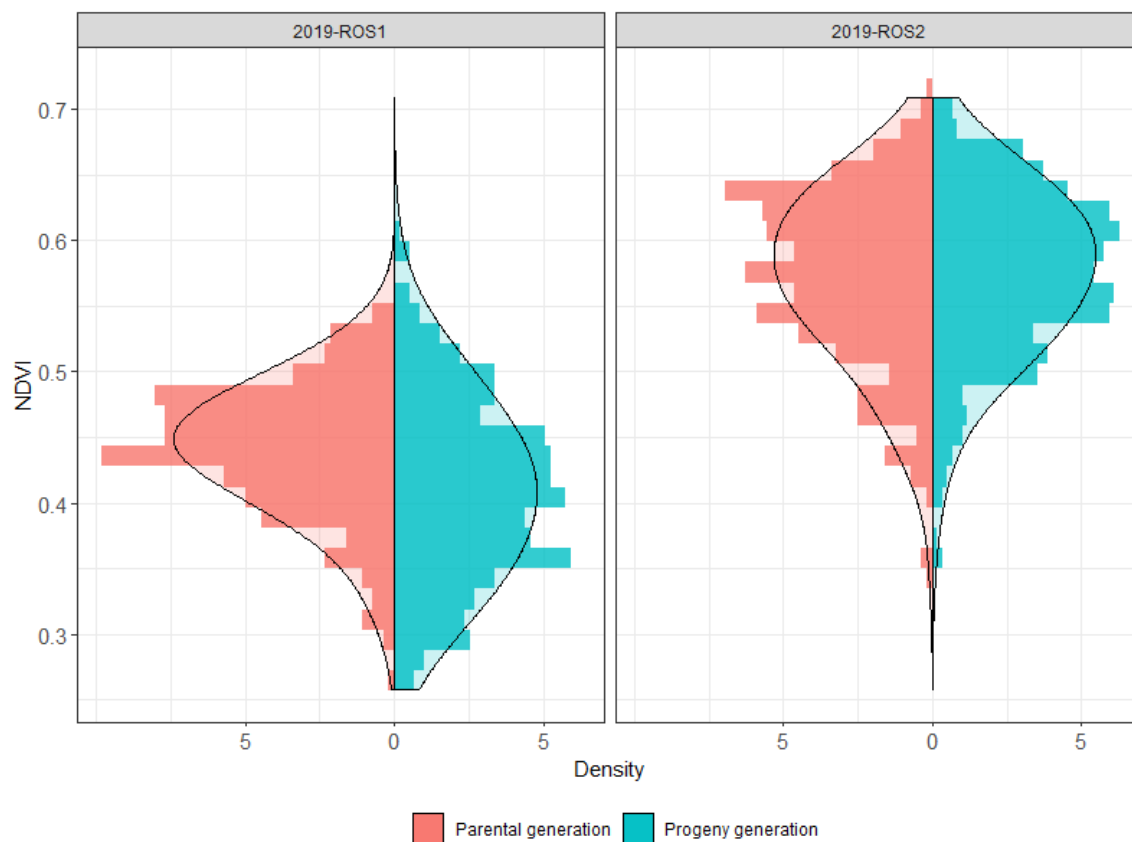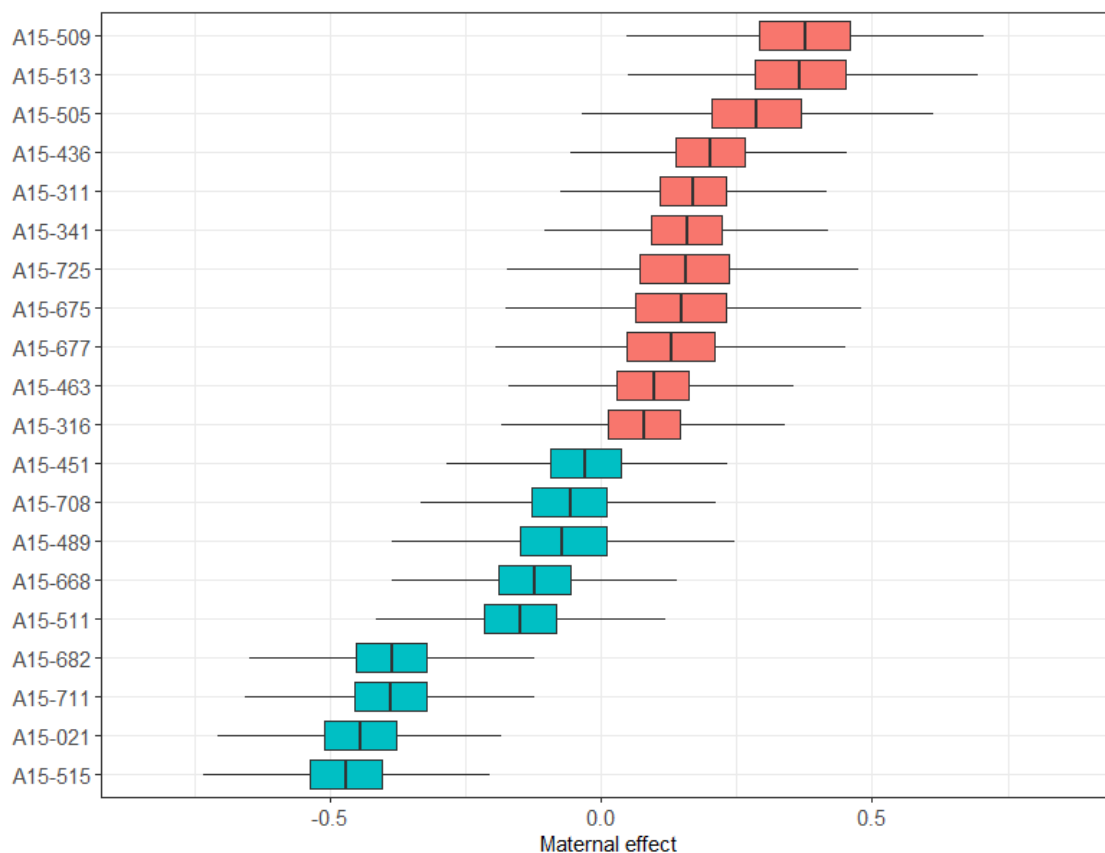 Given this heritability estimation, the theoretical prediction accuracy for parental selection, family selection, stratified mass selection, and the mass selection was calculated. The parental selection was found to have the highest mean prediction accuracy, surpassing the prediction accuracy of stratified mass selection, family selection, and mass selection. Extra attention was paid to stratified mass selection. Under the stratified mass selection method, our work demonstrated the application of different variations of best linear unbiased prediction methods (BLUP, A-BLUP, and G-BLUP) in the breeding tall fescue, and illustrated how prediction accuracy could be further improved by increasing the number of blocks or/and the implementation of A-BLUP and G-BLUP.

Keywords: BLUP, ABLUP, GBLUP, PBV, heritability, prediction accuracy.

3.1. Introduction

Global climate change presents a significant challenge for turfgrass research. The predicted rise in global temperature is expected to intensify both the frequency and severity of drought (Jacob et al., 2014; Min et al., 2011). Facing this challenge, the development of cool-season turfgrass cultivars that can thrive under drought stress remains one of the most important research objectives. Tall fescue is a predominant cool-season perennial grass in the United States. Previous studies have shown that tall fescue responds to drought stress with various mechanisms, including escape, avoidance, and tolerance (Huang & Gao, 1999; Pirnajmedin et al., 2015). Drought escape refers to the rapid phenological development of plants to complete life cycle, followed by dormancy during severe drought stress (Wang et al., 2008), while the latter two mechanisms serve to improve water uptake, minimize water loss and avoid dormancy (Pirnajmedin et al., 2015). Although escaping drought stress by dormancy may aid in the long-term survival of tall fescue, most turfgrass managers prefer to maintain a dense green surface for aesthetics, playability, and safety (Cross et al., 2013; Karcher et al., 2008).

Recent years have seen increasing applications of rainout shelter (ROS) in the selection and evaluation of turfgrass and forage cultivars (Hatier et al.,2014; Lootens et al., 2016; Schwartz et al., 2018; Steinke et al., 2010; Zhou et al., 2009). ROS allows precise control and alteration of water availability on outdoor field research plots, creating uniform selection environments (Fay et al., 2000). There are many selection methods in the breeding of tall fescue, among which mass selection, parental selection, family selection, and stratified mass selection are most commonly used. Given the low heritability of green leaf persistence under drought, parental selection is preferred over

mass selection and family selection concerning prediction accuracy for breeding values.

However, high prediction accuracy in the family selection and parental selection comes at

the cost of additional effort on production and evaluation of progenies. Stratified mass

selection provides the opportunity to maintain a sensible prediction accuracy by

increasing sample size and control of environmental variance while shortening the

breeding cycle at the same time (Gardner, 1961; Pedersen & Sleper, 1993; Walsh &

Lynch, 2018). The appropriate sample size for a study is a fundamental question in

quantitative studies. In turfgrass breeding, the sample size for an experiment can be

increased directly by asexual propagation of the same genotype in multi-environmental

trials. The genotypic values of evaluated clones can then be estimated using the best

linear unbiased prediction (BLUP) method (Henderson, 1950). The correlation of related

genotypes can be taken into account to increase the sample size further. The presumption

is that the correlation between two phenotypic values depends on the relatedness of two

individuals and the heritability of trait of interest (Raffa & Thompson, 2016). The

relatedness among individuals can be described by relationship matrices. Traditionally,

the relationship matrices are inferred from the pedigree structure, i.e., **A** matrix, and the

genetic merit of evaluated genotypes can be subsequently estimated using the best linear

unbiased prediction method based on pedigree, i.e., A- BLUP (Falconer & Mackay 1996;

Henderson, 1975). However, the open-pollination nature of most cool-season turfgrasses

and polycross practice in cultivar development inevitably lead to incomplete pedigree,

adding obstacles to the implementation of A-BLUP. More recently, marker-based

methods have been used to construct genomic relationship matrices for estimation of

breeding values, i.e., **G** matrix and G-BLUP (Gianola, Cecchinato, Naya, & Schön,

2018). The idea of genomic prediction has been proposed for the breeding of cool-season perennial grass by Talukder and Saha (2017), but few applications have been reported. Potential impediments to the widespread use of genomic prediction include marker calling in polyploidy species, calculation of unbiased relatedness matrix using low coverage sequencing, and non-Mendelian inheritance of genomic markers (Dodds et al., 2015).

In this project, 96 selected tall fescue genotypes were evaluated during the summer of 2017, 2018, and 2019, in a stratified mass selection design with ten blocks. Green persistence of each tall fescue genotype was documented using normalized difference vegetation index (NDVI). Relationship matrices were constructed based on maternal pedigree and single-nucleotide polymorphism markers (SNPs) from *de-novo* assembly of next-generation sequencing data. Collected NDVI data in combination with different relationship matrices were fitted with Bayesian mixed linear models for genetic analysis. The objectives of this project is 1) to estimate the heritability of green persistence of leaf tissue measured by NDVI in tall fescue, 2) to determine and compare theoretical prediction accuracy when using different selection methods, 3)to predict breeding values of evaluated genotypes using BLUP, A-BLUP and G-BLUP implemented under Bayesian framework, 4) and to compare the prediction accuracy of BLUP, A-BLUP, and G-BLUP.

3.2. Material and methods

3.2.1. Experiment design and data collection

The experiments were conducted at the Rutgers Plant Biology Research and Extension Farm during the summer of 2017, 2018 and 2019 in two outdoor rain-out shelters at Freehold, NJ. ROS1 is an automated double-layered canopy signaled to cover the trial area from precipitations by air moisture sensors, and ROS2 is a stationary greenhouse that protects the trial area for the entire duration of drought treatment. The soil type in both shelters is categorized as sandy loam, with a slight acidity. Mechanical analysis indicates soil from ROS1 consists of 56% sand, 26% silt, and 18% clay, while that from ROS2 consists of 70% sand, 15% silt, and 16% clay. A total number of 96 tall fescue genotypes were evaluated under drought stress in stratified mass selection design with ten blocks. All blocks were planted in the fall of the prior year and organized as spaced-plant with 30.5cm spacing between each plant. After establishment, plants were mowed weekly at 7.62cm during growing seasons. NDVI data were collected from each plant with a FieldScout TCM 500 NDVI Turf Color Meter (Item#:2975NDVI, Spectrum Technologies, 3600 Thayer Court, Aurora, IL) every week from activation to termination of drought treatment. Segregation of all plants evaluated in the same trial was monitored using the standard deviation of collected NDVI data. Once the standard deviation of the evaluated population reached the same level as in the 2017-ROS1 trial (

Figure 2.1 Standard deviation of tested populations in the rainout shelter trials.), drought stress was terminated, and the last datasets collected before termination of trials were scaled, analyzed and presented in the following section.

3.2.2.   Construction and Sequencing of ddRADseq libraries

Young leaf tissue of all 96 genotypes was collected and ground under liquid nitrogen. Genomic DNA (gDNA) was extracted following the manufacture's recommendation for the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Construction of ddRADseq libraries followed the method outlined by Poland and Rife (2012). Briefly, gDNA was double digested with the common-cutting *Msp*I (New England Biolabs, Ipwich, MA, USA) and the rare-cutting *Pst*I-HF (New England Biolabs, Ipwich, MA, USA) restriction enzymes. After ligation with *Msp*I adapter and double-stranded barcode adapter, restricted fragments were purified with the AMPure XP PCR purification kit (Agencourt Bioscience, Beverly, MA, USA) as instructed by the manufacturer. Purified samples were amplified, normalized, and pooled into two 48-plex sequencing samples. The resulting sequencing samples were submitted to GeneWiz (GeneWiz, South Plainfield, NJ, USA) for sequencing via the Illumina HiSeq 2500 sequencing platform (Illumina, San Diego, CA, USA).

3.2.3.   SNP calling and construction of relationship matrices from SNPs

The identification of SNP markers was performed using the Stacks v.1.47 pipeline (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011). A total number of 37,142 SNPs were identified. After imposing a linkage disequilibrium threshold of 0.2 and a minor allele frequency of 0.01, the number of SNPs was pruned to 970. Two SNP-based relationship matrices (unfiltered and filtered) were thereby calculated using the maximum likelihood method in R/Bioconductor Package 'SNPRelate' (Zheng et al, 2012).

3.2.4. Construction of relationship matrix from maternal pedigree

An incomplete pedigree of selected 96 tall fescue clones was available in the breeding program. Most of the sire information was missing, due to the polycross practice in cultivar development. Pedigree chart (Figure 3.1 Pedigree chart of 96 tall fescue clones evaluated under drought stress in summer of 2017, 2018 and 2019 at Freehold, NJ.) and **A** matrix were constructed using R package 'optiSel' (Wellmann, 2017), assuming the missing sires are random, independent genotypes that are not related to any other genotypes in the pedigree chart.

3.2.5. Statistical models and model implementation

Let y_{ij} denotes the scaled NDVI of the jth plant from block $i(i = 1,2, \dots, n; j = 1,2, \dots, n_j)$. y_{ij} can be expressed as the sum of an environmental value consisting of a block effect, β_i, its genotypic value, u_{ij}, and the residual environmental value, e_{ij}, that is

$$y_{ij} = \beta_i + u_{ij} + e_{ij}$$

In matrix notation,

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e} \qquad \text{Equation 3.1}$$

where **X** and **Z** are the corresponding design matrix for block effect and genotype effect. Under the Bayesian framework, all effects were assumed to follow independent normal distributions. Specifically,

$$\begin{aligned}
\boldsymbol{\beta} &\sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_\beta^2) \\
\mathbf{u} &\sim \text{MVN}(\mathbf{0}, \mathbf{K}\sigma_u^2) \\
\mathbf{e} &\sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_e^2)
\end{aligned}$$
<div align="right">Equation 3.2</div>

where $\mathbf{I}$ is the identity matrix, $\mathbf{K}$ is the causal relationship matrix; σ_β^2, σ_u^2, and σ_e^2 are the variance components ascribed to block effect, genotype effect, and residual effect, respectively. The model also assumed that all variance components followed an inverse-gamma distribution with shape and scale parameter values of 0.001. Therefore, the total phenotypic variance, σ_P^2 , can be expressed by the following equation.

$$\sigma_P^2 = \sigma_\beta^2 + \sigma_u^2 + \sigma_e^2$$

The heritability is

$$h^2 = \frac{\sigma_u^2}{\sigma_P^2} = \frac{\sigma_u^2}{\sigma_\beta^2 + \sigma_u^2 + \sigma_e^2}$$
<div align="right">Equation 3.3</div>

Four different relationship matrices were adopted to approximate the causal relationship matrix, $\mathbf{K}$, in the analysis of this study. The first was $\mathbf{I}$, which assumes all the clones are unrelated, leading to the BLUP of genotypic values. The second was $\mathbf{A}$ matrix inferred from maternal pedigree, which assumes random, unrelated paternal clones in matrix construction, providing the A-BLUP of genotypic values. The last two were $\mathbf{G}$ matrices constructed from unfiltered and filtered SNP markers respectively, providing two different estimations for G-BLUP of genotypic values.

The resampling method was applied to simulate the process of data collection given different numbers of blocks, n . Specifically, for $n = 1,2,3,\dots,10$, a sample dataset was created by randomly selecting n blocks of data from the total ten blocks of collected data. The sample dataset was then fitted with the models given in (1) in combination with

four genomic relationship matrices for estimation of predicted breeding value (PBV),

heritability, and the prediction accuracy. This process was repeated 20 times to access

the variance of estimated values.

Theoretical prediction accuracy for PBV, ρ, in stratified mass selection, was

approximated by the correlation between within-block deviation and the true breeding

value (TBV, Walsh & Lynch, 2018),

$$\rho = \rho(y_{ij} - \overline{y_i}, A_{ij}) \quad \approx (1 - \frac{1}{n})\sqrt{\frac{\sigma_u^2}{\sigma_u^2 + \sigma_e^2}} \qquad \text{Equation 3.4}$$

where $\overline{y_i}$ is the mean of block i, A_{ij} is the TBV of jth clone from block i. For comparison
with mass selection, family selection and parental selection, the corresponding theoretical
prediction accuracy for PBV was quantified based on posterior samples of h^2 estimated
using the identity matrix and all ten blocks of collected data under a postulated family
size of 20 maternal half-sibs following the experimental design in the previous chapter. In
mass selection, ρ is the square root of h^2 by definition. In parental selection and family
selection, ρ is a function of family size, m, and h^2. Specifically, in parental selection
with half-sibs,

$$\rho = \sqrt{\frac{mh^2}{4 + (m-1)h^2}} \qquad \text{Equation 3.5}$$

as described by Walsh and Lynch (2018). In family selection with half-sib, ρ takes the

correlation between half-sibs into account,

$$\rho = \frac{m+3}{2}\sqrt{\frac{h^2}{4m + m(m-1)h^2}} \qquad \text{Equation 3.6}$$

as described by Lush (1947).

For comparison of analytical results using different relationship matrices, realized

prediction accuracy was accessed using Pearson's correlation coefficient, which measures

the linear correlation between PBVs and estimations of TBVs. Estimations of TBVs from

38 out of 96 selected genotypes were provided in the previous chapter.

Posterior distributions of parameters in the model were sampled in R (R Core Team, 2019) environment with package 'R2jags' (Su & Yajima, 2015). In each model, a total number of 5 Markov chain Monte Carlo chains each with 20,000 iterations were implemented, with the first 10,000 iterations were discarded. The rest iterations were thinned by taking every 5th to reduce sample autocorrelation. The convergence of chains was visually confirmed. Visualization of analysis results was achieved using package 'tidyverse' (Wickham et al., 2019).

3.3. Results

3.3.1.   Genetic parameters and predicted breeding values (PBVs)

The 96 clones evaluated in this study traced back to 8 founders (Figure 3.1 Pedigree chart of 96 tall fescue clones evaluated under drought stress in summer of 2017, 2018 and 2019 at Freehold, NJ.). For demonstration and comparison purposes, **I** together with **A** matrix, unfiltered **G** matrix, and filtered **G** matrix was visualized in Figure 3.2 Visualization of different relationship matrices for 96 selected tall fescue clones evaluated under drought stress in summer of 2017, 2018 and 2019 at Freehold, NJ.. The **A** matrix and filtered **G** matrix showed a similar pattern, which can also be vaguely recognized in the filtered **G** matrix. Compared with **I**, more information was filled in the off-diagonal areas in the other relationship matrices, indicating the correlations of random effect **u**. Similar relationship matrices lead to close results in the estimation of variance components. The largest variance component with all four matrices is environmental variance, accounting for about 63% of the total phenotypical variance on average. The residual variance is the second-largest component, constituting

43% of the total phenotypical variance. The smallest variance component is the variance

of the genotypic effect, leading to low estimates of heritability (Table 3.1 Summary

statistics for variance components and heritability estimated from NDVI data collected

from 96 selected tall fescue genotypes evaluated in rainout shelter trials in summer of

2017, 2018, and 2019 at Freehold, NJ). The mean posterior heritability estimates using $\mathbf{I}$,

$\mathbf{A}$ matrix, unfiltered $\mathbf{G}$ matrix, and filtered $\mathbf{G}$ from ten blocks of tall fescue clones were

0.072, 0.074, 0.069 and 0.023 respectively. PBVs estimated using four matrices were

shown in Figure 3.3 Posterior mean and 95% highest density interval for predicted

breeding values of 96 selected tall fescue genotypes evaluated under drought stress in

the summer of 2017, 2018, and 2019 at Freehold, NJ.. Genotypes with high PBVs

included ROS16-079, ROS16-022, ROS16-002, ROS16-047, ROS-092, etc., while those

with low PBVs were ROS16-093, ROS16-095, ROS16-098, ROS-090, ROS-096, etc.

3.3.2. Theoretical and realized prediction accuracy

The boxplot of theoretical prediction accuracy of stratified mass selection

estimated from random subsamples of collected data in combination with different

relationship matrices was presented in Figure 3.4 Boxplot for theoretical prediction

accuracy from random subsamples of the 10 blocks of 96 selected tall fescue clones

evaluated under drought stress in the summer of 2017, 2018 and 2019 at Freehold, NJ.,

overlaid by the mean and 95% highest density interval(HDI) of theoretical prediction

accuracy in the mass selection, parental selection and family selection given previously

defined settings. Parental selection yielded the highest mean theoretical prediction

accuracy, with a mean of 0.509, and a 95% HDI of (0.369, 0.626), followed by family

selection, with a mean theoretical prediction accuracy of 0.293 and a 95% HDI of (0.212, 0.360). The mass selection was the lowest in mean theoretical prediction accuracy, with a value of 0.265 and a 95% HDI of (0.177, 0.354).

With the increase of the number of blocks, the variance of theoretical prediction accuracy in stratified mass selection estimated using all four relationship matrices decreased while the mean increased. The mean theoretical prediction accuracy in the stratified mass selection, in general, lay between that of parental selection and family selection, except for the prediction accuracy estimated using unfiltered **G** matrix. The highest mean theoretical prediction accuracy with the smallest variance was obtained when all ten blocks of data taken into account. The mean theoretical prediction accuracy and corresponding 95% HDI estimated using **I**, **A** matrix, filtered **G** matrix, and unfiltered **G** matrix was 0.392 (0.233, 0.509), 0.399(0.247, 0.519), 0.230(0.133, 0.314), and 0.384(0.232, 0.511) respectively.

To further investigate the differences in analytical results when using BLUP, A-BLUP and G-BLUP under a stratified mass selection design, realized prediction accuracy for PBVs was estimated and visualized in Figure 3.5 Estimates for realized prediction accuracy and 95% highest density interval from random subsamples of the 10 blocks of 96 selected tall fescue clones evaluated under drought stress in the summer of 2017, 2018 and 2019 at Freehold, NJ.. Given different numbers of blocks, the mean realized prediction accuracy for PBVs using all four relationship matrices fell within the range of 0.12 to 0.33, consistent with the theoretical prediction accuracy estimated using filtered **G** matrix. Similarly, realized prediction accuracy increased with the numbers of blocks and seemed to plateau after seven blocks. The adoption of maternal pedigree and

genomic relationship matrices consistently increased realized prediction accuracy. Specifically, in seven out of ten simulations, realized prediction accuracy estimated using the filtered **G** matrix yielded the highest value, compared with the values estimated using the other three matrices. Realized prediction accuracy estimated using **A** matrix and unfiltered **G** relationship matrix was, for the most part, identical, and higher than that estimated from the identity matrix.

3.4. Discussion

In Chapter 2, variance components and heritability of green persistence of tall fescue in rain-out shelter trials with half-sib designs were estimated using the genetic covariance between half-sibs. The model assumed that scaled phenotypic observations, **y**, follows a multivariate normal distribution:

$$\mathbf{y} \quad \sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_\beta^2 + \mathbf{K}\sigma_u^2 + \mathbf{I}\sigma_e^2) \qquad \text{Equation 3.7}$$

The heritability, fraction of the variance of **y** due to the genetic component, can then be estimated using $h^2 = \frac{\sigma_u^2}{\sigma_P^2} = \frac{\sigma_u^2}{\sigma_\beta^2 + \sigma_u^2 + \sigma_e^2}$ Equation 3.3. However, the causal genetic variants were unknown in practice. Four different approximations of **K** were chosen in this study. The first approximation was **I**, which assumed all evaluated genotypes were mutually independent without any correlation imposed by relatedness. This model captured not only the additive genetic effect but also epistasis and dominance effect, providing an estimation of broad-sense heritability and BLUPs for **u**. The second approximation utilizes the **A** matrix, which assumes constant and categorical covariance between related individuals. For example, full-sibs are expected

to share 50% of alleles that are identical-by-descent (IBD), while half-sibs are expected to share 25% of alleles that are IBD (Falconer & Mackay, 1996). First proposed by Henderson (1975), the A-BLUP method has been widely used to estimate the genetic merit of individuals in both animal and plant breeding programs. However, the relationship matrix derived from pedigree estimates the expected proportion of the genome that is IBD, ignoring the random sampling of the two possible alleles from each parent at each locus during meiosis, i.e., the Mendelian sampling term(Avendaño, Woolliams, & Villanueva, 2005; Zapata-Valenzuela, Whetten, Neale, McKeand, & Isik, 2013). Given this reason, two **G** matrices were also adopted to approximate **K**. Compared with the **A** matrix, **G** matrices indicate the realized and more accurate estimates of genetic covariances among relatives, constituting the core concept of G-BLUP. With the previous examples, the genomic covariance of the same full sibs can vary around a mean of 0.5, while that of the same half-sibs can vary around a mean of 0.25. As the number of markers used to construct the relationship matrix increases, the **G** matrix is expected to converge to the **A** matrix. Indeed, a similar pattern was observed in the **A** matrix and the unfiltered **G** matrix in Figure 3.2 Visualization of different relationship matrices for 96 selected tall fescue clones evaluated under drought stress in summer of 2017, 2018 and 2019 at Freehold, NJ.. Marker filtering is an essential step to reduce the number of collinear or false-positive SNPs and remains to be an active field of research. Typically applied filters check for linkage disequilibrium (LD), allele frequency, minimum and maximum read depth. (Altman et al. 2012). The mean heritability estimated from **I**, **A** matrix, and unfiltered **G** matrix was around 0.070, close to the estimation in the previous chapter. However, the mean heritability

estimated using the filtered $\mathbf{G}$ matrix was only 0.023. This discrepancy has been termed

missing heritability (Zuk, Hechter, Sunyaev, & Lander, 2012), indicating that additional

causal variants remain to be discovered. The low heritability subsequently leads to low

theoretical prediction accuracy when using the filtered $\mathbf{G}$ matrix to estimate PBVs

(Figure 3.4 Boxplot for theoretical prediction accuracy from random subsamples of the

10 blocks of 96 selected tall fescue clones evaluated under drought stress in the

summer of 2017, 2018 and 2019 at Freehold, NJ.). However, in terms of the realized

prediction accuracy obtained (Figure 3.5 Estimates for realized prediction accuracy and

95% highest density interval from random subsamples of the 10 blocks of 96 selected

tall fescue clones evaluated under drought stress in the summer of 2017, 2018 and

2019 at Freehold, NJ.), it was clear that both G-BLUP and A-BLUP increased

prediction accuracy compared with BLUP. It is also worth noting that the A-BLUP

method was implemented with the maternal pedigree. When genomic data is not

available, breeders can improve the prediction accuracy of BLUP by incorporating

partial pedigree information.

To determine the prediction accuracy of stratified mass selection relative to mass

selection, parental selection, and family selection, we also calculated the theoretical

prediction accuracy based on estimated heritability values in the rain-out shelter selection

process. Stratified mass selection with more than five blocks yield mean prediction

accuracy higher than that in family selection with 20 maternal half-sibs but still lower the

prediction accuracy than paternal selection. However, stratified mass selection may save

breeders time and effort in progeny production and evaluation, which could potentially be

more efficient in the long run. The mean theoretical prediction accuracy in stratified mass

selection showed relatively considerable variation when the number of blocks is within

the range of one to four and started to plateau after five blocks. When studying the

realized prediction accuracy, we find it did not reach a plateaued level until seven blocks.

Hence, taking both prediction accuracy and parsimony into consideration, a seven-block

stratified mass selection design combined with G-BLUP or A-BLUP method in selection

is recommended in the rain-out shelter selection of tall fescue.

Table 3.1 Summary statistics for variance components and heritability estimated from NDVI data collected from 96 selected tall fescue genotypes evaluated in rainout shelter trials in summer of 2017, 2018, and 2019 at Freehold, NJ.

Parameter	Matrix	Mean	Median	Mode	95%HDI[a]
Heritability (h^2)	Identity	0.072	0.071	0.074	(0.031,0.125)
	Pedigree-based	0.074	0.071	0.062	(0.034,0.130)
	SNP-based (filtered)	0.023	0.022	0.020	(0.010,0.041)
	SNP-based (unfiltered)	0.069	0.067	0.068	(0.031,0.124)

Variance of block effect (σ_β^2)	Identity	0.075	0.073	0.069	(0.045,0.115)
	Pedigree-based	0.078	0.075	0.070	(0.048,0.117)
	SNP-based (filtered)	0.023	0.023	0.022	(0.014,0.036)
	SNP-based (unfiltered)	0.074	0.072	0.068	(0.042,0.116)
Variance of genotype effect (σ_u^2)	Identity	0.623	0.511	0.440	(0.202,1.720)
	Pedigree-based	0.632	0.543	0.485	(0.217,1.532)
	SNP-based (filtered)	0.634	0.551	0.437	(0.220,1.496)
	SNP-based (unfiltered)	0.641	0.554	0.432	(0.206,1.541)
Variance of residual effect (σ_e^2)	Identity	0.428	0.427	0.423	(0.387,0.472)
	Pedigree-based	0.427	0.426	0.427	(0.389,0.470)
	SNP-based (filtered)	0.428	0.428	0.426	(0.389,0.470)
	SNP-based (unfiltered)	0.430	0.430	0.431	(0.389,0.472)

Note: [a] Highest density interval

Figure 3.1 Pedigree chart of 96 tall fescue clones evaluated under drought stress in summer of 2017, 2018 and 2019 at Freehold, NJ.

Figure 3.2 Visualization of different relationship matrices for 96 selected tall fescue clones evaluated under drought stress in summer of 2017, 2018 and 2019 at Freehold, NJ.

Figure 3.3 Posterior mean and 95% highest density interval for predicted breeding values of 96 selected tall fescue genotypes evaluated under drought stress in the summer of 2017, 2018, and 2019 at Freehold, NJ.

Note: [a] Highest density interval

Figure 3.4 Boxplot for theoretical prediction accuracy from random subsamples of the 10 blocks of 96 selected tall fescue clones evaluated under drought stress in the summer of 2017, 2018 and 2019 at Freehold, NJ.

Note: EBV, estimated breeding value.

Figure 3.5 Estimates for realized prediction accuracy and 95% highest density interval from random subsamples of the 10 blocks of 96 selected tall fescue clones evaluated under drought stress in the summer of 2017, 2018 and 2019 at Freehold, NJ.

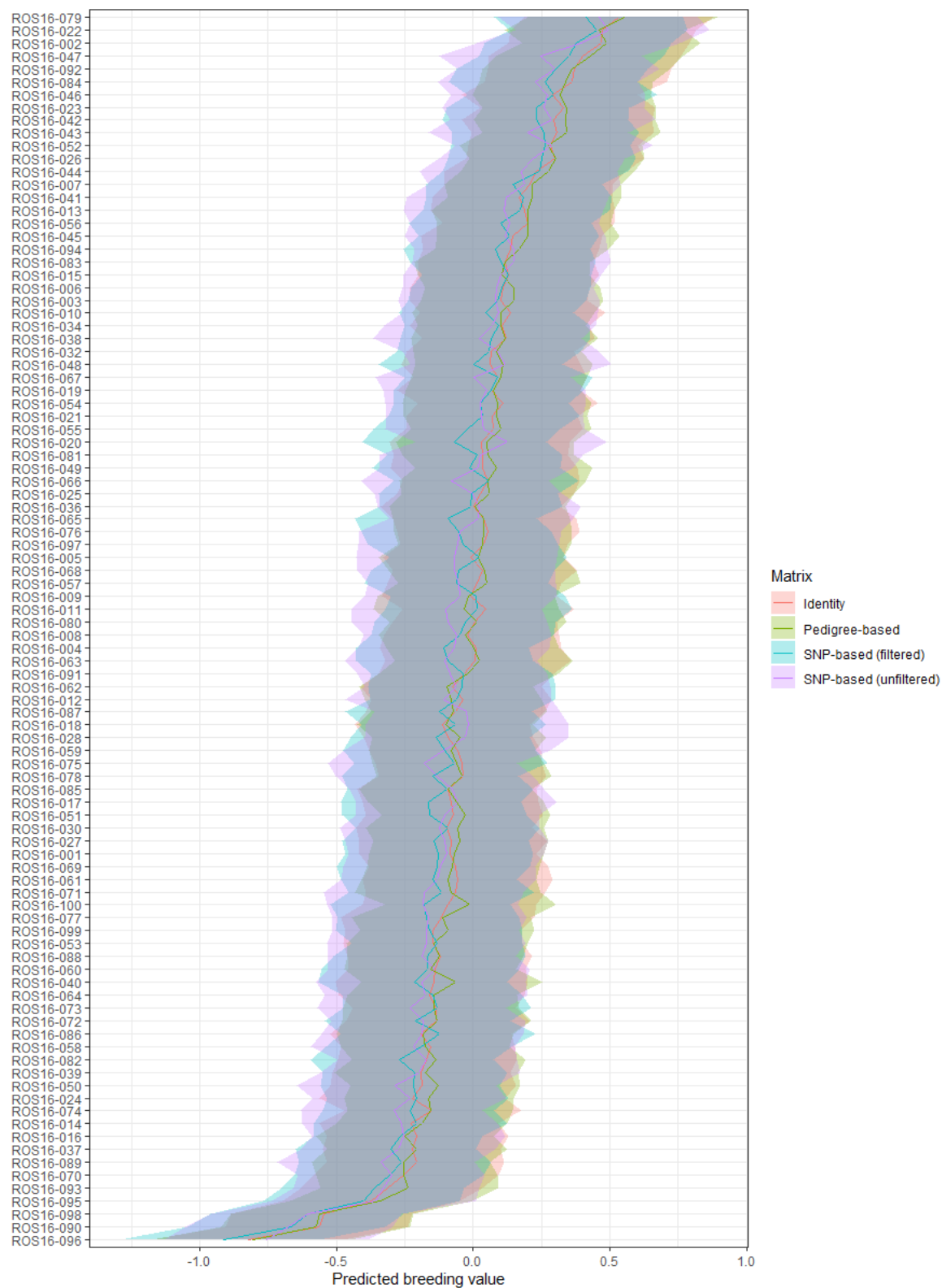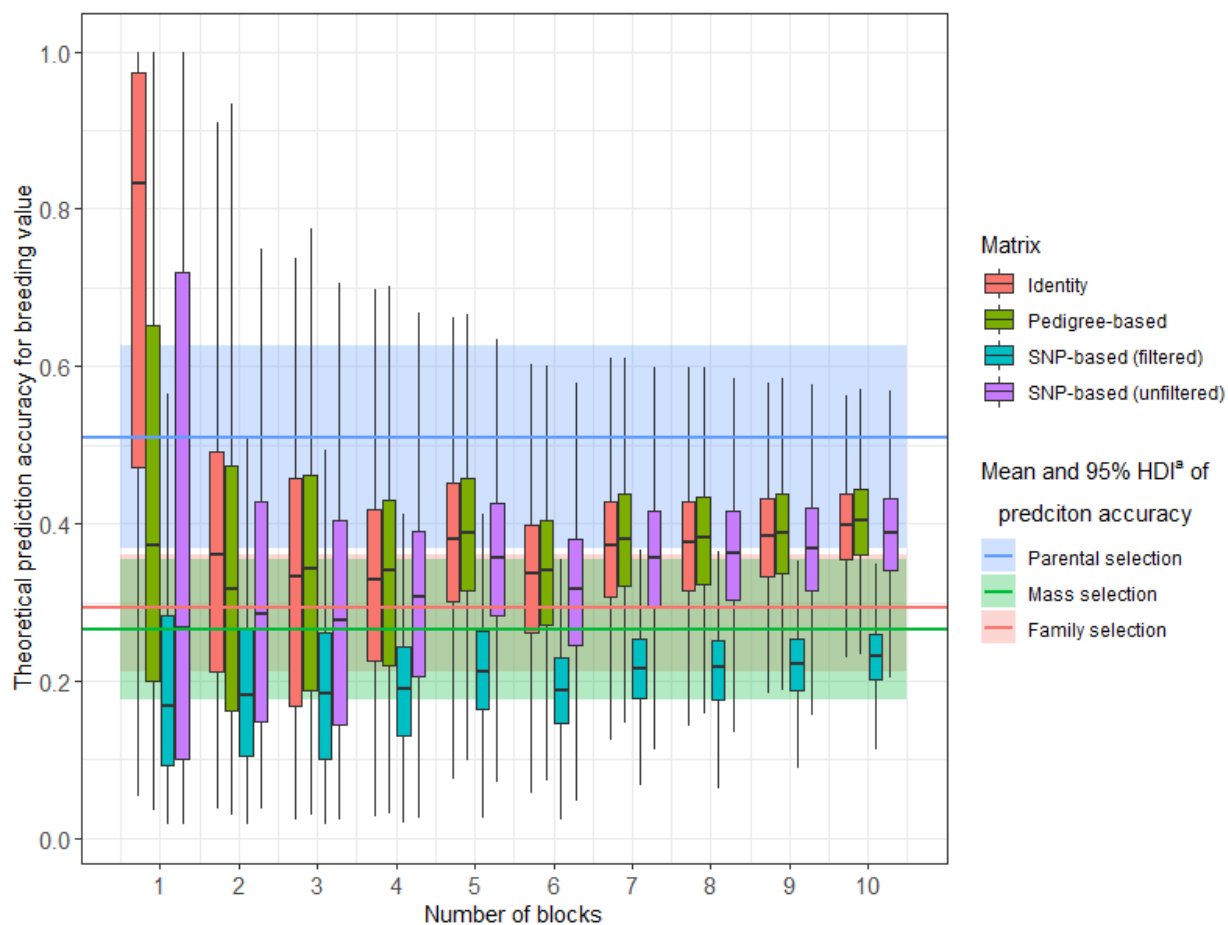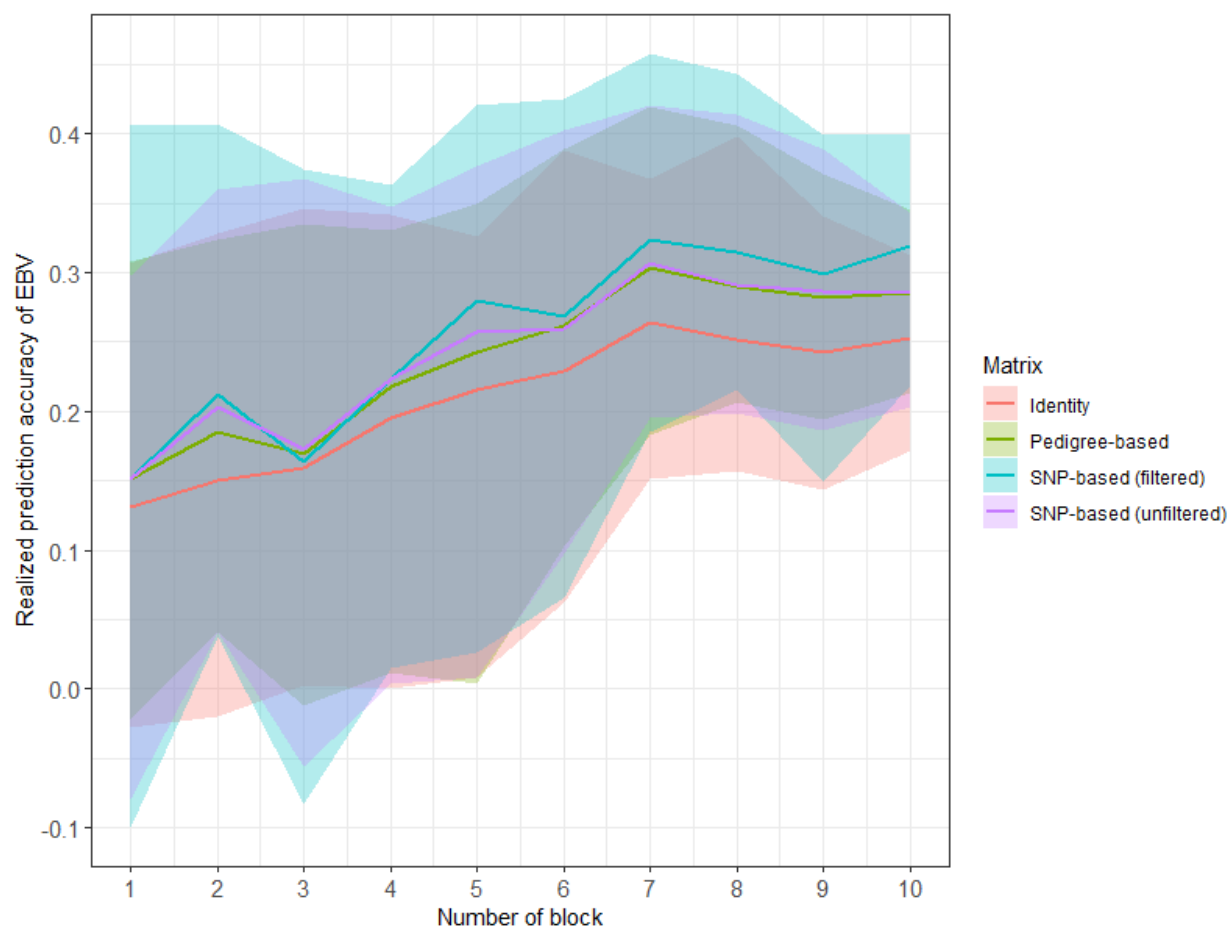4. **EVALUATION AND GENETIC ANALYSIS OF RED THREAD [***Laetisaria fuciformis* (Berk.) Burds.]DISEASE INCIDENCE IN TALL FESCUE (*Festuca arundinacea* Schreb.)**

Abstract

Red thread, caused by [*Laetisaria fuciformis* (Berk.) Burds.], is a common disease on many cool-season and some warm-season grasses throughout the world. Low reproducibility of experiments under both natural and artificial infection and inadequate understanding of the underlying genetics have limited the selection of turfgrass for resistance to red thread. This study investigated red thread disease incidence of tall fescue (*Festuca arundinacea* Schreb.) populations in two locations over multiple years, estimated disease incidence in a broad collection of tall fescue populations, and calculated heritability of disease incidence with two different experimental designs. Collections from Albania and Romania, as well as those that were recently backcrossed to adapted germplasm, were more susceptible to red thread compared with the rest of the populations, highlighting the importance of selection efforts against red thread disease in tall fescue. Narrow-sense heritability (0.52,0.74 and 0.48) estimated from different experimental designs were all in the range of moderate to high, indicating a large proportion of additive genetic variance in red thread incidence among tall fescue populations. Hence, disease incidence of red thread in tall fescue can be effectively reduced through selection and breeding. This is also the first report documenting the efficacy of family selection in reducing red thread incidence in tall fescue.

Keywords: half-sib populations; diallelic cross; variance component; heritability estimation; red thread; disease incidence; logistic regression

4.1. Introduction

Red thread, caused by *Laetisaria fuciformis* (Berk.) Burds., is a common disease on many cool-season and some warm-season grasses throughout the world (Bonos, Wilson, Meyer, & Funk, 2005; Raikes, Lepp, & Canaway, 1996; Zhang et al., 2015). Symptoms of red thread disease on turf stands are circular or irregularly shaped patches ranging from 5 to 60cm, with the presence of red, thread-like sclerotia, hence the name. In addition to the "red thread" outgrowth, small cream or pink-colored mycelial mats and flocks of arthroconidia may be produced. The sclerotia can survive adverse environmental conditions and stay viable for up to two years (Smiley, Dernoeden, & Clarke, 2005). Once conditions permit, the sclerotia germinate and start a new cycle of infection. Sclerotia and arthroconidia can be disseminated by water, mowing equipment, or foot traffic to initiate secondary inoculation (Stalpers & Loerakker, 1982). Outbreaks of red thread disease usually occur during cloudy and rainy conditions in low maintenance turf stands such as residential lawns, parks, golf course rough areas, and low budget athletic fields. Damage may be more severe on infrequently moved areas, or when low temperature or fertility slows the growth of turfgrasses. Applications of nitrogen fertilizer not only encourage faster recovery but also reduce disease incidence (Smiley et al., 2005). Other control methods include higher light intensity or light penetration (if shaded), increased ventilation, clipping removal during major infection, and use of resistant cultivars (Raikes et al., 1996; Tani & Beard, 1997). Traditionally, red thread is most common in perennial ryegrass (*Lolium perenne* L.), especially when under fertilized (Qu et al., 2005). As the popularity of tall fescue (*Festuca arundinacea* Schreb.) rapidly

expanded in the United States from 39,500 acres in 1940 to 37.1 million acres in 2013, red thread epidemics in this species have increased (Rogers & Locke, 2013). Selection of turfgrass species for resistance to red thread has not progressed as much, compared to that against other major diseases of turfgrasses, such as dollar spot caused by *Clarireedia jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch (Bonos, 2006), or gray leaf spot caused by *Pyricularia oryzae* Cavara (Bonos, Kubik, Clarke, & Meyer, 2004; Han, Bonos, Clarke, Meyer, 2006). Additionally, genetic studies have not been conducted for red thread resistance in any turfgrass species; this is primarily due to the low reproducibility of experiments carried out under natural infection and variable success with artificial inoculations (Berestetski, Ehrig, & Kastirr 2002).

One of the crucial tasks in studying disease resistance in selection experiments is to obtain reliable estimates of genetic parameters, such as genetic variances and their functions. Plant breeders have paid particular attention to additive genetic variance and its proportion in total phenotypic variance (i.e., narrow-sense heritability, denoted by h^2), as they determine the rate at which a trait of interest responds to selection. When genomic data are not available, researchers can estimate the genetic parameters from variance components given a specific mating design. The connection between variance components in a given mating design and targeted genetic parameters is the covariance of relatives (Zhu & Weir, 1996). Statistically, the covariance of relatives can be obtained from partitioning observed phenotypic variance; genetically, it can be interpreted in terms of genetic and environmental components. Hence, the estimators of genetic parameters can be derived. The study was initiated by investigating red thread disease incidence in a collection of tall fescue maternal half-sib populations and commercial synthetics during

two disease outbreaks in 2016 and 2017 induced by artificial inoculation. We then proceed to estimate disease incidence in different populations and the narrow-sense heritability of red thread incidence in tall fescue under maternal half-sib design. Based on estimated heritability and disease incidence, family selection was applied to identify resistant and susceptible genotypes. Diallelic crosses were subsequently conducted to evaluate the efficacy of family selection and to confirm the repeatability of estimated genetic parameters.

4.2. Materials and Methods

The first part of the study was conducted at the Rutgers Turfgrass Research Facility at Horticultural Farm #2 in North Brunswick, NJ. A total of 4716 tall fescue clones were arbitrarily selected from 48 experimental maternal half-sib populations (Table 4.1) and 8 commercial synthetic populations (Table 4.2). Selected tall fescue clones were subsequently planted 30.5cm apart in a spaced-plant trial in October of 2013. All populations were replicated at least three times, with the fourth replication being incomplete (i.e., the fourth replication did not include all populations). Once matured, the trial was mowed weekly at 7.62cm during the growing season, maintained with 16-0-8 granular N-P-K fertilizer at a nitrogen rate of $4.9 \text{ g} \cdot \text{m}^{-2}$ every year. In 2013, 24 isolates of *L. fuciformis* were collected from tall fescue plants exhibiting symptoms of red thread; 12 from Horticultural Farm #2 in North Brunswick, NJ and 12 from the Adelphia Farm in Freehold, NJ. Inoculum was prepared by growing isolates of *L. fuciformis* on sterilized Kentucky bluegrass (*Poa pratensis* L.) seed following the method described by Bonos, Casler, & Meyer (2003). Inoculations were repeated five times, each at a rate of 9.7 $\text{g} \cdot \text{m}^{-2}$ infested seed, until disease outbreaks were observed in the spring of 2016 and

2017. In the first three inoculation attempts, a composite of all 24 isolates were used for inoculation but with limited infections (less than 5% plants infected). In 2015, 24 strains of *L. fuciformis* were re-isolated from tall fescue clones at Horticultural Farm #2 that had been previously inoculated, and a fast-growing isolate (RTHF-C3) was selected for inoculum preparation. This led to successful inoculations in the fourth and fifth attempts (greater than 60% plant infected). Between disease outbreaks, all plants were fertilized to encourage complete recovery from the disease. Disease occurrence was recorded on individual clones when the proportion of diseased to non-diseased plants was the highest, using a binary scale, where 0 indicated a healthy clone, and 1 indicated a clone with red thread infection.

To confirm the heritability estimated from the maternal half-sibs design and to further investigate the underlying genetics of red thread disease incidence in tall fescue, genetic parameters were estimated using a diallelic cross design. A resistant genotype was defined as the clone with the least amount of disease in a maternal half-sib population with a low disease incidence, and a susceptible genotype as the one with the most amount of disease in a maternal half-sib population with a high disease incidence. Based on this family selection criterion, three resistant genotypes (R1, R2, and R3) were selected from U41-37, TA3-38, and TA3-15, respectively, and three susceptible genotypes (S1, S2, and S3) were selected from TA1-64, TA1-15, and TA2-27. Controlled diallelic crosses between these genotypes were carried out in the spring of 2017. All genotypes were used as both male and female parents to develop full-sib progenies, with seeds harvested, dried, and threshed separately. Cleaned seeds were treated with 0.2% KNO_3 to induce germination. Once germinated, seedlings were replicated and maintained

in seeding flats before transplanted to mowed space-plant field trials. In the fall of 2017, two replicated field trials were established at Horticultural Farm #2 in North Brunswick, NJ, and Adelphia Farm in Freehold, NJ. Both trials were arranged in a randomized complete block design with four replications. Each replication consisted of 12 progenies from each of the 30 cross combinations as well as six vegetative clones of the six selected parental genotypes used in the diallelic cross. Similarly, established trials were mowed weekly at 7.62cm during the growing season, maintained at an annual nitrogen rate of $4.9\text{g} \cdot \text{m}^{-2}$. Both trials were inoculated at a rate of $9.7 \text{ g} \cdot \text{m}^{-2}$ following the same method using RTHF-C3 on April 20th of 2018. Red thread symptoms appeared three weeks after inoculation, and binary disease occurrence data were collected.

4.3. Statistical Model

4.3.1. Estimation of Disease Incidence

Disease incidence of different populations, including maternal populations, commercial synthetics, and populations of replicated clones, were estimated using the Beta-Binomial model. For n independent Bernoulli data point ($Y = 0$ or 1) in a given population, the number of plants infected, y, follows a binomial distribution with parameter π on $[0,1]$. We have

$$y|\pi \sim \text{Bin}(n, \pi)$$

For comparison of different tall fescue populations, we first assume a beta distribution for π as the prior distribution, i.e.

$$\pi \sim \text{beta}(1,1)$$

Based on Bayes' theorem, the posterior distribution for π is a beta distribution as well, i.e.

$$\pi|y \sim \text{beta}(y + 1, n - y + 1)$$

Posterior mean and standard deviation for π are,

$$\mu_{(\pi|y)} = \frac{y + 1}{n + 2}$$

$$s_{(\pi|y)} = \sqrt{\frac{(y + 1)(n - y + 1)}{(n + 2)^2(n + 3)}}$$

4.3.2. Genetic Analysis

A logistic regression model was fitted to collected data for the estimation of genetic parameters. The following analysis focused on half-sib populations, excluding commercial synthetics and replicated clones. In general, for a binary vector $\mathbf{Y} = \{Y_i\}_{i=1}^N$, let $\boldsymbol{\pi} = \{\pi_i\}_{i=1}^N$ denote the probability of red thread infection. The logit transformation of $\boldsymbol{\pi}$ can be expressed by the following model.

$$\text{logit}\boldsymbol{\pi} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}$$

where N is the total number of observations, $\mathbf{b}$, $\mathbf{u}$, and $\mathbf{e}$ denote the effect of environmental component, genetic component, and unexplained residuals respectively, $\mathbf{X}$ and $\mathbf{Z}$ are the design matrices for the corresponding effects. Distributions for $\mathbf{b}$, $\mathbf{u}$ and $\mathbf{e}$ are assumed to be normal,

$$\mathbf{b} \sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_b^2)$$
$$\mathbf{u} \sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_u^2)$$
$$\mathbf{e} \sim \text{MVN}(\mathbf{0}, \mathbf{I}\sigma_e^2)$$

in which $\mathbf{I}$ is the identity matrix, σ_b^2, σ_u^2, and σ_e^2 are the variance components ascribed to the environmental component, genetic component, and unexplained residuals respectively. We further assume that all variance components follow an inverse-gamma distribution with $\alpha = \beta = 0.001$.

Under the maternal half-sib design, the environmental effects were further partitioned into year effect and replication effect, the genetic effect was mainly the maternal effect. Hence,

$$\text{logit}\boldsymbol{\pi} = \mathbf{X_t b_t} + \mathbf{X_r b_r} + \mathbf{Z_m u_m} + \mathbf{e}$$

where $\mathbf{b_t}$, $\mathbf{b_r}$, $\mathbf{u_m}$ and $\mathbf{e}$ denote year, replication, and maternal effect respectively. $\mathbf{X_t}$, $\mathbf{X_r}$, and $\mathbf{Z_m}$ are the design matrices for the corresponding effects. Assuming epistasis is of minor importance, the variance of maternal effect provides an estimation of a quarter of additive genetic variance in half-sib designs, i.e. $\sigma_m^2 = \frac{1}{4}\sigma_A^2$ (Lynch & Walsh, 1998). Therefore, the additive genetic variance, σ_A^2, for red thread incidence in tall fescue was estimated as $4\sigma_m^2$. And the narrow-sense heritability estimated from maternal half-sibs was

$$h_m^2 = \frac{4\sigma_m^2}{\sigma_t^2 + \sigma_r^2 + \sigma_m^2 + \sigma_e^2}$$

Under the diallelic cross design, the environmental effects considered were location

effect and replication effect, and genetic effects included maternal effect, paternal effect

as well as their interactions. Hence,

$$\text{logit}\boldsymbol{\pi} = \mathbf{X_l b_l} + \mathbf{X_r b_r} + \mathbf{Z_m u_m} + \mathbf{Z_p u_p} + \mathbf{Z_{mp} u_{mp}} + \mathbf{e}$$

where $\mathbf{b_l}$, $\mathbf{b_r}$, $\mathbf{u_m}$, $\mathbf{u_p}$, and $\mathbf{u_{mp}}$ denote location, replication, maternal effect, paternal

effect, and maternal × paternal interaction respectively. $\mathbf{X_l}$, $\mathbf{X_r}$ $\mathbf{Z_m}$, $\mathbf{Z_p}$, and $\mathbf{Z_{mp}}$ are the

corresponding design matrices. Additive genetic variance can be estimated likewise,

either from maternal half-sibs or paternal half-sib, i.e. $\sigma_A^2 = 4\sigma_m^2$ or $\sigma_A^2 = 4\sigma_p^2$, and

dominant genetic variance can be derived from the variance of maternal × paternal

interaction by $\sigma_D^2 = 4\sigma_{mp}^2$ (Zhu and Weir 1996). Hence, heritability estimated from

maternal half-sibs was

$$h_m^2 = \frac{4\sigma_m^2}{\sigma_l^2 + \sigma_r^2 + \sigma_m^2 + \sigma_e^2}$$

Heritability estimated from paternal half-sibs was

$$h_p^2 = \frac{4\sigma_p^2}{\sigma_l^2 + \sigma_r^2 + \sigma_p^2 + \sigma_e^2}$$

4.3.3. Model Implementation

All models were fitted in R (R Core Team, 2019). Posterior distributions of

parameters in the models of genetic analysis were sampled with package "R2jags" (Su &

Yajima, 2015). The Gibbs sampler was implemented with 5 Markov chain Monte Carlo

chains, each with 15000 iterations. The first 5000 iterations were discarded to minimize

the effect of initial values, and the rest of the iterations were thinned by taking every 10th to reduce sample autocorrelation. Convergence of chains was confirmed via visual inspection. Analysis results were visualized using the package "tidyverse" (Wickham et al., 2019).

4.4. Results

4.4.1. Evaluation of red thread incidence

Mean red thread incidence ranged from 38% to 86% in 2016 and from 31% to 100% in 2017(Figure 4.1). Despite some rank differences, data collected from both years showed a positive (Pearson's r = 0.73) and significant (p-value = 1.47e-10) correlation, suggesting a potentially sizeable genetic effect. For commercial synthetics, older cultivars, such as "Kentucky-31", "Faith", and "Rembrandt", had higher disease incidence, while "Regenerate", a newer cultivar, had a lower incidence over both years (see Table 4.2 for the longevity of cultivars in the National Turfgrass Evaluation Program). Such improvement is due to the generic selection effort of turf breeders for overall turf quality and stress tolerance throughout the years. However, substantial differences were seen among cultivars that were released in the same year, indicating differentiated selection efforts against the disease. Comparing with commercial synthetic populations, tested maternal populations exhibited a more extensive spread in mean disease incidence. Specifically, Albanian and Romanian collections (i.e., 55426-13 and 55609-13) and those that were recently backcrossed to adapted germplasm (i.e., MOR-4, MOR-8, and MOR-22) were more susceptible to red thread when compared to the rest of the maternal populations. Top-performing populations included TA3-15, TA3-38, TA2-1,

U41-37, and U41-36, while TA1-43, TA1-64, TA1-15, TA2-27and TA3-21 had red thread incidence rate greater than 70% in both years (Figure 4.1).

Disease incidence in replications of selected genotypes evaluated in 2018 at Horticulture Farm #2 and Adelphia Farm indicated the success of family selection. Significant differences were observed between resistant and susceptible genotypes. R1, R2, and R3 exhibited disease incidence lower than 50%, while S1, S2, and S3 showed disease incidence higher than 70% in both locations (Figure 4.2).

4.4.2. Genetic analysis

In both mating designs, large additive genetic variance was observed, which lead to moderate and large heritability values for red thread disease incidence in tall fescue populations. The model also provided posterior estimations of red thread incidence in the progeny populations by incorporating data collected from 2016 and 2017. Mean disease incidence demonstrated a similar trend as obtained from the Beta-binomial model. Additionally, an increase in variance was observed as disease incidence increased. Similarly, collections from Albania and Romania, as well as recently backcrossed germplasm, had the highest disease incidence (Figure 4.3). The smaller variation observed in these populations presumably resulted from the smaller numbers of progenies evaluated.

Progeny populations from Resistant $\times$ Resistant crosses showed lowest red thread incidence, ranging from 7% to 26%, while those from Susceptible $\times$ Susceptible crosses exhibited the highest red thread incidence, ranging from 70% to 90%. Disease incidence of progeny populations derived from Resistant $\times$ Susceptible and Susceptible$\times$ Resistant

crosses varied from 23% to 87%, showing a broad spread (Figure 4.4). This provided a

clear example of the association in red thread incidence between selected parental

genotypes and their progenies. When comparing reciprocal crosses, the cross matrix was,

for the most part, symmetric with respect to the diagonal line (Figure 4.4). Significant

interactions were detected in $R2 \times S2$ and its reciprocal cross, which happen to have the

highest and the lowest breeding value, as shown in Figure 5. Consequently, a small

quantity of dominance variance was seen, accounting for 6% of the total phenotypic

variance on average. Maternal and paternal effects estimated from progeny populations

(Figure 4.5) provided a measurement for breeding values of selected parental clones,

showing a high correlation with disease incidence observed from parental genotypes

(Figure 4.2).

4.5. Discussion

To evaluate the efficacy and efficiency of selection, genetic studies on traits of

interest are of great importance in plant breeding. Due to the allogamy of most cool-

season turfgrass species and the practice of polycross in cultivar development, maternal

half-sib populations are commonly evaluated in the form of turf plots and maintained as

sources of elite genotypes. Hence, maternal half-sib design is a convenient way to

conduct population genetic studies in cool-season turfgrass. In this study, disease

incidence of red thread was evaluated on a broad collection of tall fescue populations

using a half-sib design. By partitioning the phenotypic variance, a large additive genetic

variance was estimated, leading to moderate heritability. Heritability was estimated to

have a mean of 0.52 and a 95% highest density interval of (0.11, 0.89). Given this

relatively high heritability estimation, the family selection method was adopted to

identify resistant and susceptible genotypes, which subsequently served as parental

genotypes in the diallelic cross. Evaluation of replicated parental genotypes and their

progenies in 2018 confirmed the success of family selection. Heritability estimation from

maternal half-sibs and paternal half-sibs had a mean of 0.74 and 0.48, respectively,

showing consistency with the previous estimation. Other evidence supporting high

heritability of disease incidence in tall fescue include the high correlation of red thread

incidence in evaluated populations in 2016 and 2017, and consistency between estimated

breeding value (Figure 4.5) and observed phenotypic value of selected parental genotypes

(Figure 4.2).

For disease incidence of red thread, there is no published estimate of h^2 for tall

fescue nor any other turfgrass species. Other researchers have reported high heritability

estimations associated with diseases on cool-season turfgrass species. Bonos (2006)

reported narrow-sense heritability estimates of 0.79 ($\pm$0.18) in a two-year study on dollar

spot of creeping bentgrass (*Agrostis stolonifera* L.). Han et al. (2006) estimated narrow-

sense heritability of resistance to gray leaf spot in perennial ryegrass with multiple

growth chamber studies; those values ranged from 0.57 to 0.76. Bokeyer, Bonos, and

Meyer (2009) investigated the inheritance of brown patch resistance in tall fescue using a

diallelic cross design and obtained heritability estimates of 0.62 and 0.57 for two

different years. Compared with these values, our estimate of h^2 for red thread incidence

is in the moderate to high range, indicating that additive genetic effects are prevalent in

red thread disease incidence in tall fescue. However, caution must be exercised for such

comparison, as the reported heritability estimations in this study are specific for the tall

fescue population and the environments studied. The classical definition of narrow-sense

heritability is the proportion of additive genetic variance in total phenotypic variance, which measures the efficiency of response to selection (Lynch & Walsh, 1998). Given the estimated heritability in this study, disease incidence of red thread in tall fescue can be effectively reduced through selection and breeding. Prabhakaran and Jain (1987) evaluated the probabilities of inadmissible estimates of heritability (i.e. $\Pr(h^2 < 0)$ and $\Pr(h^2 > 1)$) in half-sib analysis using linear regression under the frequentist framework, and pointed out that with the true value of h^2 around 0.5, $\Pr(h^2 > 1)$ is invariably higher than $\Pr(h^2 < 0)$ for total numbers of half-sibs being greater and equal to 100. In the genetic model presented earlier, all variance components were assumed to follow Gamma distributions; hence, the probability of negative heritability is 0. But we did observe 0.68% in the half-sib design and 1.24% in the diallelic cross of posterior heritability samples with values greater than 1. This observation could potentially relate to the inbreeding and controlled crossing of studied populations. All adapted germplasm from crossing blocks has at least undergone ten cycles of selection for improved turf quality, leading to an increased amount of inbreeding. Another potential explanation could be provided from the perspective of the ploidy level. The population genetic models adopted in this study were derived from Fisher's infinitesimal model, which assuming the species of study is diploid (Jacquard, 1974; Wright, 1966). However, tall fescue is allohexaploid, whose genome is designated by $\mathbf{PPG_1G_1G_2G_2}$ (Meyer & Watkins, 2003), which could potentially inflate heritability estimation.

Although a large number of tall fescue populations were evaluated in this study, only a single isolate of *L. fuciformis* was used for the fourth and fifth inoculations in the half-sibs design and all inoculations in diallelic design. This may limit how broadly our

results can be applied to other selection processes on tall fescue against red thread disease, since one isolate does not reflect the full range of virulence among *L. fuciformis* populations. In case of quantitative disease resistance (e.g., resistance to red thread), the main objective is to improve populations utilizing additive genetic variance. This process is achieved by repetitively selecting against susceptible genotypes using virulent strains of the pathogen. In other words, strains with less virulence are less of a concern when it comes to selection experiments. While the current study clearly shows that incidence of red thread in tall fescue can be effectively reduced through selection, it would be informative to evaluate other more virulent isolates in the future to determine how tall fescue populations might perform over a range of virulence.

In summary, this study investigated red thread disease incidence in tall fescue populations, highlighting the importance of specific selection effort against red thread disease in tall fescue. We also estimated the heritability of disease incidence with two different experimental designs. Estimated results from two experimental designs are consistent, supporting the idea that additive genetic variance accounts for large phenotypic variance in red thread incidence in tall fescue populations. This is the first report documenting the efficacy of family selection in reducing red thread incidence in tall fescue.

Table 4.1 Information on Experimental Maternal Tall Fescue Populations Evaluated for Red Thread Disease Resistance in 2016 and 2017 at New Brunswick, NJ.

Maternal clone	N^a	Note	Maternal clone	N^a	Note
55426-13	48	Albanian collections	TA2-31	96	
55609-13	48	Romanian collections	TA2-34	96	
MOR-22	48	Collections from Morocco that were recently backcrossed to adapted germplasm	TA2-36	72	
MOR-4	48		TA2-37	96	
MOR-8	48		TA2-40	96	
TA1-3	96		TA2-42	96	Adapted germplasm from crossing blocks created in 2013
TA1-4	96		TA2-5A	96	
TA1-15	96		TA3-12	96	
TA1-21	96		TA3-15	96	
TA1-42	96		TA3-21	96	
TA1-43	96		TA3-38	96	
TA1-50	96		TA3-43	96	
TA1-54	96		U41-11	96	
TA1-57A	96	Adapted germplasm from crossing blocks created in 2013	U41-12	96	
TA1-64	96		U41-14	96	
TA1-68	96		U41-19	96	
TA1-72	96		U41-36	96	
TA2-1	96		U41-37	96	Adapted germplasm from crossing blocks created in 2009
TA2-23	96		U43-14	96	
TA2-25	96		U43-24	84	
TA2-26	96		U44-6	96	
TA2-27	96		W41-34	96	
TA2-28	96		W41-35	96	
TA2-29	96		W45-48	96	

[a] Number of progenies evaluated in each population.

Table 4.2 Information on commercial synthetic tall fescue populations evaluated for red thread disease resistance in 2016 and 2017 at New Brunswick, NJ.

Commercials [a]	Number of clones evaluated	Sponsor[b]	Year[c]
Kentucky-31	48	University of Kentucky	1983
Rembrandt	48	Lebanon Turf Products, Inc.	1996
Falcon IV	48	ProSeeds Marketing, Inc.	2001
Van Gogh	48	Lebanon Seaboard Corp.	2006
Mustang 4	48	Pickseed	2006
Faith	48	The Scotts Company	2006
Falcon V	48	ProSeeds Marketing	2006
Regenerate	48	Landmark Turf& Native Seed	2012

[a] all information in the table was collected from National Turfgrass Evaluation Program (NTEP; www.ntep.org)

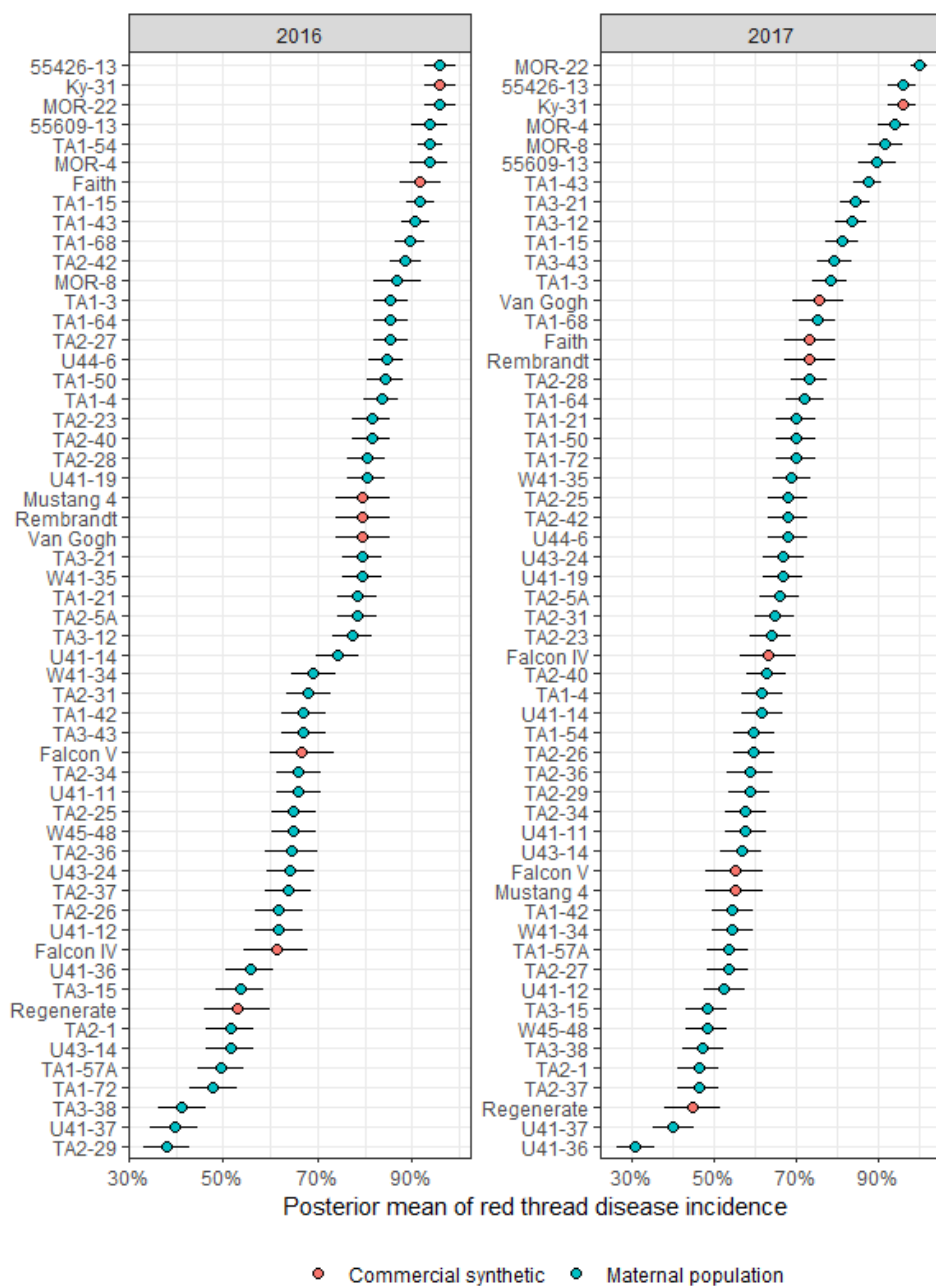[b] Sponsor of the commercial cultivar when first appeared in the NTEP.

[c] Year of first appearance in the NTEP.

Table 4.3 Variance components and heritability values estimated from tall fescue populations evaluated for red thread disease resistance in 2016, 2017 and 2018 at New Brunswick, NJ and Freehold, NJ under maternal half-sib design and diallelic cross.

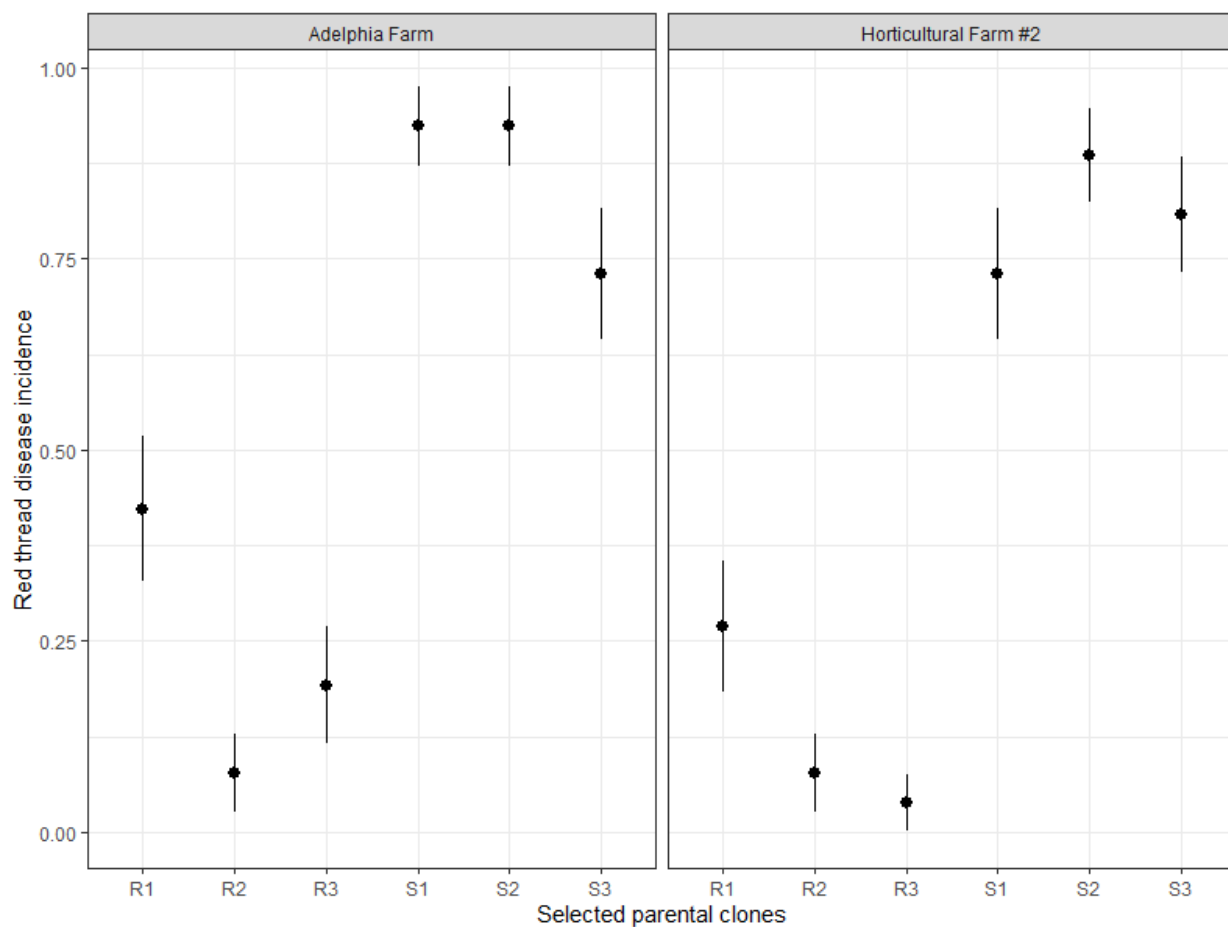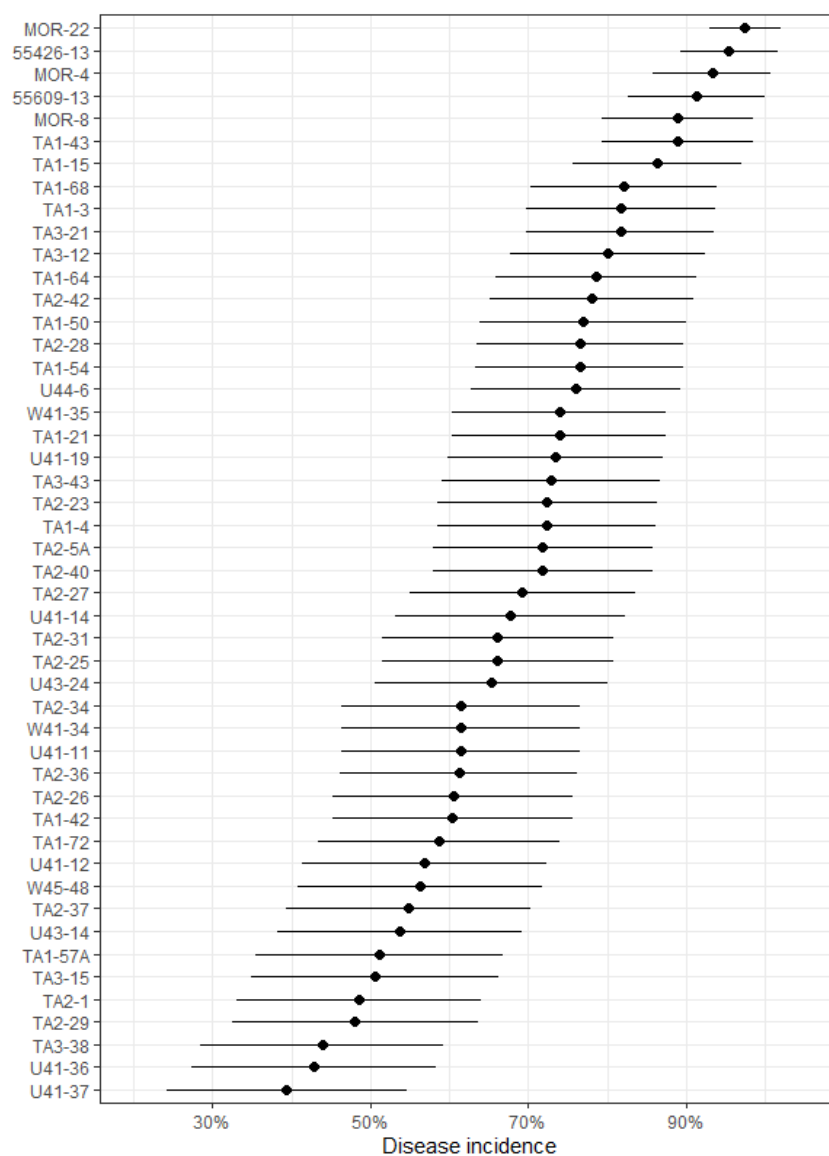| Mating design	variance component	mean	median	mode	95%HDI[a]
Half-sib design	year effect (σ_t^2)	523.91	29.18	9.23	(0.03,957.70)
	replication effect (σ_r^2)	113.23	38.51	11.84	(0.29,408.27)
	maternal effect (σ_m^2)	67.14	50.56	19.46	(1.69,184.59)
	residual (σ_e^2)	274.72	219.85	79.53	(6.94,717.14)
	additive genetic variance	268.54	202.26	77.85	(6.77,738.34)
	total phenotypic variance	979.00	420.84	442.14	(12.03,2189.21)
	Heritability estimated from maternal half-sibs (h_m^2)	0.52	0.53	0.55	(0.11, 0.89)
Diallelic cross	location effect (σ_l^2)	51.91	17.11	10.22	(0.23,168.98)
	replication effect (σ_r^2)	82.71	4.69	4.41	(0.01,133.74)
	residual (σ_e^2)	120.52	89.67	33.41	(5.04,360.09)
	maternal effect (σ_m^2)	50.75	30.25	14.56	(1.72,160.96)
	paternal effect (σ_p^2)	32.03	19.19	8.92	(0.64,99.19)
	maternal $\times$ paternal interaction (σ_{mp}^2)	4.25	2.73	1.11	(0.07,13.27)
	additive genetic variance estimated from maternal half-sibs	203.01	121.01	58.23	(6.89,643.83)
	additive genetic variance estimated from paternal half-sibs	128.11	76.78	35.69	(2.54,396.72)
	dominance genetic variance	17.00	10.92	4.42	(0.29,53.06)
	total phenotypic variance	342.17	207.00	150.16	(14.43,878.38)
	Heritability estimated from maternal half-sibs (h_m^2)	0.74	0.63	0.46	(0.16, 1.18)
	Heritability estimated from paternal half-sibs (h_p^2)	0.48	0.40	0.27	(0.11, 1.13)

Note:

[a] Highest density interval.

Note: error bars represent standard deviation of population red thread incidence

Figure 4.1 Red thread incidence of 48 maternal populations and 8 synthetic cultivars of tall fescue evaluated in 2016 and 2017 at New Brunswick, NJ after artificial inoculation with *Laetiseria fuciformis.*

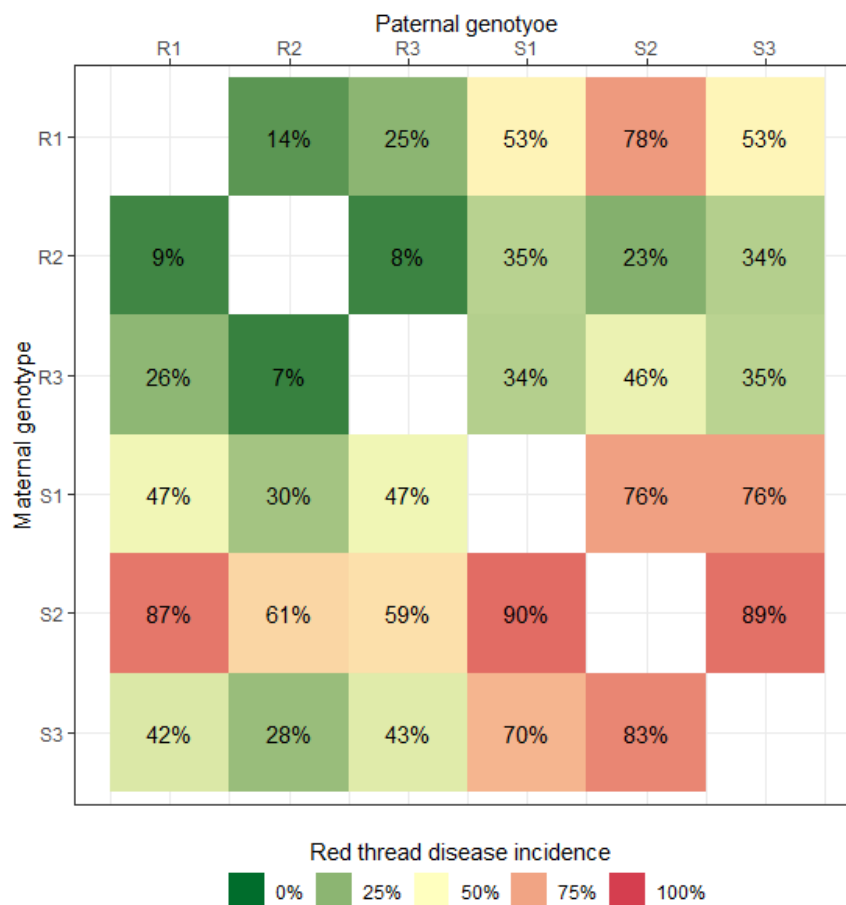Note: error bars represent standard deviation of red thread incidence

Figure 4.2 Red thread disease incidence among selected parental clones evaluated in 2018 in two field trials at New Brunswick, NJ and Freehold, NJ, after artificial inoculation with *Laetiseria fuciformis*.

Note:

Green color indicates reduced red thread incidence in progeny populations while red color indicates elevated red thread incidence in progeny populations.

Figure 4.3 Estimated red thread incidence in 48 maternal populations evaluated in 2016 and 2017 at Horticultural Farm #2 in North Brunswick, NJ after artificial inoculation with Laetiseria fuciforms.
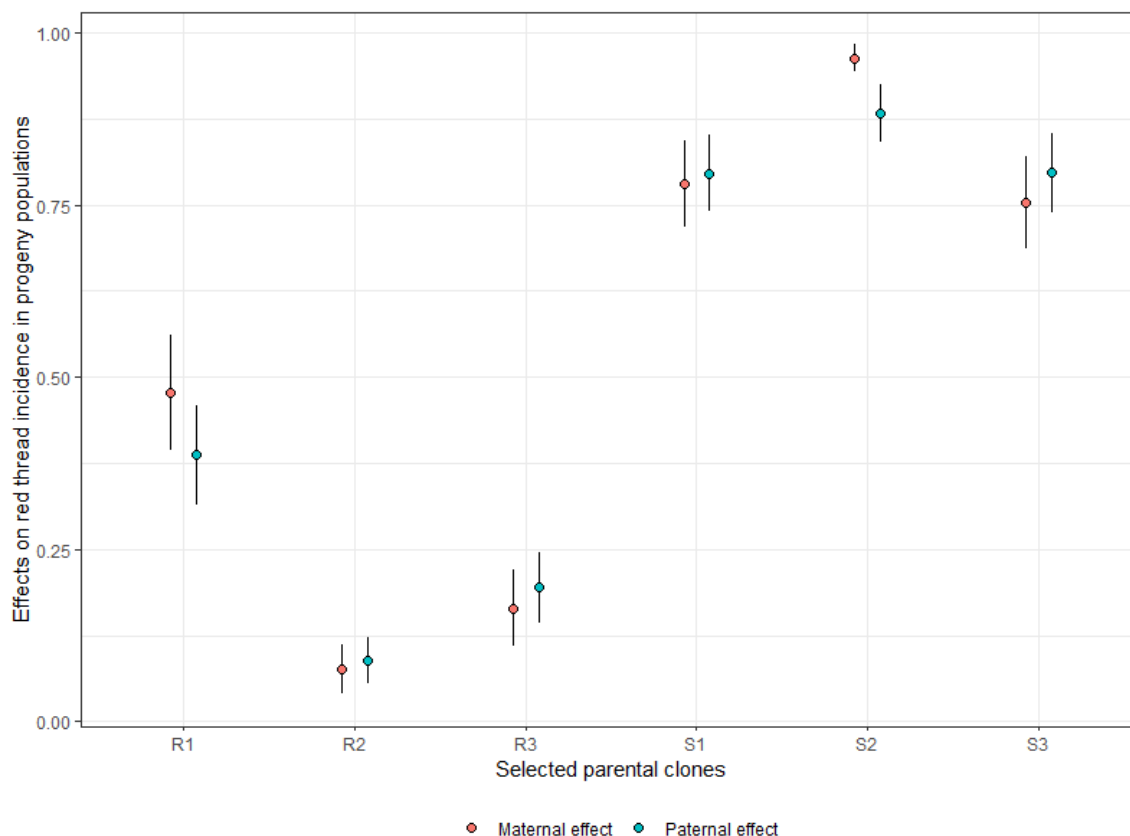
Note:

Colored blocks represent cross events with the color indicating red thread disease incidence in the progeny population of that specific cross.

R1, R2, R3 were the resistant genotypes, selected from U41-37, TA3-38, and TA3-15 respectively; S1, S2, S3 were the susceptible genotypes, selected from TA1-64, TA1-15, and TA2-27, respectively.

Figure 4.4 Disease incidence of progeny populations created from diallelic cross evaluated in 2018 in two field trials at New Brunswick, NJ and Freehold, NJ after artificial inoculation with *Laetiseria fuciformis.*

Note:

Error bars represent standard deviation of red thread incidence.

R1, R2, R3 were the resistant genotypes, selected from U41-37, TA3-38, and TA3-15 respectively; S1, S2, S3 were the susceptible genotypes, selected from TA1-64, TA1-15, and TA2-27, respectively.

Figure 4.5 Maternal and paternal effects of selected parental clones on red thread disease incidence estimated from diallelic cross progenies evaluated in 2018 in two field trials at New Brunswick, NJ and Freehold, NJ after artificial inoculation with Laetiseria fuciformis.

REFERENCES

Aastveit, A. H., & Aastveit, K. (1990). Theory and application of open-pollination and polycross in forage grass breeding. *Theoretical and applied genetics*, *79*(5), 618-624. https://doi.org/10.1007/BF00226874

Avendaño, S., Woolliams, J. A., & Villanueva, B. (2005). Prediction of accuracy of estimated Mendelian sampling terms. *Journal of Animal Breeding and Genetics*, *122*(5), 302-308. https://doi.org/10.1111/j.1439-0388.2005.00532.x

Barton, N. H., Etheridge, A. M., & Véber, A. (2017). The infinitesimal model: Definition, derivation, and implications. *Theoretical population biology*, *118*, 50-73. https://doi.org/10.1016/j.tpb.2017.06.001

Berestetski, A., Ehrig, F., & Kastirr, U. (2002). Preliminary evaluation of turfgrass (perennial ryegrass and red fescue) cultivars for resistance to red thread disease using artificial infection. *Plant breeding*, *121*(6), 493-500. https://doi.org/10.1046/j.1439-0523.2002.00763.x

Bokmeyer, J. M., Bonos, S. A., & Meyer, W. A. (2009). Broad-sense Heritability and Stability Analysis of Brown Patch Resistance in Tall Fescue. *HortScience*, *44*(2), 289-292. https://doi.org/10.21273/HORTSCI.44.2.289

Bolaños, J., & Edmeades, G. O. (1996). The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. *Field Crops Research*, *48*(1), 65-80. https://doi.org/10.1016/0378-4290(96)00036-6

Bonos, S. A. (2006). Heritability of dollar spot resistance in creeping bentgrass. *Phytopathology*, *96*(8), 808-812. https://doi.org/10.1094/PHYTO-96-0808

Bonos, S. A., Casler, M. D., & Meyer, W. A. (2003). Inheritance of dollar spot resistance in creeping bentgrass. *Crop Sci.*, *43*(6), 2189-2196. https://doi.org/10.2135/cropsci2003.2189

Bonos, S. A., Clarke, B. B., & Meyer, W. A. (2006). Breeding for disease resistance in the major cool-season turfgrasses. *Annual Review of Phytopathology*, *44*, 213-234. https://doi.org/10.1146/annurev.phyto.44.070505.143338

Bonos, S. A., Kubik, C., Clarke, B. B., & Meyer, W. A. (2004). Breeding perennial ryegrass for resistance to gray leaf spot. *Crop Sci.*, *44*(2), 575-580. https://doi:10.2135/cropsci2004.5750

Bonos, S. A., Wilson, M. M., Meyer, W. A., & Funk, R. C. (2005). Suppression of red thread in fine fescues through endophyte-mediated resistance. *Appl. Turfgrass Sci.*, *2*(1), 1-7. https:// doi:10.1094/ATS-2005-0725-01-RS

Carrow, R. N., & Duncan, R. R. (2003). Improving drought resistance and persistence in turf-type tall fescue. *Crop Science*, *43*(3), 978-984. https://doi.org/10.2135/cropsci2003.9780

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes, Genetics*, *1*(3), 171-182. https://doi.org/10.1534/g3.111.000240

Cattivelli, L., Rizza, F., Badeck, F. W., Mazzucotelli, E., Mastrangelo, A. M., Francia, E., ... & Stanca, A. M. (2008). Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *Field Crops Research*, *105*(1-2), 1-14. https://doi.org/10.1016/j.fcr.2007.07.004

Chen, Z., Wang, M. L., Waltz, C., & Raymer, P. (2009). Genetic diversity of warm-season turfgrass: seashore paspalum, bermudagrass, and zoysiagrass revealed by AFLPs. *Floriculture and Ornamental Biotechnology*, *3*(1), 20-24.

Condorelli, G. E., Maccaferri, M., Newcomb, M., Andrade-Sanchez, P., White, J. W., French, A. N., ... & Tuberosa, R. (2018). Comparative aerial and ground based high throughput phenotyping for the genetic dissection of NDVI as a proxy for drought adaptive traits in durum wheat. *Frontiers in Plant Science*, *9*, 893. https://doi.org/10.3389/fpls.2018.00893

Cross, J. W., Bonos, S. A., Huang, B., & Meyer, W. A. (2013). Evaluation of heat and drought as components of summer stress on tall fescue genotypes. *HortScience*, *48*(12), 1562-1567. https://doi.org/10.21273/HORTSCI.48.12.1562

Dodds, K. G., McEwan, J. C., Brauning, R., Anderson, R. M., van Stijn, T. C., Kristjánsson, T., & Clarke, S. M. (2015). Construction of relatedness matrices using genotyping-by-sequencing data. *BMC genomics*, *16*(1), 1047. https://doi.org/10.1186/s12864-015-2252-3

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). London, UK: Prentice Hall.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. In E. Lichtfouse, M. Navarrete, P. Debaeke, S. Véronique, C. Alberola (Eds.), *Sustainable agriculture* (pp. 153-188). Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2666-8_12

Fay, P. A., Carlisle, J. D., Knapp, A. K., Blair, J. M., & Collins, S. L. (2000). Altering rainfall timing and quantity in a mesic grassland ecosystem: design and performance of rainfall manipulation shelters. *Ecosystems*, *3*(3), 308-319. https://doi.org/10.1007/s100210000028

Fisher, R. (1919). XV.—The Correlation between Relatives on the Supposition of Mendelian Inheritance. *Transactions of the Royal Society of Edinburgh*, *52*(2), 399-433. https://doi.org/10.1017/S0080456800012163

Fu, J., Fry, J., & Huang, B. (2007). Tall fescue rooting as affected by deficit irrigation. *HortScience*, *42*(3), 688-691. https://doi.org/10.21273/HORTSCI.42.3.688

Fu, J., & Huang, B. (2004). Leaf characteristics associated with drought resistance in tall fescue cultivars. *Acta Horticulturae*, *661*, 233-239. https://doi.org/10.17660/ActaHortic.2004.661.29

Gianola, D., Cecchinato, A., Naya, H., & Schön, C. C. (2018). Prediction of complex traits: robust alternatives to best linear unbiased prediction. *Frontiers in Genetics*, *9*, 195. https://doi.org/10.3389/fgene.2018.00195

Han, Y., Bonos, S. A., Clarke, B. B., & Meyer, W. A. (2006). Inheritance of resistance to gray leaf spot disease in perennial ryegrass. *Crop Sci.*, *46*(3), 1143-1148. https://doi.org/10.2135/cropsci2005.07-0217

Hatier, J. H. B., Faville, M. J., Hickey, M. J., Koolaard, J. P., Schmidt, J., Carey, B. L., & Jones, C. S. (2014). Plant vigour at establishment and following defoliation are both associated with responses to drought in perennial ryegrass (*Lolium perenne* L.). *Journal of Experimental Botany*, *65*(20), 5823-5834. https://doi.org/10.1093/jxb/eru318

Henderson, C. R. (1950). Estimation of genetic parameters. Annals of Mathematical Statistics, *21*, 309–310.

Henderson, C. R. (1975). Use of relationships among sires to increase accuracy of sire evaluation. *Journal of Dairy Science*, *58*(11), 1731-1738. https://doi.org/10.3168/jds.S0022-0302(75)84777-1

Huang, B., & Gao, H. (1999). Physiological responses of diverse tall fescue cultivars to drought stress. *HortScience*, *34*(5), 897-901. https://doi.org/10.21273/HORTSCI.34.5.897

Jacob, D., Petersen, J., Eggert, B., Alias, A., Christensen, O. B., Bouwer, L. M., ... & Georgopoulou, E. (2014). EURO-CORDEX: new high-resolution climate change projections for European impact research. *Regional Environmental Change*, *14*(2), 563-578. https://doi.org/10.1007/s10113-013-0499-2

Jacquard, A. (1974). Genetic relationships between relatives. In *The Genetic Structure of Populations* (pp. 102-140). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-88415-3_7

Jiang, Y., & Huang, B. (2001). Physiological responses to heat stress alone or in combination with drought: A comparison between tall fescue and perennial ryegrass. *HortScience*, *36*(4), 682-686. https://doi.org/10.21273/HORTSCI.36.4.682

Jiang, Y., Wang, X., Yu, X., Zhao, X., Luo, N., Pei, Z., ... & Garvin, D. F. (2017). Quantitative trait loci associated with drought tolerance in *Brachypodium distachyon*. *Frontiers in Plant Science*, *8*, 811. https://doi.org/10.3389/fpls.2017.00811

Karcher, D. E., Richardson, M. D., Hignight, K., & Rush, D. (2008). Drought tolerance of tall fescue populations selected for high root/shoot ratios and summer survival. *Crop Science*, *48*(2), 771-777. https://doi.org/10.2135/cropsci2007.05.0272

Karnieli, A., Agam, N., Pinker, R. T., Anderson, M., Imhoff, M. L., Gutman, G. G., ... & Goldberg, A. (2010). Use of NDVI and land surface temperature for drought assessment: Merits and limitations. *Journal of Climate*, 23(3), 618-633. https://doi.org/10.1175/2009JCLI2900.1

Khosravi, H., Haydari, E., Shekoohizadegan, S., & Zareie, S. (2017). Assessment the effect of drought on vegetation in desert area using landsat data. *The Egyptian Journal of Remote Sensing and Space Science*, *20*, S3-S12. https://doi.org/10.1016/j.ejrs.2016.11.007

Lootens, P., Maes, W. H., De Swaef, T., Aper, J., Mertens, K. C., Steppe, K., ... & Roldán-Ruiz, I. (2016). UAV-based remote sensing for evaluation of drought tolerance in forage grasses. In *Breeding in a world of scarcity* (pp. 111-116). New York, NY: Springer. https://doi.org/10.1007/978-3-319-28932-8_16

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits* (Vol. 1, pp. 535-557). Sinauer, Sunderland, MA.

Mathew, I., Shimelis, H., Mwadzingeni, L., Zengeni, R., Mutema, M., & Chaplot, V. (2018). Variance components and heritability of traits related to root: shoot biomass allocation and drought tolerance in wheat. *Euphytica*, *214*(12), 225. https://doi.org/10.1007/s10681-018-2302-4

Merewitz, E., Belanger, F., Warnke, S., Huang, B., & Bonos, S. (2014). Quantitative trait loci associated with drought tolerance in creeping bentgrass. *Crop Science*, *54*(5), 2314-2324. https://doi.org/10.2135/cropsci2013.12.0810

Meyer, W. A., & Watkins, E. (2003). Tall fescue (*Festuca arundinacea*). In M. D. Casler & R. R. Duncan (Eds), *Turfgrass biology genetics and breeding* (pp. 107-127). John Wiley & Sons, Hoboken, NJ.

Min, S. K., Zhang, X., Zwiers, F. W., & Hegerl, G. C. (2011). Human contribution to more-intense precipitation extremes. *Nature*, *470*(7334), 378. https://doi.org/10.1038/nature09763

Nguyen, H. T., & Sleper, D. A. (1983). Theory and application of half-sib matings in forage grass breeding. *Theoretical and Applied Genetics*, *64*(3), 187-196. https://doi.org/10.1007/BF00303763

Nyquist, W. E., & Baker, R. J. (1991). Estimation of heritability and prediction of selection response in plant populations. *Critical Reviews in Plant Sciences*, *10*(3), 235-322. https://doi.org/10.1080/07352689109382313

Pedersen, J. F., & Sleper, D. A. (1993). Genetic manipulation of tall fescue. *Agriculture, Ecosystems & Environment*, *44*(1-4), 187-193. https://doi.org/10.1016/0167-8809(93)90046-R

Pirnajmedin, F., Majidi, M. M., & Gheysari, M. (2016). Survival and recovery of tall fescue genotypes: association with root characteristics and drought tolerance. *Grass and Forage Science*, *71*(4), 632-640. https://doi.org/10.1111/gfs.12231

Poland, J. A., & Rife, T. W. (2012). Genotyping-by-sequencing for plant breeding and genetics. *The Plant Genome*, *5*(3), 92-102. https://doi.org/10.3835/plantgenome2012.05.0005

Prabhakaran, V. T., & Jain, J. P. (1987). Probability of inadmissible estimates of heritability from regression and half-sib analyses. *Biom. J.*, *29*(2), 219-230. https://doi.org/10.1002/bimj.4710290219

Qu, Y., Mohr, M. M., Bara, R. F., Smith, D. A., Szerszen, E., Bonos, S. A. & Meyer, W. A. (2015). Performance of perennial ryegrass cultivars and selections in New Jersey turf trials. In A. B. Gould (Ed.), 2015 Turfgrass (pp. 125-147). Atlantic City, New Jersey: Rutgers University.

Raffa, J. D., & Thompson, E. A. (2016). Power and effective study size in heritability studies. *Statistics in Biosciences*, *8*(2), 264-283. https://doi.org/10.1007/s12561-016-9143-2

R Core Team (2019). R: A language and environment for statistical computing [Computer software manual]. Vienna, Austria. Retrieved from http://www.R-project.org

Rosielle, A. A., & Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environment. *Crop Science*, *21*(6), 943-946. https://10.2135/cropsci1981.0011183X002100060033x

Raikes, C., Lepp, N. W., & Canaway, P. M. (1996). The effect of red thread (*Laetisaria fuciformis*) incidence on wear tolerance of monostands of perennial ryegrass (*Lolium perenne*) cultivars under three nitrogen levels. *J. of the Sports Turf Research Institute*, *72*, 61-66.

Schwartz, B. M., Hanna, W. W., Baxter, L. L., Raymer, P. L., Waltz, F. C., Kowalewski, A. R., ... & Milla-Lewis, S. R. (2018). 'DT-1', a Drought-tolerant Triploid Turf Bermudagrass. *HortScience*, *53*(11), 1711-1714. https://doi.org/10.21273/HORTSCI13083-18

Smiley, R. W., Dernoeden, P. H., & Clarke, B. B. (2005). Compendium of Turfgrass Diseases. APS press, St. Paul, MN

Smith, A. B., Cullis, B. R., & Thompson, R. (2005). The analysis of crop cultivar breeding and evaluation trials: an overview of current mixed model approaches. *The Journal of Agricultural Science*, *143*(6), 449-462. https://doi.org/10.1017/S0021859605005587

Stalpers, J. A., & Loerakker, W. M. (1982). Laetisaria and Limonomyces species (Corticiaceae) causing pink diseases in turf grasses. *Canadian J. Bot.*, *60*(5), 529-537. https://doi.org/10.1139/b82-071

Steinke, K., Chalmers, D., Thomas, J., White, R., & Fipps, G. (2010). Drought response and recovery characteristics of St. Augustinegrass cultivars. *Crop Science*, *50*(5), 2076-2083. https://doi.org/10.2135/cropsci2009.10.0635

Su, Y. S., & Yajima, M. (2015). R2jags: Using R to run 'Jags'. *R package version 0.5-7*.

Talukder, S. K., & Saha, M. C. (2017). Toward genomics-based breeding in C3 cool-season perennial grasses. *Frontiers in Plant Science*, *8*, 1317. https://doi.org/10.3389/fpls.2017.01317

Tani, T., & Beard, J. B. (1997). Color Atlas of Turfgrass Diseases. John Wiley & Sons, Inc.

Tuberosa, R. (2012). Phenotyping for drought tolerance of crops in the genomics era. *Frontiers in Physiology*, *3*, 347. https://doi.org/10.3389/fphys.2012.00347

Tucker, C. J. (1979). Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sensing of Environment*, *8*(2), 127-150. https://doi.org/10.1016/0034-4257(79)90013-0

Walsh, B., & Lynch, M. (2018). *Evolution and selection of quantitative traits*. Oxford University Press.

Wang, J. P., Bughrara, S. S., & Nelson, C. J. (2008). Morpho-physiological responses of several fescue grasses to drought stress. *HortScience*, *43*(3), 776-783. https://doi.org/10.21273/HORTSCI.43.3.776

Wellmann, R. (2017). optiSel: optimum contribution selection and population genetics. *R Package. version 0.9*, *1*.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., ... & Yutani H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wright, J. A. (1966). Estimation of components of genetic variance in an open-pollinated variety of maize using single and three-way crosses among random inbred lines. https://doi.org/10.31274/rtd-180813-494

Zapata-Valenzuela, J., Whetten, R. W., Neale, D., McKeand, S., & Isik, F. (2013). Genomic estimated breeding values using genomic relationship matrices in a cloned population of loblolly pine. *G3: Genes, Genomes, Genetics*, *3*(5), 909-916. https://doi.org/10.1534/g3.113.005975

Zhang, W., Hu, M., Liu, G., Gao, Z., Li, M., & Nan, Z. (2015). Investigation and characterization of red thread and pink patch on warm-season turfgrasses in Hainan Province, tropical China. *European J. Plant Pathol.*, *141*(2), 311-325. https://doi.org/10.1007/s10658-014-0543-1

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326-3328. https://doi.org/10.1093/bioinformatics/bts606

Zhou, Y., Lambrides, C., Kearns, R., Ye, C., Cao, N., & Fukai, S. (2009). Selecting for drought tolerance among Australian green couch grasses (*Cynodon* spp.). *Crop and Pasture Science*, *60*(12), 1175-1183. https://doi.org/10.1071/CP09172

Zhu, J., & Weir, B. S. (1996). Mixed model approaches for diallel analysis based on a bio-model. *Genetics Research*, *68*(3), 233-240. https://doi.org/10.1017/S0016672300034200

Zuk, O., Hechter, E., Sunyaev, S. R., & Lander, E. S. (2012). The mystery of missing heritability: Genetic interactions create phantom heritability. *Proceedings of the National Academy of Sciences*, *109*(4), 1193-1198. https://doi.org/10.1073/pnas.1119675109