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Research Article

A Preliminary Evaluation on the Performance of Tall fescue F1 Hybrids

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ABSTRACT

Within the Lolium-Festuca genome complex there is a need for modern breeding approaches to facilitate the rapid development of improved cultivars. Traditional recurrent or mass-selection methods for population or synthetic development are labor-intensive and time-consuming. The recent development of an approach to produce dihaploid or homozygous lines of tall fescue offers an opportunity to achieve such new and improved cultivars. Dihaploid inducer lines of annual ryegrass (Lolium perenne L. subsp). multiflorum (Lam.) Husnot (syn. Lolium multiflorum Lam.) which exhibit genome loss when hybridized with tall fescue (Festuca arundinacea Schreb. syn. Lolium arundinaceum (Schreb.) Darbysh.) were previously hybridized to an array of tall fescue germplasm which resulted in the generation of numerous dihaploid tall fescue lines. Four of these lines were utilized to perform a preliminary evaluation on the performance of F1 hybrid tall fescue. Results of the performance trials suggests F1 hybrid tall fescue can exhibit heterosis, hybrid vigor and provide forage yields that are superior or competitive to check cultivars. A comparison of F1 forage production to the parental DH was also included in the study to determine the potential for a hybrid vigor and a heterotic response. Preliminary molecular analysis of the DH lines and their pairing for hybrid production also suggests that genetic distance measures may be useful in identifying those DH parental lines which maximize hybrid vigor and display a heterotic response. Though these are preliminary data, utilizing only experimental materials, the results suggest that the gains in production when developing F1 hybrids can be applied to tall fescue improvement programs.

Keywords: Dihaploid; Hybrid; Tall fescue

INTRODUCTION

A double haploid or dihaploid (DH) is a genotype formed when haploid cells undergo chromosome doubling. The generation of DH has great value in many aspects of plant breeding and plant genetics by enabling a shortened time to produce homozygous lines when compared with traditional breeding approaches [1]. Forster B, 2005 have reported that methods and procedures for generating doubled haploid lines through breeding are available for over 250 crop species [2]. According to the authors, over 300 cultivars, across 12 species, have been derived from double haploid methods. Generally, homozygous or DH lines are generated through a few well established methods which are traditional selfing [3], microspore or ovule culture [4,5] and various genetic related DH generation methods [6]. The production of homozygous lines in tall fescue is not new. Earlier efforts generated near homozygous lines by traditional selfing and such recovered lines were used for palatability studies [7]. In addition, methods focused on the production of F1 hybrid ryegrass, a close relative to tall fescue, have been suggested [8]. Recently, tall fescue research has identified a low frequency DH generation approach which can produce DH tall fescue lines in as little as one generation by utilizing a set of ryegrass IL lines [9,10]. From this approach, several DH tall fescue lines have been generated and have been used to evaluate the potential performance of single-cross, F1 hybrid tall fescue.

MATERIALS AND METHODS

In 2003, DH inducer lines IL1 and IL2 [9, 11] were used as maternal parents and hybridized in bulk to an array of tall fescue cultivars and populations. In 2004, the IL × TF hybrid seed were sown in trays and DH were recovered by an approach described elsewhere [12]. Several of these first generation DH lines, including DH16, DH22, DH23 and DH36 which were used in this study to generate two F1 hybrids by intercrossing DH23 × DH16=DHTF1 and DH22 × DH36=DHTF2. These and other DH lines were submitted to a 14 EST-SSR molecular marker evaluation by DNA LandMarks (84 Rue Richelieu, Saint-Jean-sur-Richelieu, QC J3B

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6 × 3, Canada). Genetic distance (GD) scores were determined across the available DH lines using the GenAlEx6 Genetic Analysis in Excel software package [13]. The GD score between DH23 and DH16 was 14.64 and the GD value between DH22 and DH36 was 9.732. From 2006-2008, additional phenotypic data were gathered in the greenhouse on flowering date, plant color and over all plant vigor. Compatible flowering dates for successful cross-pollination was a major component for the selection of the DH parental lines since a lack of flowering compatibility would make the generation of hybrids unsuccessful. Selfing for each DH line was performed in the greenhouse to produce small quantities of DH seed stock material for an un-replicated DH performance field study.

The F1 hybrids were generated by hand-pollination in both directions to simulate the open-pollinated, cross pollination tendency of the species. Following pollination, each inflorescence was bagged to retain the seed. Inflorescences were allowed to mature and seed were harvested and cleaned in small lots and packaged for eventual sowing to the field nursery. The DHTF1 and DHTF2 hybrid seed and their respective DH parents were sown to a three replicate, field nursery on September 24, 2008 at the USDA-ARS, Agronomy Farm, Grazinglands Research Laboratory, El Reno, OK. Check cultivars were also sown in the three replicate trials while the selfed seed of DH16, DH22, DH23 and DH36 were sown in an adjacent, non-replicated, nursery plot. Check varieties for the trial consisted of cv Dover, cv Nanyro and cv Demeter tall fescue cultivars. Drover was provided by Barenbrug Seeds, Albany, OR, USA. The Nanyro cultivar was provided by the Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan and Demeter was provided by the Tasmanian Institute of Agriculture, Hobart, AU. The check cultivars of Drover, Nanyro and Demeter each had been previously identified as good tall fescue varieties with adaptation and persistence in the central Oklahoma environment. All checks and experimental F1 represent the Continental type tall fescue. The soil type at the trial location is described as a Brewer silty clay soil type (fine, mixed, superactive, thermic Undertic Arguiustoll). The Fishers multiple comparison test statistic with ranking was applied to forage yield data (Tables 1-3).

Table 1: Forage yield of the DHTF1 and DHTF2 hybrids compared to check varieties. Forage harvest clippings were obtained on May 25, 2009. Fishers multiple comparison test statistics with rankings are provided only for yield.

Name	%Moisture	Yield Kg/ha
DHTF1	51.15	3845.64 [a]
Drover	49.88	3824.34 [a]
Nanyro	55.10	3778.39 [a]
DHTF2	48.48	3230.29 [b]
Demeter	58.07	2056.76 [c]
LSD[1]=563.10 (p=0.05)		
SED[2]=249.96		
[1]LSD represents the Least Significant Difference statistic		
[2]SED represents the Standard Error of Difference statistic		

Table 2: Forage yield of the DHTF1 and DHTF2 hybrids and the check varieties. Percent moisture and forage dry weight clippings were obtained on May 3, 2010. Fishers multiple comparison test statistics with rankings are provided only for yield.

Name	%Moisture	Yield Kg/ha
Nanyro	54.19	5185.24 a
DHTF2	48.88	4773.29 ab
DHTF1	50.87	5236.91 ab
Drover	49.02	4673.11 b
Demeter	56.98	1866.13 c
LSD[1]=536.10 (p=0.05)		
SED[2]=249.96		
[1]LSD represents the Least Significant Difference statistic		
[2] SED represents the Standard Error of Difference statistic		

Table 3: Forage yield performance of DH30, DH38, DH1 and DH23 compared to their hybrids DHTF1 and DHTF2 obtained during the 2010 season. DH was harvested on May 5, 2010. DHTF1 and DHTF2 data were obtained on May 3, 2010. Fishers multiple comparison test statistics and rankings are provided only for yield.

Name	%Moisture	Yield Kg/ha
DH16	60.4	3381.34 c
DH23	58.6	1675.96 f
DHTF1	50.87	5236.90 a
DH22	58.6	2508.24 e
DH36	58.6	3161.92 d
DHTF2	48.88	4773.29 b
LSD[1]=12.75 (p=0.05)		
SED[2]=4.96		
[1]LSD represents the Least Significant Difference statistic		
[2]SED represents the Standard Error of Difference statistic		

Granular nitrogen was applied at a rate of approximately 36 kg/ha for the trial on October 15, 2008 and October 6, 2009. A single forage clipping was performed on the F1 performance trial on May 25, 2009 and May 3, 2010. The DH performance trial was harvested once on May 5, 2010. The forage trials were clipped using a Hege, Model 212 small plot forage harvester.

RESULTS

Forage yield performance data are provided in (Tables 1 and 2). Comparisons of the check varieties to the experimental hybrids clearly indicate that the DHTF1 and DHTF2 F1 hybrids are competitive. Comparison of the forage production of DH lines DH16, DH22, DH23 and DH36 to their respective hybrids DHTF1 and DHTF2 suggest higher forage productivity in the hybrids than the DH lines themselves (Table 3). This is not necessarily unexpected as homozygous DH lines are inbred lines and it has been known for decades that inbred lines across species have a consistently lower production potential than the hybrids they are used to generate [14,15]. However, it was unexpected to observe that the percent moisture levels in the DH lines were approximately 10% higher than the F1. At this time, we have no data or studies to hypothesize or understand the higher levels of moisture in the DH lines when compared to their respective F1 hybrids.

It was observed that in 2010, the entire nursery plots of DHTF2 became fully infested with a race of leaf rust. Leaf rust is rarely a problem in this dryland region; however, 2010 was particularly wet during the cool spring growing season and this was followed by a long period of elevated temperature and high humidity. As there were no genetic differences between the individuals comprising the DHTF2 hybrid, all individuals exhibited the same susceptibility. This observation reinforces the knowledge that germplasm with a high level of genetic uniformity will succumb in a uniform fashion to any particular susceptibility. The lack of genetic diversity found in an F1 raises the issue of a potential problem with F1 development, and the observation of uniform susceptibility for one F1 evaluated in this study should be considered accordingly.

However, even understanding the potential faults associated with uniformity, the approach offers an opportunity to develop DH lines specific to environments or geographic locations with the eventual development of designer F1 hybrids suitable to those locations or environments. Similar to the hybrid corn industry where particular inbred lines produce specific hybrid genotypes that are optimized for particular locations or environments, the potential development of selected tall fescue DH lines and the production of their respective F1 tall fescue hybrids could show optimal performance to particular locations or environments. This advantage may prove to be more beneficial to commercial seed producers who presently attempt to market a few genetically, broad based cultivars to fulfill the needs across multiple geographic locations and environments.

DISCUSSION

Though these data represent preliminary results conducted only on two F1 hybrids generated from experimental material and evaluated at one location (Figure 1), the results indicate the positive development of F1 tall fescue as a commercial product and the potential occurrence of a heterotic response regarding biomass productivity. Though this study suggests a potential for the future production of F1 hybrid tall fescue, there are several criteria that will need to be addressed prior to commercialization. First, the DH lines need to be adapted to the region where the hybrids are to be utilized. This may be addressed by applying the methodology of gamete selection to DH development [11]. Second, the DH lines need to be self-compatible and vigorous enough to produce an adequate supply of seed, both for their own maintenance and F1 hybrid seed production. Tall fescue is generally considered an out-crossing, self-incompatible species; however, we have seen in this study that self-compatible genotypes are generated. Third, generation and identification of DH parental lines utilized in F1 generation should be of an adequate genetic distance to maximize the potential for hybrid heterosis. The performance of traditional general combining ability and specific combining ability tests, applied prior to any molecular marker distant studies, may provide a simpler and lower cost approach to solving this component.

As mentioned this study, the DHTF2 hybrid exhibited a uniform susceptibility to a form of leaf rust. In a hybrid situation, increased production will come at a cost of decreased genetic diversity and increased genetic uniformity. Any susceptibility in an F1 genotype will be one-hundred percent across all individuals.

Though the experimental F1 hybrids produced in this study were generated by hand in the greenhouse, there are several methods that may be utilized to develop more commercially viable methods for larger scale hybrid development. One approach, similar to that used to maintain line purity by reducing outcrossing or pollen contamination is through the use of appropriate nylon mesh or netting [16,17]. F1 seed generation can be greatly expanded beyond limited greenhouse space into field nurseries by placing a selected pair of DH lines under a nylon pollen exclusion netting with a pore size of <.035 mm and allow the cross-pollination to occur naturally, (Figure 2). This is a low maintenance approach that has been subsequently applied toward generating many experimental small lots of hybrid seed for testing.

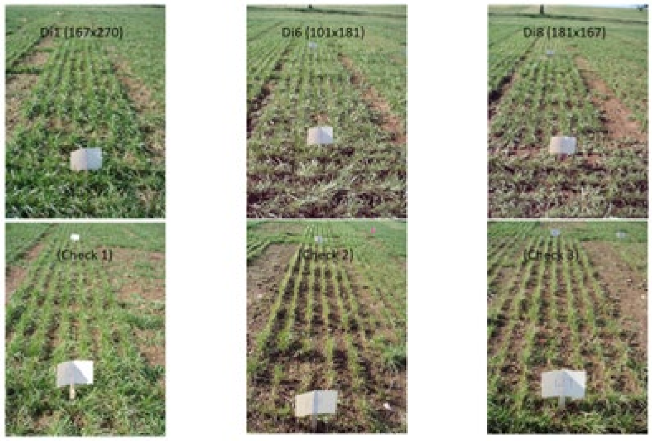

Figure 1: Performance plots of three experimental F1 hybrid tall fescues compared to three commercial check varieties. Trial was conducted at the Animal Forage Production Research Unit, USDA-ARS, and Lexington, KY. Checks are unnamed in the figure due to their proprietary nature.

Figure 2: An image showing several DH × DH pollinations being produced within a field nursery at the Barenbrug West Coast Research Center, Albany, OR.

CONCLUSION

A novel method has been identified that allows the production of true F1 hybrids of tall fescue. Preliminary evaluation of the F1 hybrids indicates hybrid vigor, heterosis and competitive performance. Though there are obstacles to this approach such as wind, insect and elevated humidity, F1 hybrids have been generated and are under evaluation using this approach. Future studies focused on both traditional and novel approaches in generating hybrids within a generally, self-incompatible species will be necessary to determine the optimum method for large scale commercial production of F1 hybrid tall fescue.

It is hoped that this early study in production and evaluation of F1 hybrid tall fescue will be used as a teaching and educational tool and that the potential for such hybridity in tall fescue may eventually produce cultivars with elevated yields and performance not currently available or observed in today's genetically wide based cultivars.

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