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## Sampling the Genetic Diversity of Tall Fescue Utilizing Gamete Selection

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### 1. Introduction

Tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire) ( $2n=6x=42$ ) represents the predominant perennial cool-season grass forage in the USA. Its wide adaptation, excellent spring, summer and fall production, deep root system, tolerance to heat and persistence over summer conditions makes this a highly desirable species for hay, pasture and turf. Tall fescue tolerates short-term flooding, moderate drought and heavy livestock grazing and machinery traffic. It responds well to fertilizer but can maintain itself under limited fertility conditions and is adapted to moderately acid and wet soils (Jennings et al., 2008). Approximately two-thirds of the total annual growth of tall fescue occurs during the spring and about one-third occurs during summer and fall making it nearly a full season forage. A wide array of phenotypic and genotypic variation exists in tall fescue and cultivar development has traditionally used recurrent selection techniques. For this review, we focus primarily on tall fescue as a forage or hay producing species; however, methods discussed can be equally applicable to turf programs. Although tall fescue is a complex hexaploid species, it can be hybridized with annual ( $2n=2x=14$ ) or perennial ryegrass ( $2n=4x=28$ ) (*Lolium perenne* L. subsp. *multiflorum* (Lam.) Husnot (syn. *Lolium multiflorum* Lam.) for the development of festuloliums (Yamada and Takamizo, 2004). In some instances, induced androgenesis has been utilized to generate useful germplasm (Vagera et al., 1998).

Gamete selection as originally defined by Stadler (1944) is based on the principal that selection exerted at the gametophytic level can increase desirable allelic frequencies detectable at the sporophytic level. If superior gametes can be recognized with certainty through a selection cycle, then such a system would be theoretically more efficient than one based on zygotic selection (Richey, 1947). In practice, gamete selection ordinarily involves two steps: 1) selection on the basis of outcross performance testing of individual plants of a variety or population, and 2) a similar controlled selection for outstanding individuals exhibiting desirable agronomic attributes. Following the identification of superior genotypes, such individuals would undergo continued selfing, followed by phenotypic selection, to generate a homozygous line, fixed for the desired agronomic characteristics. In instances where haploids can be generated through microspore culture, followed by genome doubling, homozygous or dihaploid lines can be obtained.

Breeding methods that utilize haploids can be varied and often provide materials exhibiting particular usage in genetic analysis and breeding systems when properly exploited (Dunwell, 2010). In addition, gametophytic selection approaches can lead to a correlated sporophytic response that can involve the segregation of important agronomic traits (Mulcahy, 1971). When applicable, gamete selection has emerged as a superior and efficient form of selection and the feasibility of this approach appears to be a promising method for facilitating the incorporation of desirable alleles within a short period of time. Stadler (1944) is among the earliest who reviewed its potential application in corn breeding. More recently, the importance of a gamete form of selection, utilizing early generation selection, has demonstrated successful simultaneous selection of multiple traits, including quantitative trait loci for characteristics such as seed yield, maturity, and tolerance to disease (Singh, 1994; Ravikumar et al., 2004). In these instances, gamete selection was found to be superior in its efficiency to conventional pedigree, single seed descent, and mass selection methods (Singh, 1994). A gamete selection approach may also be equally effective on complex traits such as biotic and abiotic stress, herbicide tolerance, general and specific combining ability, and the selection of complex quantitative traits such as yield, drought tolerance, disease resistance, etc. Traditionally applied to commercial grain crops such as corn (Gordilli and Geiger, 2008; Chang and Coe, 2009), wheat (Barclay, 1975), barley (Kasha and Kao, 1970; Shugar, 1989) and beans (Singh et al., 1998) gamete selection often utilizes the induction of haploids and the subsequent generation of double haploid breeding approaches. With the use of these methods, various degrees of success in gamete selection have been attained. The generation of haploid or monoploid individuals is typically achieved through microspore culture methods (Mohan et al., 1997; Maheshwari et al., 1980; Tuvešson et al., 2008), wide hybridization (Inagaki, 1997), or through a pollen/sperm nucleus utilizing a haploid inducing system (Chang and Coe, 2009). This approach has even been applied to tall fescue (Kasperbauer et al., 1980; Bai & Qu, 2000). Each of these methods relies on the haploid individual normalizing its chromosome number either through the utilization of mitotic inhibitors such as colchicine, or through spontaneous doubling. This chromosome doubling event results in the generation of individuals that are homozygous for the genotype conferred by the single contributing gamete. By using a paternal monoploid or dihaploid generation process, gamete selection is a proven and efficient method of selection that has yet to be applied to forage grass breeding (Stadler 1944; Lu, et al., 1996; Rotarencu and Chalyk, 2000). Dihaploid (DH) breeding methods have been suggested and employed as a means to develop new germplasm in the *Festuca-Lolium* complex of polyploid grasses (Humphreys et al., 2003; Guo and Yamada, 2004; Guo et al., 2005). Previous research utilizing homozygous lines to study the inheritance of palatability has suggested that selection within homozygous lines can represent a useful methodology for the improvement of tall fescue (Henson and Buckner, 1957; Buckner and Fergus, 1960a). As a consequence, when the gamete selection approach is utilized in a breeding program, the generation of DH tall fescue lines should result in the development of efficient breeding advances and superior germplasm (Bouchez and Gallais, 2000). Additional research also indicates that genetic mapping studies focused on the elucidation of a variety of complex genetic traits can be more readily accomplished through the use of homozygous lines (Riera-Lizarazu et al., 2010). The application of gamete selection to tall fescue could be accomplished if an efficient system of haploid induction followed by parthenogenesis were available.

Sporophytically expressed traits are transmitted as genetic information through the gametes (sperm or egg nuclei) and contain half the information that is contained in the sporophytic tissue. In some instances, gametophytic genes can regulate both gamete and sporophytic traits and this is called genetic overlap. In the presented gamete selection approach, a slight modification of the methodology as presented by Stadler and his contemporaries is utilized. Employing the method of gamete selection considered in this text, traits transmitted by the gamete are under the control of genes that are expressed specifically in the sporophyte. A single gamete provided by the tall fescue parent fertilizes the egg of an inducer line (IL) that results in genome loss and a parthenogenic response. Individuals derived from this hybridization event, followed by genome loss and the parthenogenic response, are recovered ryegrass, tall fescue or varied intermediate genotypes of ryegrass-fescue (e.g. *festulolium*). Selection applied at the F1 sporophyte results in a form of gamete selection.

The USDA-ARS has recently developed an approach utilizing *Lolium multiflorum* lines (IL1 and IL2) that are uniquely characterized by their ability to lose either a ryegrass or tall fescue genome following hybridization and the expression of a low frequency of parthenogenic behavior (Kindiger, 2009). Parthenogenesis involves development of the egg cell within the embryo sac without involvement of the sperm nucleus and such behavior has been characterized across numerous species, especially following wide hybridizations (Kendall, 1934). In this review, a low frequency parthenogenic response represents the first reported incidence of such behavior in ryegrass x tall fescue hybrids. The frequency of this behavior is difficult to determine, however, it is estimated to occur less than 1% which is typical for other species (Kendall, 1934). This frequency can and does vary across tall fescue genotypes hybridized with the IL lines.

Two ryegrass (*Lolium perenne* L. subsp. *multiflorum* (Lam.) Husnot (syn. *Lolium multiflorum* Lam.) ( $2n=2x=14$ ) genetic stocks, identified as IL1 and IL2 (Kindiger and Singh, 2011; Kindiger, 2011), are characterized by a genome loss phenomenon following hybridization with tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire (syn. *Festuca arundinacea* Schreb.) ( $2n = 6x = 42$ ), followed by a low level of parthenogenic development. The IL1 and IL2 genetic stocks exhibit few advantageous agronomic characteristics and are essentially notable only for their ability to induce chromosome or genome loss following hybridization. Both lines are free of the fungal endophytes *Epichloë* sp. or *Neotyphodium* sp. (Carroll, 1988; Moon et al., 1994; Pederson & Sleper, 1988). Recurrent selection remains a widely utilized approach for breeding most cereal and forage species (Gallais, 1993) and has also been applied utilizing DH lines (Bouchez and Gallais, 2000). Incorporation of a gamete selection methodology should provide an equally successful approach for tall fescue improvement.

In the approach, hybrids are generated utilizing genetic stocks IL1 or IL2 as the maternal parent and a single tall fescue individual or population as the paternal parent. Varietal or individual plant sampling within improved tall fescue cultivars or populations will yield a myriad of segregating gametes possessing a wide array of advantageous and disadvantageous alleles. Each pollen sperm nuclei will contribute a unique genotype to each hybrid and as a result, each hybrid represents a random genotypic combination of the IL maternal line and tall fescue pollen parent. Since the IL1 and IL2 lines are narrow based, agronomically inferior, unimproved genetic stocks, any potential advantageous contribution they may provide is considered minimal, notwithstanding unknown potential heterotic



effects in the F1 that may occur between the *Lolium* and *Festuca* genomes. The primary focus of the approach is placed on the gametic contribution of the paternal tall fescue parent and its expression in the hybrid sporophyte. Through this process, the breeder or geneticist is essentially sampling the genetic diversity of the paternal individual on a single pollen grain basis and examining each contribution in the hybrids.

## 2. Methodology

Pollinations are generated by hand utilizing the appropriate IL genetic stock as the maternal parent. Hybrid seed is easily generated in quantity. Following germination of the hybrid seed, numerous ryegrass-tall fescue hybrids are now available with each F1 hybrid being derived from a single tall fescue pollen grain sperm nucleus fertilizing the IL egg. The chromosome number of the hybrids is typically  $2n=2x=28$  with each hybrid possessing one genomic contribution from each parent (ryegrass ( $n=7$ ) and tall fescue ( $n=21$ )). Hybrids are generally sterile, but a low incidence of fertility can occasionally occur (Buckner, 1960b; Buckner et al., 1961). The generated seed can be sown in low density space planting nurseries, grown in the greenhouse, or planted to a spaced planted nursery where various induced or natural selection pressures can be applied to the hybrid individuals (Figures 1a, 1b, 1c). Multiple locations are desirable to focus selection on the particular attributes such as stress or rust tolerance in a region where the germplasm is planned to be released (Figures 2a, 2b). If disease tolerance is a selection criterion, then the hybrids should be grown in a region where the specific disease under study is prevalent. Once exceptional hybrid individuals are identified, the F1's can be transferred to the greenhouse to exclude any chance of cross-pollination with any tall fescue pollen in the field. If an abundance of tall fescue pollen is not a problem in the nursery, then the hybrids can remain in the nursery for a future harvest of each selected plant inflorescence. Hybrid individuals that do not possess an appropriate genotype contribution from the paternal tall fescue parent are culled from the nursery. If multiple years of selection are to be performed, seed heads are not to be retained until the final year of selection.



Fig. 1. a (left) Low density nursery planting of IL X tall fescue seed; USDA-ARS, Grazinglands Research Laboratory, El Reno, OK, USA. Figure 1b (center) Space planted nursery of IL x tall fescue hybrids. Figure 1c (right) Stress tolerant and stress intolerant hybrids from the IL x tall fescue hybrid space planted nursery.



Fig. 2. a (left) IL x tall fescue F1 drought selection nursery at the Mound Valley Unit, Kansas State University, Southeast Agricultural Research Center, KS in July, 2010. Circled individuals identify F1 plants exhibiting superior drought tolerance and plant vigor. Figure 2b (right) IL x tall fescue F1 rust tolerance selection nursery at Barenbrug Seeds, West Coast Research Center, Albany, OR in August, 2011.

The surviving hybrids are allowed to flower; then inflorescences are gathered at maturity, broken up by hand or machine and sown to trays. A light cleaning is applied to remove stems. The cleaned seed heads are then placed in germination trays for identification and selection of either recovered ryegrass or tall fescue seedlings. Typically, following two weeks of germination, a few seedlings will appear and are allowed to grow to appropriate size for transplanting (Figure 3). The germinating seedlings will generally be ryegrass recoveries possessing a chromosome number of  $2n=2x=14$ , tall fescue recoveries possessing a chromosome number of  $2n=6x=42$ , or various tall fescue DH recoveries with ryegrass introgression or ryegrass recoveries with tall fescue introgression. Since the seedlings obtained from the sterile F1 hybrids are generated through parthenogenic development following spontaneous chromosome doubling, each recovery will possess a full genome contribution of either the ryegrass or tall fescue parent. Genome loss, spontaneous doubling followed by parthenogenic development represent the important and essential contributions of the IL1 and IL2 lines. All recovered lines derived from this process will have all genes, alleles or quantitative loci conferring a trait fixed in the DH recovery (Kindiger and Singh, 2011; Kindiger 2011). Essentially, the process generates homozygous or DH lines from the IL x tall fescue F1 hybrids.

Each recovered individual will be free of the *Lolium* sp. fungal endophytes since the IL1 and IL2 lines do not possess endophyte. Recovered tall fescue DH lines can then be evaluated under additional selection schemes and eventually inter-crossed to perhaps generate a synthetic possessing various advantageous attributes, or be utilized as breeding lines for the development of cultivars.

### 3. Example 1: Gamete selection for crude protein

The development of forages with superior nutritional qualities is an ongoing and time consuming breeding effort often hindered by the complex genetics associated with each quality trait (Casler, 2001; Bouton, 2009). Since grazing animals have specific and differing





Fig. 3. Germination of dihaplod seedlings from a selected IL x tall fescue F1 hybrid. These individuals will represent DH tall fescue recoveries.

nutritional requirements, the qualities of the forage should be adjusted to fit that particular need (Brummer and Casler, 2009). Intergeneric hybridization between *Lolium* and fescue

has been a common method to understand and improve the genetics underlying the forage quality (Naganowska et al., 2001; Cogan et al., 2005). In addition, applying approaches such as introgression mapping facilitated with either genomic *in situ* hybridization techniques or molecular markers, provide an additional set of powerful tools for forage quality improvements (Cardinal et al., 2003). The close homology between the *Lolium* sp. and *Festuca* sp. genomes can also allow either recombination or a full substitution of a *Lolium* chromosome for a *Festuca* chromosome (Kopecký et al., 2009) and such events could affect the expression of a forage quality. It may also be useful to incorporate molecular markers during the breeding process or during backcross generations to identify any ryegrass segments that might have been introgressed into the fescue genome (Humphreys, 2004).

In a preliminary demonstration utilizing the gamete selection approach, 14 IL x tall fescue hybrids were evaluated for their crude protein (CP) quantity via available nitrogen in plant leaf tissue. The need for forages to possess adequate levels of CP must complement the grazing animal's nutritional requirements. Typically, CP estimates reflect the level of nitrogen and amino acids in forages. CP content is considered a quantitative trait in most forages (Fei et al., 2006.) and is typical of a trait exhibiting low heritability and is, as a consequence, difficult to transfer rapidly and effectively (Vogel et al., 1981). Leaf samples

from 14 IL x tall fescue F1 hybrids were obtained from the nursery in March 2011 and were run on an Elementar varioMacro flash combustion instrument (Elementar Americas, Inc., Mt. Laurel, NJ). In addition, DH lines derived from particular IL x tall fescue hybrids were assayed for N, along with the check tall fescue cultivars Nanyro, Retu, Drover and Barcarella. CP concentration of samples was estimated by multiplying total nitrogen (N) concentration by 6.25 (Hersom, 2007)(Table 1). Multiplying the N concentration by 6.25 to estimate CP level is performed because protein molecules contain an average of 16% N ( $1/16 = 6.25$ ). This N and CP estimation represents the standard approach utilized for evaluating beef cattle protein requirements (Hersom, 2007). Results presented in Table 1 indicate that hybrids high in CP content provide DH recoveries high in CP content. DH23A, a DH exhibiting low CP content, was obtained from a hybrid exhibiting low CP content. A

| IL x Tall Fescue | Crude Protein | Description       |
|------------------|---------------|-------------------|
| LF12-8           | 18.5          | IL X TF Hybrid    |
| LF12-9           | 10.6          | IL X TF Hybrid    |
| LF13-1           | 14.3          | IL X TF Hybrid    |
| LF13-3           | 11.7          | IL X TF Hybrid    |
| LF14-1           | 17.9          | IL X TF Hybrid    |
| LF14-2           | 13.1          | IL X TF Hybrid    |
| LF15-2           | 17.1          | IL X TF Hybrid    |
| LF16-1           | 11.7          | IL X TF Hybrid    |
| LF16-2           | 16.2          | IL X TF Hybrid    |
| LF16-3           | 14.4          | IL X TF Hybrid    |
| LF800-4          | 15.6          | IL X TF Hybrid    |
| LF800-8          | 12.0          | IL X TF Hybrid    |
| LF800-9          | 19.4          | IL X TF Hybrid    |
| LF900-1          | 19.2          | IL X TF Hybrid    |
| Syn1             | 21.0          | Blend of DH lines |
| DH8B             | 18.7          | LF12-8 recovery   |
| DH9B             | 18.4          | LF12-8 recovery   |
| DH36D            | 21.1          | LF900-1 recovery  |
| DH15B            | 19.2          | L F800-9 recovery |
| DH14B            | 18.6          | LF14-1 recovery   |
| DH23A            | 12.2          | LF16-1 recovery   |
| Nanyro           | 21.4          | Cultivar Check    |
| Drover           | 19.8          | Cultivar Check    |
| Barcarella       | 15.5          | Cultivar Check    |
| Retu             | 18.4          | Cultivar Check    |
|                  |               |                   |

Table 1. Crude protein estimates across IL x tall fescue hybrids (LF), their dihaploid recoveries (DH) and standard cultivar checks.



program focused on developing a high CP cultivar would simply require the identification of agronomically superior DH lines exhibiting high CP estimations. This approach is superior to traditional recurrent selection as there are no segregating alleles that could confer lower or segregating CP levels. All lines utilized in the breeding process are fixed for the high or low CP genes. This approach removes much of the random segregation of alleles governing CP or other traits of low heritability. Following identification of high CP materials, eight high CP DH lines were combined to generate an experimental synthetic (Syn1). In limited performance trials, Syn1 has demonstrated itself to be a DH synthetic exhibiting high CP and superior agronomic attributes. As a consequence, a selection program focused on identifying hybrid genotypes exhibiting high CP content, then deriving high CP content DH lines from those hybrids should be an efficient and effective method to concentrate quantitative trait loci defining elevated CP content via gamete selection.

#### **4. Example 2: Gamete selection for stress tolerance**

A primary objective of the forage research program at the USDA-ARS, Grazinglands Research Laboratory is the development of tall fescue forage possessing tolerance to the environmental extremes of the Southern Plains Region. Typically, these environmental extremes involve heat, drought and low nitrogen inputs. To achieve this goal, a three year program of natural selection was conducted with numerous IL x tall fescue hybrids. Selection criteria were to evaluate the hybrids in a high stress environment on the Southern and Central Plains of the USA consisting of high summer temperatures, high wind, non-irrigated, low nitrogen input (40 lbs/ac) conditions (Figures 1a, 1b, 2a, 2b). Trials were conducted at the USDA-ARS, Grazinglands Research Laboratory, El Reno, OK and at the Mound Valley Research Unit, Kansas State University, Southeast Agricultural Research Center, Parsons, KS. Seed generated from the initial IL x tall fescue crosses were sown in a greenhouse and individuals were transferred to spaced plot field selection nurseries in the fall of 2006 (El Reno, OK) and 2008 (Mound Valley, KS). IL x tall fescue hybrids that did not possess a genotype adaptable to these conditions either died or, if the individual hybrids exhibited unsatisfactory agronomics, were physically removed from the nursery on a year to year basis (Figure 4). In the fall of 2009, seed heads were removed from the El Reno, OK nursery and in the fall of 2010, seed heads were removed from the surviving IL x tall fescue hybrids at the Mound Valley, KS location. Seed heads were threshed as described and sown to germination trays in the greenhouse.

Recovered DH tall fescue lines were removed from the germinating trays and transplanted to pots. In some instances, DH lines were selfed for seed increase. Though tall fescue is considered an obligate out-crossing species we have observed that this is not the case for many recovered DH lines and successful selfing of tall fescue has been observed and utilized in prior tall fescue research programs (Buckner and Fergus, 1960a). Seed generated through selfing recovered DH lines were sown in small, unreplicated plots in the nurseries at the USDA-ARS, Grazinglands Research Laboratory, El Reno, OK. Single DH plant selections were transplanted to the Kansas State University, Southeast Agricultural Research Center, Parsons Unit, Mound Valley, KS in 2010. The selection criteria remained unchanged at both locations for DH evaluation. During the summer of 2011 both locations experienced extended severe to extreme drought and heat conditions (National Oceanic and Atmospheric Administration, National Climatic Data Center, 2011). The DH tall fescue

recoveries have exhibited good to excellent tolerance, superior adaptation to and persistence under these record-setting drought conditions at both locations. This study indicates that the gamete selection approach is effective and efficient in identifying genotypes that can tolerate high environmental stress conditions such as drought, heat and low nitrogen inputs. These and additional DH lines generated from this gamete selection approach will be utilized to form a foundation of tall fescue germplasm with particular adaptation to high stress sites across the Southern Plains and Midwest regions of the USA.



Fig. 4. Example of a stress intolerant IL x tall fescue F1 hybrid (left) and a stress tolerant hybrid (right).

## 5. Confirming recovery of DH tall fescue lines

There are two methods that can be performed to indicate or verify that the recovered tall fescue materials are DH homozygous recoveries. The first step is to self-pollinate the particular DH individual for seed increase and perform nursery grow outs. If the offspring from the selfing do not segregate for size, inflorescence morphology, maturity or other obvious phenotypic characteristics, they are likely DHs (Figure 5).

A second approach is to utilize molecular markers known to exhibit a banding pattern that is consistent with disomic inheritance and co-dominant expression. That is, each marker exhibits a maximum of two bands for each individual when two alleles at a particular locus are present in a heterozygous state. When one band is present, the allele at that locus would be in a homozygous condition. These markers will be most useful since segregation will be predictable and such markers are easy to score (Stift et al., 2008). Tall fescue, having a polyploid genome constitution (Alderson and Sharp, 1994), has a considerable level of genome duplication. As a consequence, it is important to utilize markers that are known to amplify only a single or specific site in the tall fescue genome. When such markers are utilized, only a single amplification product will be formed at that locus since that locus would be homozygous. If the site were heterozygous, the amplification of multiple alleles would be visualized. Following the approach of Saha et al. (2004), selected DH lines were evaluated utilizing a set of twelve EST-SSR markers with the analyses being performed by DNA LandMarks (Quebec, Canada). The results of the analysis indicated that DH47

exhibited a high level of allelic variability when compared to the other DH recoveries (Table 2). From this analysis, DH47 is indicated to be non-homologous for all marker loci and is likely a rogue. DH lines 41, 42, 44 and 57 are indicated to be true DH recoveries, homozygous at the evaluated marker loci. Similar approaches may be equally effective in polyploids thought to possess a high degree of genome duplication (Esselink et al., 2004).



Fig. 5. A plot in a DH phenotypic uniformity trial at the USDA-ARS, Grazinglands Research Laboratory.

| EST-SSR | DH41 | DH42 | DH44 | DH47        | DH57 |
|---------|------|------|------|-------------|------|
| NFA004  | 285  | 285  | 285  | 298         | 285  |
| NFA030  | 199  | 199  | 199  | 199         | 199  |
| NFA039  | 293  | 293  | 293  | 291         | 293  |
| NFA047  | 244  | 244  | 244  | 241/244/250 | 244  |
| NFA067  | 130  | 151  | 175  | 147         | 136  |
| NFA068  | 254  | 254  | 254  | 251         | 254  |
| NFA073  | 236  | 236  | 246  | 236         | 246  |
| NFA074  | 244  | 244  | 244  | 241/244/250 | 244  |
| NFA095  | 187  | 187  | 187  | 182/187     | 187  |
| NFA104  | 218  | 218  | 218  | 238         | 218  |
| NFA115  | 236  | 236  | 236  | 220/230     | 236  |
| NFA133  | 150  | 150  | 150  | 145/155     | 150  |

Table 2. Base-pair marker size segregation of twelve EST-SSR markers across five potential dihaploid lines. The absence of allelic variation across markers indicates a dihaploid recovery, homozygous for the locus (e.g. DH41, DH42, DH44, DH57). An excessive level of allelic marker variability suggests a non-homozygous condition and the lack of a homozygous DH recovery (DH47). The DH47 line would be discarded.



## 6. Conclusion

The generation of haploid or dihaploid lines through a gamete selection approach can be of critical importance in breeding and genetic analysis research (Duwell, 2010). The materials and procedures described above apply directly to the breeding and selection of superior tall fescue germplasm and could be expanded to other fescue species. The expansion of research programs utilizing this approach should find the availability of DH lines useful when objectives are focused on plant genomics, fine mapping of DH derived populations and/or marker-assisted selection of genes that confer agronomically favorable traits. One advantage to the described method is that microspore methods are not utilized and the researcher is not limited to genotypes amenable to microspore culture techniques. The sampling of hundreds of thousands of pollen grains, each segregating for a myriad of genotypes from a single tall fescue individual or population requires less labor input and represents a low cost, rapid selection strategy that can be implemented across diverse environments. The simultaneous selection of multiple traits or complementary traits can be applied quite effectively and the prescribed approach also does not require any prior genetic information regarding the inheritance or expression of the quantitative trait. Though molecular markers can be applied, the approach does not require the utilization of molecular markers for marker-assisted selection. Generated DH lines possessing superior genotypes are maintained through selfing, retaining the fixation of the qualitative or quantitative trait of interest.

Though IL1 and IL2 can be utilized to generate DH lines, the frequency of generation is low, less than 1%. However, the ability to generate IL x tall fescue hybrids is rapid and inflorescences on the hybrids are abundant. When numerous F1 inflorescences are utilized from the F1, the recovery of DH lines is quite efficient. Depending on the quality and number of inflorescences, it is not unusual to obtain one to eight seedlings from each IL x tall fescue hybrid. When multiple hybrids are screened and placed in a commercial line production situation, hundreds of DH recoveries may be obtained each season. What the approach may lack in DH generation frequency, is compensated by its efficiency in recovering DH lines. It is anticipated that when applied correctly, this approach would be effective for development of tall fescue lines exhibiting quality traits such as days to flowering, drought tolerance, grazing persistence, CP, NDF, ADF, RFQ, lignin, forage yield, etc. The gamete selection approach should also be advantageous to tall fescue breeders requiring efficient and rapid methods of developing tall fescue germplasm adapted to the unknown parameters of global climate change (Humphreys et al, 2006).

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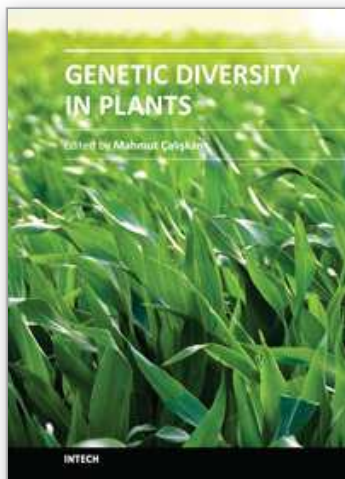
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## **Genetic Diversity in Plants**

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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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