Genetic Characterization of Fine-leaved Festuca valesiaca Germplasm and Evaluation of Their Relationship to the F. ovina complex

Yingmei Ma
Utah State University

Follow this and additional works at: https://digitalcommons.usu.edu/etd

Part of the Plant Sciences Commons

Recommended Citation
Ma, Yingmei, "Genetic Characterization of Fine-leaved Festuca valesiaca Germplasm and Evaluation of Their Relationship to the F. ovina complex" (2012). All Graduate Theses and Dissertations. 1352. https://digitalcommons.usu.edu/etd/1352

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.
GENETIC CHARACTERIZATION OF FINE-LEAVED *FESTUCA VALESIACA* GERMLASM

AND EVALUATION OF THEIR RELATIONSHIP

TO THE *FESTUCA OVINA* COMPLEX

by

Yingmei Ma

A thesis submitted in partial fulfillment
of the requirements for the degree
of

MASTER OF SCIENCE
in

Plant Science

Approved:

Dr. Paul G. Johnson
Major Professor

Dr. Jack E. Staub
Committee Member

Dr. Steven R. Larson
Committee Member

Dr. Mark R. McLellan
Vice President for Research and
Dean of the School for Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2012
Copyright © Yingmei Ma 2012

All Rights Reserved
ABSTRACT

Genetic Characterization of Fine-leaved Festuca valesiaca Germplasm and Evaluation of Their Relationship to the F. ovina complex

by

Yingmei Ma, Master of Science
Utah State University, 2012

Major Professor: Dr. Paul G. Johnson
Department: Plants, Soils, and Climate

Fine-leaved Festuca valesiaca possesses abiotic stress tolerances. However, their agronomic performances in the western United States and its genetic relationship to species of the Festuca ovina complex have not been investigated. Also, natural hybridization due to open pollination presents difficulties in distinguishing them for closely related taxa using morphological analysis. Given the species’ agronomic potentials, a project was designed to identify Festuca valesiaca accessions possessing high biomass production and seed yield for possible low-maintenance applications and to examine their relatedness to taxa of the Festuca ovina complex by multi-locus AFLP genotyping and chloroplast DNA sequence analysis using primer combinations designed from three intergenic spacers.

Plant vigor, height and width, total biomass, and seed weight and seed number of Festuca valesiaca accessions were evaluated from 2009 to 2011 at Blue Creek, Utah in a random complete block design with six replications. The Festuca valesiaca accessions examined produced abundance of small seeds. Seed production was significantly (P = 0.001) correlated ($r^2 = 0.84$) with the total biomass, plant height, and plant vigor rating. The Festuca valesiaca
accessions examined possessed lower height than the control ‘Cascade’ but higher biomass, spring green-up, and seed production. Given their morphological attributes, *Festuca valesiaca* accessions PI 659923, W6 30575, and W6 30588 should be considered for low-maintenance applications and use in plant improvement.

The AFLP-based neighbor-joining analysis indicated that *Festuca valesiaca* is a closely related subcluster of *Festuca ovina* and should be considered as one species. *Festuca trachyphylla* is a subcluster under *Festuca ovina* and *Festuca valesiaca*. *Festuca idahoensis* has a close relationship with *Festuca roemeri* but not with *Festuca ovina*. Low admixture was detected between the *Festuca rubra* and *Festuca trachyphylla* accessions examined, while a comparative high admixture was detected among the commercial cultivars examined.

Chloroplast sequences data reconfirmed that the *Festuca ovina* complex genetically differed from *Festuca rubra* and the other reference taxa examined. *Festuca valesiaca* and *Festuca ovina* possessed the same maternal lineage based on chloroplast DNA sequence analysis. One *Festuca valesiaca* accession, W6 30537, was genetically similar to the *Festuca rubra* examined and should be putatively reclassified as *Festuca rubra* pending further taxonomic analysis.
PUBLIC ABSTRACT

Genetic Characterization of Fine-leaved Festuca valesiaca Germplasm and Evaluation of Their Relationship to the F. ovina complex

Yingmei Ma

Fine-leaved Festuca valesiaca has stress tolerance. However, its agronomic performance in the western United States and its genetic relationship to species of the Festuca ovina complex has not been investigated. Also, natural hybridization makes them difficult to identify. Given the species’ potentials, our project was designed to identify Festuca valesiaca accessions that possess high biomass and seed yield and to examine their relatedness with the Festuca ovina complex.

The Festuca valesiaca accessions examined produced many small seeds. Seed production was correlated with the total biomass, plant height, and plant vigor. The Festuca valesiaca accessions examined were shorter than the control ‘Cascade’ with higher biomass, spring green-up, and seed production. Given their morphological attributes, Festuca valesiaca accessions PI 659923, W6 30575, and W6 30588 should be considered for low-maintenance applications and use in plant improvement. Broad-leaf species (Festuca arundinacea, Festuca pratensis, and Lolium perenne) were different from fine-leaved Festuca species in genetic analysis. Festuca valesiaca is closely related to Festuca ovina and should be considered as one species. Festuca trachyphylla is a subcluster under Festuca ovina and Festuca valesiaca. Festuca idahoensis is closely related to Festuca roemeri but not to Festuca ovina. The Festuca ovina complex is genetically different from Festuca rubra and the other reference taxa.
ACKNOWLEDGMENTS

I thank the Joint U.S.-China Biotechnology Research and Extension program in the College of Agriculture at Utah State University and the U.S. Department of Agriculture, Agricultural Research Service Forage and Range Research Laboratory who made this fine-leaved fescue germplasm evaluation and genetic relationship analysis possible by providing financial support and by contributing fine-leaved fescue collections. I am indebted to a number of people who were involved in this project. Without them, I could never have completed this project. I would like to thank my academic advisor, Dr. Paul G. Johnson, who spent time and effort on helping me get through all of the courses selection and arrangements which gave me great learning experiences while at USU and guidance for completing my degree. I am also grateful for his help on correcting my thesis and providing suggestions for its improvement. I am thankful to him for giving me the opportunity to attend the scientific meeting. At the same time, I would like to thank my research advisor, Dr. Jack E. Staub, who provided me this amazing study opportunity at Utah State University to obtain a master’s degree. I am also thankful for support for attending scientific meetings, his numerous project contributions, and for designing and helping to carry out the experiment and thesis corrections. I am grateful for his encouragement and inspiration during the study period to finish the thesis statement and teaching me to be a stronger woman in the future. Furthermore, I want to thank my committee member, Dr. Steven R. Larson, for his sincere and unique teaching methods on molecular data analysis, which helped me to learn a lot of the data analysis skills and the ways of doing research. He is a great person full of knowledge and I am grateful for his help on data analysis, correcting the thesis, and the ways of dealing with situations and issues in scientific research areas. In addition, I thank Dr. Matthew D. Robbins for his incalculable and timely assistance both on the research project and life in Logan. I also thank Dr. Douglas Johnson who made this work possible by contributing a fine-leaved fescue collection, Dr. Joseph Robins for morphological data analysis consultation, and Carolyn Jaussi for ordering all
the seeds I needed for this project. I acknowledge Dr. Matthew D. Robbins, Dr. Shaun B. Bushman, Lisa Michaels, Kimberly Thorsted, Linear Johnson, and Ningling Ying for their technical support during the molecular analysis. I am also grateful to Dr. Matthew D. Robbins, Aaron Newhall, Spencer Derr, Brian Anderson, and Riley Wyatt for helping with plant traits data collection of the fine-leaved fescue in the field. I appreciate Amanda Wilhelm, Dale C. Nielson, and Ryan Nelson for helping me run the flow cytometer. Additionally, I would like to give my special gratitude to those who (Tammy Roper, Teryl Roper, Frany Staub, Kimberly Thorsted, Lisa Michaels, Matthew D. Robbins, Mary E. Barkworth, Wengang Xie, Liujun Wen, Ye Yao, and Lijun Wang) helped me with so much besides research by giving me inspiration and encouragement to finish this work. Moreover, I would like to show my appreciation to my previous advisor, Dr. Feishi Luan, and all of classmates during my graduate studying period as well as six of my roommates in China for their support and comfort during my difficulties by reminding me of the happiness of obtaining fuels in the snow. Lastly, and perhaps most importantly, I thank my parents and younger brother for their sincere support my choice to continue my education.

Yingmei Ma
# CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT .................................................................................................................................... iii</td>
</tr>
<tr>
<td>PUBLIC ABSTRACT ...................................................................................................................... v</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS .............................................................................................................. vi</td>
</tr>
<tr>
<td>LIST OF TABLES .......................................................................................................................... xi</td>
</tr>
<tr>
<td>LIST OF FIGURES ...................................................................................................................... xiii</td>
</tr>
<tr>
<td>CHAPTER ....................................................................................................................................... 1</td>
</tr>
<tr>
<td>1. OVERVIEW............................................................................................................................. 1</td>
</tr>
<tr>
<td>GENERAL INTRODUCTION......................................................................................................... 1</td>
</tr>
<tr>
<td>Festuca species ......................................................................................................................... 2</td>
</tr>
<tr>
<td>Festuca filiformis .......................................................................................................................... 2</td>
</tr>
<tr>
<td>Festuca idahoensis ....................................................................................................................... 3</td>
</tr>
<tr>
<td>Festuca roemeri ........................................................................................................................... 5</td>
</tr>
<tr>
<td>Festuca ovina .............................................................................................................................. 6</td>
</tr>
<tr>
<td>Festuca trachyphylla .................................................................................................................... 8</td>
</tr>
<tr>
<td>Festuca valesisaca ......................................................................................................................... 9</td>
</tr>
<tr>
<td>Forage.......................................................................................................................................... 10</td>
</tr>
<tr>
<td>Turf Usage ................................................................................................................................. 13</td>
</tr>
<tr>
<td>Biofuels Usage ........................................................................................................................... 14</td>
</tr>
<tr>
<td>Molecular Markers ...................................................................................................................... 17</td>
</tr>
<tr>
<td>PROBLEM STATEMENT ............................................................................................................. 19</td>
</tr>
<tr>
<td>OBJECTIVES ............................................................................................................................ 19</td>
</tr>
<tr>
<td>REFERENCES ............................................................................................................................. 20</td>
</tr>
<tr>
<td>2. MORPHOLOGICAL TRAITS EVALUATION OF FESTUCA VALESISACA FOR LOW MAINTENANCE TURF APPLICATION ............................................................................ 33</td>
</tr>
<tr>
<td>ABSTRACT .................................................................................................................................... 33</td>
</tr>
<tr>
<td>INTRODUCTION .......................................................................................................................... 34</td>
</tr>
<tr>
<td>Turf Usages in United States ........................................................................................................ 35</td>
</tr>
<tr>
<td>Problem Statement and Potential Solutions ................................................................................. 36</td>
</tr>
<tr>
<td>MATERIALS AND METHODS ...................................................................................................... 37</td>
</tr>
</tbody>
</table>
Plant Materials ............................................................................................................... 37
Morphological Trait Evaluation ..................................................................................... 38
Phenotypic Analysis ....................................................................................................... 39

RESULTS .............................................................................................................................. 41

Climate at Blue Creek, Utah during Experimentation ................................................... 41
Morphological Trait Description .................................................................................... 41
  Total Biomass ......................................................................................................... 42
  Height ..................................................................................................................... 43
  Width ...................................................................................................................... 44
  Vigor Rating ........................................................................................................... 45
  Seed Weight ............................................................................................................ 45
  Seed Number .......................................................................................................... 47
Principal Component Analyses ...................................................................................... 48
  Principal Component Analysis of 2009 Data ......................................................... 48
  Principal Component Analysis of 2010 Data ......................................................... 49
  Principal Component Analysis of Combined 2009-2011 Data ............................... 50

DISCUSSION ........................................................................................................................ 51

Breeding of Festuca valesiaca ....................................................................................... 51
Morphological Traits in Festuca Species ....................................................................... 52
  Plant Height in Festuca Species ............................................................................. 53
  Plant Width in Festuca Species ............................................................................. 54
  Vigor Rating in Festuca Species ............................................................................. 54
  Seed Production in Festuca Species ....................................................................... 55
Interpretation of Principal Component Analyses ........................................................... 56

CONCLUSIONS ................................................................................................................... 57

REFERENCES ...................................................................................................................... 58

3. GENETIC RELATIONSHIP BETWEEN FESTUCA VALESIACA AND FESTUCA OVINA
   COMPLEX BASED ON AFLP MARKER ........................................................................... 76

ABSTRACT .......................................................................................................................... 76

INTRODUCTION ................................................................................................................. 77

MATERIALS AND METHOD ............................................................................................. 78
  DNA Extraction and PCR Amplification .................................................................... 78
  AFLP-based Clustering Analyses .............................................................................. 80
  Ploidy Estimation in Festuca Species ...................................................................... 81

RESULTS .............................................................................................................................. 81
  AFLP-based Neighbor-joining Tree Analyses ......................................................... 81
  AFLP-based Population Structure ...................................................................... 82
DISCUSSION

Genetic Clustering of Fine-leaved *Festuca* Species ........................................ 85
Genetic Clustering of *Festuca trachyphylla* ...................................................... 86
Genetic Clustering of *Festuca valesiaca* and *Festuca ovina* ......................... 86
Ploidy Level Differences Among Fine-leaved *Festuca* Species ............................ 87

CONCLUSIONS ............................................................................................................. 88

REFERENCES .................................................................................................................. 89

4. GENETIC RELATEDNESS OF *FESTUCA OVINA* COMPLEX TO THE REFERENCE TAXA BY CHLOROPLAST DNA SEQUENCES ......................................................... 105

ABSTRACT ..................................................................................................................... 105

INTRODUCTION .......................................................................................................... 106

MATERIALS AND METHODS ...................................................................................... 107

- DNA Extraction, PCR Amplification, and DNA Sequencing ............................ 107
- Phylogenetic Analysis ............................................................................................ 108

RESULTS ......................................................................................................................... 109

- Chloroplast DNA Sequence .................................................................................. 109
- Chloroplast DNA Sequence-based Cluster Analyses .......................................... 109

DISCUSSION .................................................................................................................. 110

- Over-arching Genetic Relationships among *Festuca* Species ......................... 110
- Genetic Relationships among *Festuca rubra* and *Festuca trachyphylla* .......... 112

CONCLUSIONS ............................................................................................................. 112

REFERENCES .................................................................................................................. 113

5. SUMMARY .................................................................................................................. 122

APPENDICES .................................................................................................................. 124
LIST OF TABLES

Table                                                                                                                                            Page

2-1 Probability values of type III error tests of fixed effects in a split plot in time design of plant traits used to assess *Festuca* species examined over multiple years (2009-2011) .......... 63

2-2 Probability values of type III error tests of fixed effects (accession) evaluated using a randomized block design for plant traits of *Festuca* species accessions examined over multiple years (2009-2011) at Blue Creek, Utah .......................................................... 64

2-3 Mean values of phenotypic traits of *Festuca* species accessions evaluated over three years at Blue Creek, Utah .................................................................................................................... 65

2-4 Pearson correlation coefficients ($r^2$) with associated significance (P) value as superscript between traits of *Festuca* species accessions evaluated in randomized block design in 2009 67

2-5 Pearson correlation coefficients ($r^2$) with associated significance (P) value as superscript between traits of *Festuca* species accessions evaluated in randomized complete block design in 2010 (below diagonal) and 2011 (above diagonal). ................................................................. 68

2-6 Pearson correlation coefficient ($r^2$) with significance (P) value as superscript between traits of *Festuca* species accessions evaluated in randomized complete block design over three years (2009-2011) ................................................................................................................................................................. 69

3-1 *Festuca* species germplasm used for morphological traits evaluation and genetic relationship analyses .................................................................................................................................................. 93

3-2 Analysis of molecular variance of seven fine-leaved *Festuca* species (*F. valesiaca, F. ovina, F. rubra, F. idahoensis, F. trachyphylla, F. roemeri, and F. filiformis*) partitioned into five clusters (*Festuca ovina, Festuca idahoensis, Festuca trachyphylla, Festuca Festuca rubra, and Festuca valesiaca*) based on pair wise genetic distance matrix constructed using 1,689 AFLP markers (*EcoR I* and *Mse I*). ............................................................................................................ 97

3-3 Pair wise matrix of percentage the genetic variation among accessions of seven fine-leaved *Festuca* species as assessed with 1,689 AFLP markers (*EcoR I* and *Mse I*). .................................................................................................................. 98

3-4 Matrix of corrected (below diagonal) average number of pair wise differences between seven *Festuca* species (populations), within species (diagonal), and total difference between species (above diagonal) as assessed by 1,689 AFLP markers ........................................................................................................ 99

4-1 Chloroplast DNA sequence order of six primers used for amplification of three cpDNA intergenic spacer regions in fine-leaved *Festuca* species .................................................................................................................. 117

A-1 A statistical model summary of a split plot in time design used to assess differences among *Festuca* species accessions over multiple years (2009-2011) at Blue Creek, Utah ............................................... 125

A-2 A statistical model summary of a split plot in time design where year is the whole plot factor for the assessment of morphological traits of *Festuca* species accessions examined over multiple years (2009-2011) at Blue Creek, Utah ........................................................................................................ 126
A-3 A statistical model summary of a randomized complete block design and data analysis of morphological traits (plant height, width, vigor rating, total biomass, seed weight, and seed number) per plant of *Festuca* species accessions examined over multiple years (2009-2011) at Blue Creek, Utah. .................................................................................................................. 127

A-4 Taxonomical traits descriptions of five *Festuca ovina* complex species as Ruemmele et al. (2003) complied. ................................................................. 128
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1 Monthly mean of temperature (panel A), relative humidity (panel B), precipitation (panel C), and solar radiation (panel D) over three years (2009-2011) at Blue creek, UT. (Data from Mesco West at the University of Utah, Salt Lake City, Utah).</td>
<td>70</td>
</tr>
<tr>
<td>2-2 Morphological trait means of <em>Festuca valesiaca</em> accessions and <em>Festuca</em> species commercial cultivars (Controls) assessed over multiple years at Blue Creek, UT. Asterisks indicate significant (P&lt;0.05) difference when compare to ‘Cascade’, and horizontal lines signify the average value of ‘Cascade’ by trait (Plant height (Panel A), plant width (Panel B), vigor rating (Panel C), total biomass (Panel D), seed weight (Panel E), seed number (Panel F); F.V. = <em>Festuca valesiaca</em>; F.R. = <em>Festuca rubra</em>; F.O. = <em>Festuca ovina</em>; F.A. = <em>Festuca arundinacea</em>; F.T. = <em>Festuca trachyphylla</em>; L. P. = <em>Lolium perenne</em>); PI and W6 = Plant introduction number according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN)</td>
<td>71</td>
</tr>
<tr>
<td>2-3 Principal component analysis plot of the first two principal components assessing <em>Festuca valesiaca</em> accessions and <em>Festuca</em> species commercial cultivars (Controls) based on eight morphological traits examined in 2009 (Panel A), 2010 (Panel B), and 2011 (Panel C) at Blue Creek, UT; PI and W6 = Plant introduction and collection number according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN); Principal component 1: percentage of variation explained; Principal component 2: percentage of variation explained</td>
<td>74</td>
</tr>
<tr>
<td>2-4 Principal component analysis plot of the first two principal components assessing <em>Festuca valesiaca</em> accessions and <em>Festuca</em> species commercial cultivars (Control) based on eight traits examined over three years (2009-2011) at Blue Creek, UT; PI and W6 = Plant introduction and collection number according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN); Principal component 1: percentage of variation was explained; Principal component 2: percentage of variation was explained</td>
<td>75</td>
</tr>
<tr>
<td>3-1 A genographer gel showing amplification of DNA fragments of 48 <em>Festuca</em> species using AFLP markers (Derived from EcoR I and Mse I restriction digestion) from 50 bp to 500 bp at 100% resolution. Each blue band in the row indicates one marker and each column demonstrates every marker for one sample</td>
<td>100</td>
</tr>
<tr>
<td>3-2 Thumbnail of 48 <em>Festuca</em> species and each of them represents the signal intensity (peak) for a specific individual’s AFLP (EcoR I and Mse I) marker as present (A) or absent (B)</td>
<td>101</td>
</tr>
<tr>
<td>3-3 Genetic relationships among <em>Festuca valesiaca</em> (va), <em>Festuca rubra</em> (ru), <em>Festuca idahoensis</em> (id), <em>Festuca ovina</em> (ov), <em>Festuca trachyphylla</em> (tr), <em>Festuca roemerii</em> (ro), and <em>Festuca filiformis</em> (fi) accessions based on NeiLi’s genetic distance among AFLP profiles after 1000 bootstrap permutations (Swofford 1998)</td>
<td>102</td>
</tr>
</tbody>
</table>
| 3-4 Natural log (Ln) probability of clustering seven fine-leaved *Festuca* species (*Festuca valesiaca*, *Festuca ovina*, *Festuca rubra*, *Festuca idahoensis*, *Festuca trachyphylla*, *Festuca filiformis*, and *Festuca roemerii*) over K values as inferred by genetic AFLP-based Bayesian
3-5 Colored bar graphs depicting the inferred ancestry coefficients from Bayesian cluster analysis of AFLP genotypes from *Festuca valesiaca* (va), *Festuca rubra* (ru), *Festuca idahoensis* (id), *Festuca ovina* (ov), *Festuca trachyphylla* (tr), *Festuca roemeriana* (ro), and *Festuca filiformis* (fi) accessions with 100000 burn-in where Pop 1, Pop 2, Pop 3, Pop 4, Pop 5, and Pop 6 are population numbers as defined within each K value according to Pritchard et al (2000) .... 104

4-1 Graphic depiction of sequence chromatogram showing evenly-spaced peaks presented of a baseline (‘noise’) from six *Festuca* species accessions ................................................................. 118

4-2 Sequences alignment from *Festuca* species as depicted using software from Staden Package v1.50 by optimally aligning with polymorphic sites (indicated with blue highlights) and manually adjusted nucleotide (indicated by lowercase letter). ......................................................... 119

4-3 Chloroplast DNA sequence strict consensus tree obtained from 1,000 heuristic parsimony bootstrap search for 36 *Festuca* (*Festuca valesiaca* (va), *Festuca rubra* (ru), *Festuca idahoensis* (id), *Festuca ovina* (ov), *Festuca trachyphylla* (tr), and *Festuca filiformis* (fi)) taxon ..................................................................................................................................... 120

4-4 Neighbor-joining tree constituted from pairwise comparisons of NeiLi’s distance for 36 *Festuca* (*Festuca valesiaca* (va), *Festuca rubra* (ru), *Festuca idahoensis* (id), *Festuca ovina* (ov), *Festuca trachyphylla* (tr), and *Festuca filiformis* (fi)) taxon based on chloroplast DNA sequences analysis ............................................................................................................ 121
CHAPTER
1. OVERVIEW

GENERAL INTRODUCTION

Species of the *Festuca* genus are used worldwide in lawn, golf courses, and forage applications as pasture and hay (Ianniello 2011). In tropical Africa, *Festuca* species are important as essential forage grasses for wild animals and controlling soil erosion in mountainous areas (Namaganda et al. 2006). They have also been utilized in soil erosion control programs (Schardl and Leuchtmann 2005). For instance, *F. arundinacea* (tall fescue) was used in land reclamation projects during the “Dust Bowl” period of the 1930s in the United States (Schardl and Leuchtmann 2005).

There are an estimated 450 broad- and fine-leafed *Festuca* species that grow in polar, alpine, and temperate regions (Clayton et al. 1986). Fine-leafed *Festuca* species are characterized by their fine and relatively narrow (less than 1 mm) leaves (Beard 1997). Five such species [*F. filiformis* Pourret (2n = 14), *F. idahoensis* Elmer (2n = 14), *F. ovina* L. ssp. *hirtula* (Hackel ex Travis) M. Wilkinson (2n = 14), *F. trachyphylla* (Hackel) Krajina (2n = 14), and *F. valesiaca* Schleich. ex Gaudin (2n = 14)] form the *Festuca ovina* aggregate that is often called the “ovina complex” (Table 3-5; Ruemmele et al. 2003). Species within the *F. ovina* complex are cross-compatible and, thus, hybrids between these and other *Festuca* species can occur frequently in nature (Schmit et al. 1974).

Fine-leaved *Festuca* species have both agronomic and horticulural attributes. Some species possess drought and shade tolerance, but are not necessarily heat tolerant (Beard 1997; Hanson et al. 1982; Ruemmele et al. 1995). Likewise, they can grow in poorly-drained soils, but many do not respond well to high nitrogen fertilization (Beard 1997; Davis 1967; Meyer 1993). Thus, their usage is usually targeted for specific applications. For instance, *ovina* complex species are
frequently grazed by ruminants because of their growth characteristics and broad temporal adaptation (sheep and cattle) on rangelands (Fırrıncıoglu et al. 2009). Although they possess typical bunch grass morphology, they have turfgrass qualities and, thus, have been used for some turf applications (Weibull et al. 1991). For example, *Festuca* species have been considered for use in low maintenance roadside and golf course applications, railway embankments, and reclamation on disturbed landscapes (Weibull et al. 1991). The horticultural use of *Festuca* species may be either broad or narrow in scope. For instance, lawn and wild flower mixtures may include diverse representative species of the *ovina* complex (Dewey et al. 2006). Some species, such as *F. ovina* ssp. *hirtula* and *F. glauca*, however, have principal value as ornamentals in mixed-species garden plantings (Weibull et al. 1991). In contrast, some diploid *F. ovina* species possess lead tolerance making them useful for some reclamation purposes (Patra et al. 2004), while other diploid species possess drought tolerance and low nitrogen requirements that allows for their use in arid environments in nutrient-poor soils (Weibull et al. 1991).

**Festuca species**

*Festuca filiformis*

*Festuca filiformis* has a wide distribution that extends from western and central Europe to Asia and North Africa (Aiken et al. 1996; Smit et al. 2001). *F. filiformis* was introduced into New Zealand as a lawn grass for its nonflowering turf characteristics and growth potential under acid, sandy, dry soil conditions (Aiken et al. 1996).

*Festuca filiformis* is relatively drought-resistant and withstands mowing as low as 1.2 cm (Ruemmele et al. 1995). It has tolerance to “moderate” shade, thus, is often found in dense woodlands (Ruemmele et al. 2003). *F. filiformis* differs from other *F. ovina* by its light brown, persistent leaf sheaths, comparatively narrower blades, long lemmas and lacking of short-awns (Aiken et al. 1996). Although, in cross-section the blade of *F. filiformis* has no ribs, a continuous
band of sclerenchyma tissue is evident at the leaf midrib (Aiken and Darbyshire 1990).

Ruemmele et al. (2003) described taxonomic characteristics of *F. filiformis* as follows:

Plants lack rhizomes, making them densely tufted. Leaves are bluish or yellowish-green. Culms grow to 15 to 55 cm. Intergroups are strongly scabrous or puberulent. Sheaths open to the base while dead sheaths remain prominent at base of plants rather split with age. Upper sheaths appear glabrous or finely scabrous-hirsute, rarely with any purple coloration. Ciliate ligules are 0.1 to 0.3 mm in length. Blades are scabrous with 3 large veins, 0-4 small veins, and one central rib. Sclerenchyma tissue forms a continuous or almost continuous abaxial ring. The inflorescence may be 1 to 7 cm long. At maturity, it is open or narrowly contracted with scabrous branches. Yellowish-green spikelets extend 3 to 6.5 mm with 2 to 6 florets. Glumes appear glabrous or apically scabrous. The first glume is 1 to 2.5 mm long and 1 veined while the second glume is 1.7 to 3.9 mm long and 3 veined. Lemmas are 2.3 to 4.4 mm long and glabrous or apically scabrous. Awns may be 0 to 0.4 cm. (pp 158)

There is one released cultivar of *Festuca filiformis*, ‘Barok’, which was developed as a European cool-season bunchgrass. It possesses fine, light-green, hair-like leaves with dense prostrate growth habit, and is adapted to dry and shady conditions (Alderson et al. 1995). It is commonly used in turf-grass mixtures in high traffic and low-maintenance areas that are prone to drought and high heat. In the United States, it is primarily used in cemeteries and in reclaimed areas having steep slopes (e.g., southern California).

*Festuca idahoensis*

Blue bunch fescue is the common name associated with *F. idahoensis* Elmer (Darbyshire and Warwick 1992; Huff et al. 1998). It is endemic to grasslands including subalpine meadows, and nonshaded dry forest opening areas that are adjacent to grasslands (Ruemmele et al. 2003; Pavlick 1983b). This species has been observed from southwestern Saskatchewan west to British Columbia in Canada, and in southern Colorado and central California (Ogle et al. 2010), and at relatively high elevations in Montana, Utah, and Idaho in the United States (Hanson 1982). Belsky and Moral (1982) also documented its distribution in alpine meadows of the Olympic Mountains in Washington State in the United States.

*Festuca idahoensis* is best adapted to deep, fertile, silt and clay soils (Gavin and Brubaker 1999; Lynch 1998). This species, however, tolerates alkaline, saline, and acidic soils, surviving on
as little as 25 cm of rainfall and cool climates. At high altitudes, *F. idahoensis* is competitive with other turf type grasses at maturity (Ruemmele et al. 2003), and it has been found that it was two times more abundant in unburned than burned areas on rangelands (Bowker et al. 2004). Thus, this species is potentially an important component in seed mixtures for restoring degraded rangelands in dry habitats (Goodwin et al. 1995), where it can be established by drill seeding (Sheley et al. 2006). It also has potential as a long-lived species, with some individuals persisting in excess of 60 years (Liston et al. 2003). However, as weedy species increase (i.e., competition), survival and growth of *F. idahoensis* on rangeland often decrease (Ewing 2002), especially where nitrogen is limiting (Mangold et al. 2004).

Ruemmele et al. (2003) describes the taxonomic characteristics of *F. idahoensis* Elmer as follows:

*Festuca idahoensis* Elmer is a densely turfed perennial that lacks rhizomes. Leaves are blue or yellowish-green. Leaves are 30 cm long and 0.6 to 1.0 mm wide. They are conduplicate, adaxially glabrous or pubescent, scabrous, and often glaucous or pruinose with 3-5 large veins and 2 to 5 small veins. Culms grow to 30-100 cm. And sheaths are open, glabrous or scabrous, and persistent. Ligules are short, about 0.3 to 0.6 mm in length. Sclerenchyma tissue is located in broad irregular strands. The inflorescence grows to 7-16 cm. Scabrid branches are erect or spreading. Spikelets may be 7.5 to 13.5 mm long with 4-9 florets where the first glume is 2.4 to 4.5 mm long and single veined while the second glume is 3.0 to 6.0 mm long and three-veined. Anthers are 2.5 to 4.0 mm long and the ovary apex is glabrous. Lemmas are 5.0 to 8.5 mm long with scabrid apices and awns are 3.0 to 6.0 mm long. Paleas have distinct pubescence between veins. (pp 159)

There are two released commercial cultivars of *F. idahoensis*, P-6435 and Trident (Alderson et al. 1995). Cultivar of P-6435 originated as a selection by R. J. Olson and J. L. Schwendiman at the Plant Materials Center, SCS, and Pullman, WA from a collection made by D. Hendrick near Winchester, ID in 1938 (Alderson et al. 1995). This cultivar is a vigorous, long-lived, bunch type fescue that was originally selected among 61 accessions and improved by mass selection. It possesses dark-green, basal, and abundant leaves. Trident was developed and released in 1988 by International Seeds Inc., Halsey, OR from original collections made from old turf sites in the southern and eastern U.S., along with derivatives of commercial cultivars (Alderson et al. 1995).
These collections were poly-crossed and evaluated in a spaced plant nursery, where six parental clones were selected to produce the synthetic variety, Trident. Trident is dark green, upright growing, and possesses a late heading characteristic. In addition to these cultivars, there are natural *F. idahoensis* x *F. roemeri* hybrids and two another released cultivars that are hybrids between *F. roemeri* and *F. idahoensis*, ‘Joseph’ and ‘Nezpurs’ (Barkworth et al. 2007). ‘Nezpurs’ was selected at the Plant Materials Center, SCS, Pullman, WA by J. L. Schwendiman from a native collection made in 1935 that was found eight kilometers south of White Bird, ID (Alderson et al. 1995). Plants are relatively large, and erect with robust stems producing broad, flat, abundant leaves. ‘Joseph’ is more ornamental than the common green varieties because of its unique color and its form that makes it a desirable source as a western native species (http://www.bluestem.ca/Festuca-joseph.htm). The blue-leafed cultivar ‘Siskiyou Blue’ was recently released and distributed in the horticulture industry for landscape design and garden use because of its broad adaptation to regions of the arid west.

**Festuca roemeri**

Roemer’s fescue was initially designated as a subspecies of *F. idahoensis* (Pavlick 1983a), but was later elevated to the species’ level by Wilson (2007). Although, the taxonomic status of this species is in question, the morphological distinction between *F. idahoensis* and *F. roemeri* rests on small differences in leaf sheath, blade width, the leaf shape, margin disposition, the number of leaf nerves, the vestiture on the adaxial leaf ribs, and leaf sclerenchyma patterns. Pavlick (1983a) also indicated that *F. roemeri* supports larger tussocks and courser leaves than *F. idahoensis*. Aiken et al. (2000), however, suggested that *F. roemeri* should be considered as subspecies of *F. idahoensis* [*F. idahoensis* subsp. *Roemer* (Pavlick)] based on analysis of unpublished evidence from seed protein banding profiles. Distinct taxa differences are supported by Jones et al. (2008) based in dissimilarities in their nuclear constitution, but not their cytoplasmic genomes. Their data indicated that the variation detected was consistent with the
hypothesis that natural populations of cross-compatible *F. idahoensis* and *F. roemeri* display considerable overlap with regards to their morphology and specific individuals within populations display common attributes over a large geographic area.

The taxonomic traits of *Festuca roemeri* was described in the following by Barkworth et al. (2007):

Plant is densely cespitose without rhizomes. Culms 50-90 cm, erect glabrous, smooth. Sheaths closed for less than 1/2 their length, glabrous, hirsute, or scabrous, persistent; Collars glabrous; ligules 0.1-0.5 mm; blades 0.5-1mm in surfaces glabrous or puberulent, adaxial surfaces sometimes scabrous, glabrous or pubescent, veins 7-9, ribs 5-9, well defined. Abaxial sclerenchyma in 5-7 wide strands, sometimes confluent into a single band; adaxial sclerenchyma absent. Inflorescences 8-20 cm long, loosely to densely contracted with 1-2 branches per Cluster; branches erect to slightly spreading, lower branches with 2 spikelets. Spikelet is 9-13.5 mm with 4-6 florets. Glumes exceeded by the upper florets, ovate-lancelate, smooth or scabrous distally; lower glumes 2.5-5 mm; upper glumes 4-6.2 mm. Lemmas 5-7 mm, scabrous near the apices, awns 3-5 mm, terminal, usually more than 1/2 as long as the lemma bodies; paleas is about as long as the lemmas, intercostals region scabrous or puberulent distally; anthers 2.8-3.6 mm; ovary apices glabrous. (pp 440)

*Festuca ovina*

*Festuca ovina* is the most common *Festuca* species in western Britain, but becomes relatively scarce in the mountains of northern and western Scotland, where it is replaced by *F. vivipara* (L.) Sm. (Wilkinson and Stace 1991). In the U.S., it is also scattered from North Dakota to Washington and Alaska, south to California and is found in Arizona, New Mexico, and isolated sites to the east into Texas (Barkworth et al. 2007).

Subspecies of *F. ovina* L. are commonly referred to as sheep fescue (Bonos et al. 2001; Ruemmele et al. 2003). Weibull et al. (1991) noted that it possesses excellent tolerance to drought and low levels of soil fertility, where it requires minimal nitrogen for vegetative growth. The subspecies prefers acidic, coarse textured low fertility soils and withstands close mowing and heavy grazing, but possesses low heat tolerance (Ruemmele et al. 2003). Short days and cool temperatures induce flowering in some *F. ovina* subspecies (Cooper and Calder 1964; Heide 1994). For instance, flowering of *F. ovina* ssp. *hittula* occurs in May and early June in the British
Isles (Ruemmele et al. 2003). *F. ovina* species are wind-pollinated, largely self-sterile and produce relatively “light” seeds (Auquier 1977; Ghatnekar 1999). Endophytic fungi play a role in affecting plant morphology in *F. ovina* species. For instance, endophyte-infected (E+) *F. ovina* seedlings possess a shorter length and higher root: shoot ratios when compared to their endophyte-free (E-) counterparts (Wäli et al. 2009).

This species can be used in turfgrass applications (Johnson 2003). For instance, a mixture of buffalo grass and fine fescue grasses (*F. ovina*) can provide for a low-maintenance turf that has low irrigation requirements with season-long green color and growth. The popular *F. ovina* turfgrass ‘Bighorn’, which was introduced commercially in 1987, resulted from three cycles of phenotypic recurrent selection for improved turf quality and color uniformity from a heterozygous and heterogeneous base population (Meyer et al. 1993).

Chromosome numbers in *F. ovina* L. species, however, range from 2n = 14 to 2n = 70 (Fuente 2001). In the broad-sense, however, only two ploidy levels have been extensively documented in *F. ovina* L. species: diploid 2n = 14 and tetraploid 2n = 2x = 28 (Šmarda and Köe 2003).

Ruemmele et al. (2003) describe the taxonomic characteristics of *F. ovina* L. as follows:

*Festuca ovina* L. distinguished by its very fine leaf texture, turf typed growth habit, and bluish-green stiff leaves. It is a densely turfed perennial with culms about 10 to 45 cm in height. Sheaths are open for over half their length, appearing pubescent or glabrous occasionally. Leaves are 2 to 10 cm long and 0.2 to 0.7 mm wide and are also adaxially pubescent, where they have five obscure veins and one rib. The green or slightly glaucous not pruinose are conduplicate and circular to oval in cross section mostly. Sclerenchyma tissue forms a thin broken ring or an unbroken ring 1-2 cells thick sometimes. The inflorescence is 5 to 10 cm long, but remains contracted, spreading only anthesis. Anthers are 1.6 to 2.5 mm long, yellow or purple, and usually more than half as long as the palea. Spikelets are 4.0 to 6.0 mm long. Distal margins of the leaves are ciliate where the first glume is 1.7 to 2.5 mm long with single vein; the second glume is 2.2 to 4 mm long possesses three veins. Lemmas typically measure 3.0 to 4.0 mm in length and five veined, green, glaucous, or tinged with reddish violet on the upper portion, and pubescent or scabrid in the distal half (they are rarely galbrous). Awns are 0.7 to 2.0 mm long. (pp 160)

The *F. ovina* cultivar ‘Covar’ is used commercially for rangeland restoration on disturbed landscapes in regions with 35-70 cm of annual precipitation (Alderson et al. 1995). ‘Covar’ was
selected at the Plant Materials Center, SCS, and Pullman, WA by J.L. Schwendiman from PI 109497 that originated from south Konya, Turkey. The development of this cultivar and its release in 1977 involved space plantings of the PI, where aberrant types were eliminated (Washington Agricultural Research Center, ID, Oregon and Idaho Experiment Stations, and the Plant Materials Center, SCS, Pullman, WA).

**Festuca trachyphylla**

*Festuca trachyphylla* is native to open forests and forest edge habitats of central Europe. It has been introduced and naturalized throughout many temperate regions, including France, Great Britain, and Scandinavia (Ruemmele et al. 2003). It was firstly common in the eastern United States and southeastern Canada, but is now widely established in North America.

This species is recommended for turf usage and sites reclamation where less mowing is preferred, such as roadsides, railway banks, parks, and sports grounds, and home lawns (Henensal et al. 1977). While this species tolerates well-drained stony and sandy soils (Ruemmele et al. 2003), its drought tolerance is considered less than that of *F. ovina* L. while greater than *F. rubra* Ruemmele et al. (2003) noted leaf blades are slightly thicker and awns are longer than *F. ovina*.

Ruemmele et al. (2003) describe the taxonomic characteristics of *F. trachyphylla* as follows:

Plants are perennial and densely tufted without rhizomes. Culms extend (9) 20 to 75 cm in length. Sheaths are open, closed only at the base. They are pubescent or occasionally glabrous. Auricles and ligules are short and minutely ciliate. Leaves reach 3.5 to 19 cm in length and 0.4 to 1 mm in width. They are conduplicate, green or subpruinose, scabrous or puberulent, 5 to 7 vein with 5 to 7 usually well-developed adaxial ribs. Sclerenchyma tissue is unevenly thickened, usually forming three tailing islets at the midrib and margins. They are 1 to 4 cells thick and rarely continuous. The inflorescence may be 3 to 9.5 cm long, erect to nodding, and contracted with branches usually scabrid on the angles. Spikelet is 5.5 to 9 mm long and yellow-green, blue-green or purple, with 3 to 8 florets. The rachilla is commonly visible between florets. Glumes are generally glabrous, although they may be scabrous apically, or even pubescent. The first glume is 2 to 3.5 mm long and 1-veined, while the second glume is 3 to 5.5 mm long and 3-veined. Lemmas are 3.8 to 5 mm long; 5-veined; glabrous, scabrous or pubescent apically; rarely entirely pubescent; and usually with apically ciliate margins. The awn is 0.5 to 2.5 mm long. Yellow or purple anthers may be 2.5 to 3.4 mm long. They are usually more than half as long as the palea. The ovary apex is glabrous. (pp 161)
**Festuca valesiaca**

*Festuca valesiaca* is typically a “dwarf” or low-growing species that has a broad distribution, being found in central Germany, north central Russia, and the Pyrenees mountains, central Italy, south central Greece, and in northern Asia where it grows in steppes, dry meadows, and open rocky or sandy areas (Ogle et al. 2010). Although *F. valesiaca* is considered to have an Asian origin, collections of this species have been made in Montana, Wyoming, New Mexico, and Kansas (Aiken et al. 2000). It has been sold in the North American seed trade as *F. pseudovina* Hack. *ex* Wiesb., apparently having become established from man-directed, deliberate seeding. Henensal et al. (1977) stated that *F. valesiaca* possesses a fine-leafed bluish or greenish leaf and is adapted to dry meadows, open rocky and sandy well-drained environments with at least 25 cm of precipitation annually.

The taxonomy of the *Festuca valesiaca* is controversial with different authors naming morphological variants and polyploid populations within it.

Ruemmele et al. (2003) describe the taxonomic characteristics of *F. valesiaca* as follows:

Plant is a densely turfed without rhizomes perennial blue grass. Culms reach 20 to 50 cm in height. Sheaths are open to the base and glabrous, smooth or sparsely scabrid. The ligule is short, less than 0.5 mm. Leaves are 0.2 to 0.6 mm wide, conduplicate, glaucous or pruinose, scabrid, 5-veined, and have 1 to 5 adaxial ribs. Leaf blades are sometimes deciduous. Sclerenchyma tissue occurs in 3 stout strands, rarely with additional small strands. The inflorescence is 3 to 10 cm long and contracted, with branches sparsely scabrid and erect or spreading. Pruinose spikelet ranges from (5.5) 6 to 6.7 mm long with 3 to 8 florets. The first glume is 2 to 2.5 mm long, while the second glume is 2.6 to 3.9 mm long. Lemmas may be 3.4 to 5.2 mm long, appearing glabrous or ciliate. Awns are 1 to 2 mm long. Anthers are 2.2 to 2.6 mm long. The ovary apex is glabrous. (pp 163)

It is an uncommon species in the commercial trade worldwide, where most cultivars possess bright blue and narrow leaves (Arndt 2008). For instance, blue-leafed cultivar of ‘Elijah Blue’ is used as an ornamental in mass plantings as repeated elements in garden borders. The cultivar ‘Glaucantha’ possesses stiff blue leaves that are upright and often brighter than ‘Elijah Blue’. In contrast, the growth habit and morphological characteristics of cultivar ‘Nefer’ are similar to ‘Elijah Blue’ and ‘Glaucantha’, but its leaves are light silver blue in mid-summer and turquoise
green in the winter.

**Forage**

Forage is defined as “edible parts of a plant other than separated grain, that can provide feed for animals, or that can be harvested for feeding” (Richard et al. 2010). Grass used as the forage sustains millions of dairy, beef cattle, horses, sheep, other livestock, and countless wild animals (Wang et al. 2001). Some fescue species have potential for providing forage for ruminants. For instance, in the western U.S., *F. idahoensis* provides excellent forage for livestock, elk, and sheep throughout the rocky mountain regions, especially in early spring, fall, and winter ([http://www.agf.gov.bc.ca/range/RangeID/Plants/FestIdah.html](http://www.agf.gov.bc.ca/range/RangeID/Plants/FestIdah.html)). Likewise, the extensive root system of *F. ovina* lends to its early season vigor and enhances its competitiveness with other grasses and it is fairly resistant to drought and trampling by herbivores such as sheep (Monsen et al. 2004).

Animal stature and performance are products of an interaction between numerous environmental factors (e.g., ecological in the case of free-ranging grazers) and nutritional resources (Barboza et al. 2009). ‘Body condition’ is a visual indicator of an animal’s health, and is directly related to its nutritional intake (Barboza et al. 2009). Moreover, feeding demand affects many aspects of an animal’s juvenile growth rate, adult mass gain, pregnancy probability, over-winter survival, timing of parturition, and neonatal birth mass and survival. In fact, nutrient intake provides a critical link between food resources and animal performance (Parker et al. 1996), where even small differences in food value can have large impacts on animal performance (White et al. 1983). Consequently, an animal’s energy and nutrient requirements are directly related to forage source (i.e., nutritional composition) and intake (quantity consumed) (Barboza et al. 2009; Karasov and Martínez 2007). Thus, forage grass must be nutritious and available at critical times during animal maturation (Marley et al. 2010).
Animal performance is a reflection of a number of nutritional and digestive factors such as dry matter intake and feed digestibility (Schroeder 1994). Dry matter intake (DMI) is estimated using percent neutral detergent fiber (NDF) that defines animal forage consumption (Schroeder 1994). Neutral detergent fiber (NDF) is the total cell wall cellulose, which is comprised of acid detergent fiber (ADF) fraction and total hemicelluloses (Schroeder 1994). The NDF value reflects the amount of forage that the animal can consume (Schroeder 1994). Feeding studies have shown that as the percent of NDF increases in forages, animal consumption decreases (Schroeder 1994). Thus, enhanced NDF digestibility is a critical component of forage quality (Van Saun 2006). In general, NDF forage digestibility significantly increases dry matter intake (DMI) and milk yield, where one unit increase of NDF digestibility is typically associated with increase of 0.37 lb, in dry matter intake and 0.51 lb, increase in milk yield in dairy cows (Oba and Allen 1999).

Crude protein (CP) is described as the nitrogen (N) content of the forage which is quantified by CP = N % X 6.25 (Schroeder 1994). Although both true protein and nonprotein nitrogen are included in CP, ruminants can utilize both to vary degrees (Schroeder 1994). The digestible protein (DP) value of forage (e.g., 70%-72%) is estimated as: CP x 0.908-3.77 (Schroeder 1994). Both CP and N vary in fescues depending on species and the environment in which they are grown (pasture and rangeland) (Schroeder 1994).

Relative feed value (RFV) is an index that combines the important nutritional factors of intake and digestibility (Schroeder 1994). Although RFV has no units, the index allows for comparisons between and among legume, grass, and legume-grass forages for their comparative feed value. Fescue species vary in RFV depending on genotype and the environment in which they are grown (pasture and rangeland). As the ADF increases, forage digestibility usually decreases, thus, the percent ADF and NDF decreases in forage, the RFV will increase (Schroeder 1994). The dry matter intake (DMI) potential is not always reported, but is often used to calculate RFV. The calculation of RFV combines dry matter intake and digestible dry matter (DDM) values.
of the forage as $RFV = DDM\% \times DMI\% \times 1.29$ (Schroeder 1994).

Digestible nutrient content can influence growth, survival, and reproduction (Shipley 2007). The higher the nutrient density (diet quality) in a diet, the less an animal will voluntarily ingest (Barboza et al. 2009; Karasov and Martinez 2007). Highly productive cows typically cannot, however, meet energy requirements of maximum milk yield from forage alone (Conrad and Martz 1985). The fiber level of forage can limit production and concentration since it typically comprises 400 to 600 g/kg of the diets of lactating dairy cows.

Chemical characteristics such as cell wall concentration and degree of lignifications affect the nutrient amount and composition of forage (Jung 1993). Fine fescue grasses possess different degrees of lignifications (Jung 1993). As lignin increases, digestibility, intake, and animal performance usually decrease and the percentage of ADF and NDF increase (Schroeder 1994). Estimates of ADF and NDF are consequently used to estimate the cell wall portions of forage that are composed of cellulose and lignin and are directly related to animal digestibility (Schroeder 1994).

Fiber content and the amount of lignifications affect particle break down during digestive mastication and rumination, which, in turn, influences the suitability of nutritive particles available to the ruminant (Murphy and Colucci 1999). Grazing forage fiber provides the foundation for cow and calf production systems throughout the world (Schroeder 1994), where some minimum fiber threshold is required in diets of dairy cows to maintain maximum dry matter (DM) and energy requirements. In dairy cows, for instance, without the appropriate amount of fiber in the diet, the fat content of milk can be relatively low (Johnson et al. 2003).

Forage grasses provide different levels of protein to ruminants which are dependent on the type of grass (genetics) and the season of the year in which it is produced (environment) (Robbins and Robbins 1979). Protein requirements are increased during fetal growth, particularly when the fetal is deposited (Robbins 1993; Robbins and Robbins 1979). Although protein requirements for
animals are typically highest during periods of rapid body growth and maturity, nonlactating beef cows have relatively low protein and energy requirements (Landete-Castillejos et al. 2001; 2003). Hence, requirements for much of the production cycle are often met with forages of low to medium quality (Wilson and Watson 1985). Given the fact that fescue species differ in their forage quality, deficiencies (i.e., minerals and/or protein) can occur in lactating cows (Lalman et al. 1993). Forage consumption increases with increasing calf age so that forage consumption is relatively high in calves (Lalman et al. 1993).

Winter hardiness and fall dormancy ratings are determined by the amount of re-growth visually after a mid-September cutting (Richard et al. 2010). Since forage composition and ruminant DM and CP degradation are affected by forage species and maturity, plant maturity at harvest has the greatest influence on NDF digestibility (Balde et al. 1993; Coblentz et al. 1998). As forage matures, NDF digestibility can decline more than 40%. Thus, earlier cutting dates and the addition of acid to herbage before ensiling can increase silage DM intake by beef cattle.

**Turf Usage**

A “lawn” is defined as an area of aesthetic and recreational land planted with grasses or other durable, low-growing plants, which usually are maintained at a relatively low and consistent height (Ruemmele et al. 2003). Turfgrass is the major vegetative ground cover in American landscape. In fact, it is the most widely used ornamental crop in the United States, and the United States and Canadian turf grass industry is a multi-million dollar business, where it is one of the fastest growing segments of the horticulture industry (Emmons 2000).

Turf-grass acts as a vegetative ground cover that is mowed regularly in lawns, highways, and golf courses. Apart from the direct economic benefits realized from turfgrass (Johnson et al. 2006), it serves to prevent soil erosion by having extensive and dense root systems and abundant top growth that knit and hold the soil together (Emmons 2000). Turfgrass also provides an
aesthetic feature to landscapes because of its attractive green color and uniform appearance. Turfgrass also provides an ideal surface for sports fields and other recreational facilities because of its ability to withstand sustained, rigorous use (Emmons 2000). Some Festuca species (e.g., *Festuca ovina*) have particular applications for low-input lawn operations and in the reclamation of degraded landscapes (Weibull et al. 1991). Additionally, turfgrass has a cooling effect on the environment via transpiration by releasing a substantial amount of oxygen into the air (Emmons 2000).

**Biofuels Usage**

The continuing growth of a global economy directly or indirectly affects climate change, current and future energy supplies, and the environment (Mussa et al. 2010). Attempts to mitigate these effects have generated interest in the cultivation of bio-energy crops for use as biofuels (Wrobel et al. 2009). Biofuels may provide an important source of renewable alternatives to reduce society’s dependence on fossil fuels, lower CO₂ emissions, and support developing local agricultural economics (Goldemberg 2007; Groom et al. 2008). In fact, the U.S. Biomass Technical Advisory Committee has suggested that 30% of the U.S. current fossil use will be replaced with biofuels by the year 2030 (Perlack 2005). This would require the production of approximately 907 million tons of dry biomass feedstock annually (Wrobel et al. 2009). It was also estimated that by 2050 biomass might provide nearly 38% of the world’s direct fuel use and 17% of the world’s electricity (Demirbas 2009). This goal can theoretically be achieved through the growth of crops for use as biofuels as well as the utilization of residue from crops already grown. Renewable forms of energy of biomass are the world’s fourth largest energy source worldwide, following coal, oil, and natural gas (Demirbas 2009). Biomass also appears to be an attractive feedstock because of its productivity, renewability, sustainability and positive environmental properties (Demirbas 2009). Biomass is biological material derived from living, or
recently living organisms (BEC 2012). Thus, the annual yield of biomass is important information for an engineer to estimate the total amount of land that must be put into production of biomass crops and how far crops must be transported to a facility (Brown 2003).

Harvested biomass was also used to show the capacity of native grasses to compete with invasive populations in Central Valley of California (Lulow 2006). Additionally, increased grass production via mixed management practices has been shown from biomass harvest estimations (Eekeren et al. 2010). As an energy source, biomass can either be used directly, or converted into other energy products (Xu et al. 2011). Biomass production can be influenced by land management (David et al. 2007), irrigation, and surrounding species (interplant competition; Robins 2010). Mineral nutrition, such as soil copper concentration, can affect biomass production in Elephant grass (*Pennisetum purpureum* Schumach), Vetiver grass (*Vetiveria zizanioides*) and the upland reed (*Phramites vulgaris*) (Liu et al. 2009). In addition, salt concentration (pH) is another factor which influences biomass production in some grasses such as Buffelgrass (*Cenchrus ciliaris* L. Syn. *Pennisetum ciliare* Link) (Griffa et al. 2010). Fertilizer, especially nitrogen, can significantly influence biomass production (tiller number) in winter wheat (*Triticum aestivum* L.) (Aravindhakshan et al. 2011; Heinsoo et al. 2011; Rao and Northup 2011). Post-fire survival (revegetation) of *Stipa speciosa* and *Festuca pallescens* is calculated based on their biomass production (Gittins et al. 2011).

Efficient and effective utilization of biomass energy technologies requires an understanding of chemical or energetic yields during the bioconversion process and agricultural economics (McLaughlin et al. 1996). The primary industrial endpoint for the processing of energy crops is ethanol production (transportation fuels), direct combustion or gasification (production of heat or electricity), or thermo chemical conversion (McLaughlin et al. 1996). Ethanol production is initially a product of the breakdown or energetic conversion of lignocelluloses cell walls to sugars, where the content of cellulose and other structural cell walls polysaccharides are the primary
determination of ethanol yield (Sladden et al. 1989). The suitability of energy crops for combustion or gasification is based on moisture content and ash chemistry during bio-energy conversion (McLaughlin et al. 1996).

The molecular characteristics of ethanol determine the maximum amount of heat that can be recovered and the potential electricity that can be generated during the energy conversion process (McLaughlin et al. 1996). Thus, several indices that reflect energy content, density, and ease of recovery have been developed to indicate the suitability of energy generating crops for either their conversion into fuels or the release of energy through combustion (McLaughlin et al. 1996). The indicators of the energy content of dried material are the moisture content of the plant at harvest, the plant’s structural density, and potential energy (i.e., bio-energy conversion characteristics) of a particular crop species (McLaughlin et al. 1996). Combustion fuel characteristics of the bio-energy conversion process includes a plant’s ash content, ash fusion temperature, and sulfur content can be used to develop indices of potential atmospheric pollution (McLaughlin et al. 1996). Ash content is important in the combustion process because it can contribute to slag development on internal boiler surfaces, which leads to formation of carbon deposits that reduce boiler efficiency and increase maintenance costs (Jenkins et al. 1998). The critical ash characteristic which promotes “slagging” is the alkali content and the presence of associated silicates in plants used for bio-energy consumption (McLaughlin et al. 1996).

A significant amount of crop residue has been used as biofuels in China (Li et al. 2001). Likewise, 27% of Canada’s current energy needs are being supplied by crop residues, mill wastes, and other biodegradable substances sources (Biocap Canada 2005). Ethanol is most commonly produced by the bio-energy conversion of wheat (*Triticum* spp.), sugarcane (*Saccharum* L.), and corn (*Zea mays* L.). Canary grass (*Phalaris arundinacea* L.) (Carlson et al. 1996) and switchgrass (*Panicum virgatum*) (McLaughlin and Kszos 2005) have been cited as promising perennial grass candidates for energy production. However, many annual grasses are not potential sources of
biofuels production because of their comparatively large root systems, relatively low requested agricultural inputs, stable agricultural commodity pricing, and harvesting ability (Wrobel et al. 2009). Therefore, it has become attractive to develop biofuel crops from perennial grasses (Demirbas 2009). More than one harvest can be obtained per year from perennial grasses because they can be grown vegetatively and reestablish rapidly after harvesting (Klass 1998). Fescue species have been differing levels of moisture content depending on their genotype, the environment in which they are grown, and their inherent potential value for ethanol production has not been explored.

**Molecular Markers**

Environmental effects may influence selection based on phenotypic traits and true “genotypic value” may be masked by genotype and environment interactions (Amini et al. 2011). Molecular marker technologies and their appropriate application possess great potential for breeding (Farooq and Azam 2002) and defining plant genetic diversity (Kibria et al. 2009). One type of molecular marker variation based on primers of random sequence is amplified fragment length polymorphism (AFLP) (Zhang et al. 2006). This type of technology (AFLP) analysis is relatively inexpensive, technically easy, comparatively rapid to perform, and reliable.

The AFLP technology is based on the selective PCR amplification of restriction fragments obtained from genomic DNA (Jones et al. 2008; Vos et al. 1995). More specifically, DNA is cut with restriction enzymes, and double-strand adapters are ligated to the ends of the restricted DNA fragments to generate template DNA for amplification. Genotyping is accomplished by inspection of band presence or absence at specific regions on an electrophoresis gel based on a fragment’s (band) weight (Vos et al. 1995).

The AFLP technology has many applications, where it is reliable and effective for genetic mapping, DNA finger printing, genetic diversity measurement, and the development of genomic

Amplified fragment length polymorphisms (Vos et al. 1995) have been used to study genetic diversity, to facilitate breeding, and in genome mapping of economically important traits in *Festuca* species (Fjellheim and Rognli 2005a; b; Mian et al. 2002, 2005; Skibinska et al. 2002). Likewise, AFLP analysis has been used in wheat (*Triticum* spp.) to define genotypes and to identify accession origin by geographical area (Pakniyat et al. 1997). AFLP marker technologies are routinely used for quickly and efficiently estimating relationships between lines and populations of many plant species (Lage et al. 2003). Although the inability of discerning the heterozygote is the drawback of this marker technology (Cresswell et al. 2001), AFLP markers have been used to investigate if the key agronomic traits in tall fescue progeny derived from genetically diverse parents (Amini et al. 2011).

AFLP marker technologies have been used successfully for diversity assessment in wheat (Almanza-Pinzon et al. 2003; Barrett et al. 1998; Bohn et al. 1999). Fine fescue grasses are often difficult to distinguish based on their morphology (Bhandari et al. 2004). AFLP marker technologies have been used effectively to characterize genetic differences in fescue populations (Jones et al. 2008). Thus, genotyping *ovina* complex species and *F. valesiaca* accessions using AFLP markers might allow for the elucidation of their genetic relationships.
PROBLEM STATEMENT

Fine-leafed fescue grasses have application for rangelands, pastures, and low-input turfgrass usages (Ruemmele et al. 2003; Bertin et al. 2009). Both natural and introduced fine-leaf *Festuca* species are important contributors to agriculture in the western U.S. (Ruemmele et al. 2003; Bertin et al. 2009). Historically, plants have been collected from their area of origin to provide needed genetic resources for germplasm enhancement (Ruemmele et al. 2003; Bertin et al. 2009). Collections of *F. valesiaca* were recently made in Kyrgyzstan by Douglas A. Johnson of the U.S. Department of Agriculture, Agricultural Research Service, Forage and Range Research Laboratory, Logan, Utah (personal communication, Johnson et al. 2010; Kyrgyzstan Plant Expedition Trip Report) and some of these accessions were initially evaluated for turfgrass performance in the Great Basin of the western U.S. (Johnson et al. 2010; Kyrgyzstan Plant Expedition Trip Report). More recently (2010), expeditions to Russia (Johnson et al. 2010) facilitated the collection of additional *F. valesiaca* accessions [Germplasm Resources Information Network (GRIN); www.ars-grin.gov]. Accessions (30) of *F. valesiaca* resident (Regional Plant Introduction Station, Pullman, WA) have not been evaluated for their horticultural or agronomic potential for low-maintenance applications (roadways, recreational turf, and rangeland reclamation). Genetic relationships among accessions of *F. valesiaca* resident in the U.S. National Plant Germplasm System (Regional Plant Introduction Station, Pullman, WA) and other *Festuca* grasses are not well defined.

OBJECTIVES

Even though some *F. valesiaca* germplasm has proven commercially important (Firincioglu et al. 2009), genetic relationships among such germplasm and to those species of the *ovina* complex have not been clearly defined. Elucidation of phenotypic and genotypic relationships *F. valesiaca* germplasm would allow for their more effective use in plant improvement programs.
Moreover, an understanding of the phylogenetic relationships among fescue species of the *ovina* complex would enhance the understanding of evolutionary relationships among *Festuca* species. Therefore, a project was designed to characterize the genetic nature of recently collected and resident *F. valesiaca* accessions in the NPGS (Table 3-1) by assessing their morphological and genotypic variation, and then, using these data, compare them to that of other *Festuca* species of the *ovina* complex (42 exotic germplasm and cultivars) and other more distantly related *Festuca* taxa (34 accessions) (Table 3-1). The following hypotheses were tested to evaluate the agronomic potential of these *F. valesiaca* accessions and to define their genetic relationships:

**H₀₁:** Morphological differences do not exist among *F. valesiaca* Schleicher ex Gaudin collections resident in the NPGS.

**H₀₂:** Nuclear genetic differences, as assessed by AFLP markers, do not exist between *F. valesiaca* collections resident in the NPGS.

**H₀₃:** Nuclear genetic differences, as assessed by AFLP markers, do not exist between *F. valesiaca* collections resident in the NPGS and other members of *Festuca* species germplasm of the *ovina* complex.

**H₀₄:** Cytoplasmic genetic differences, as assessed by cytoplasmic markers, do not exist between *F. valesiaca* collections resident in the NPGS and a diverse array of *Festuca* species germplasm of the *ovina* complex.

REFERENCES


Bohn M, Utz HF, Melchinger AE (1999) Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, SSRs and their use for predicting progeny variance. Crop Sci 39: 228-237


genera using chloroplast DNA restriction site variation. Can J Bot 70: 2415-2429


and ASA and CSSA, Madison, Wisconsin, pp 391-400


Liu XH, Shen YX, Lou LQ, Ding CL, Cai QS (2009) Copper tolerance of the biomass crops Elephant grass (Pennisetum purpureum Schumach), Vetiver grass (Vetiveria zizanioides) and the upland reed (Phragmites australis) in soil culture. Biotechnol Advances 27:633-640


Festuca species compared to the European Festuca complex. Theor Appl Genet 113:1529-1538


Robbins CT, Robbins BL (1979) Fetal and neonatal growth patterns and maternal reproductive effort in ungulates and sub-ungulates. Amer Natural 114:101-116

Robins JG (2010). Cool-season grasses produce more total biomass across the growing season than do warm-season grasses when managed with an applied irrigation gradient. Biomass and Bioenergy 34: 500-505


2. MORPHOLOGICAL TRAITS EVALUATION OF *FESTUCA VALESIACA* FOR LOW MAINTENANCE TURF APPLICATION

ABSTRACT

Fine-leaved *Festuca valesiaca* possesses varied abiotic stress tolerances. However, its agronomic performance in the semiarid western United States has not been investigated. Therefore, a project was designed to identify *Festuca valesiaca* accessions possessing high biomass production and seed yield for possible low-maintenance applications and future plant improvement. Twelve *Festuca valesiaca* accessions originating from Kyrgyzstan and eight commercial cultivars were transplanted to a field nursery at Blue Creek, Utah in a random complete block design (RCBD) with six replications. Plant height and width, total biomass, and seed weight and seed number per plant were evaluated from 2009 to 2011. Morphological trait evaluation indicated that the plant height, plant width, and total biomass of the *Festuca valesiaca* accessions examined were equal to the control ‘Cascade’. The plant vigor and seed weight of accessions PI 659923, PI 659932, W6 30575, and W6 30588 were, however, significantly higher than ‘Cascade’. Principal component analysis using all traits as loading factors suggested that these accessions were distinct from the majority of the accessions examined. The *Festuca valesiaca* species examined possessed abundant seed of small seed. Seed production was significantly correlated with the total biomass ($r^2 = 0.84$, $P = 0.001$), plant height ($r^2 = 0.58$, $P = 0.05$), and plant vigor rating ($r^2 = 0.83$, $P = 0.001$). *Festuca valesiaca* accessions were smaller than the control ‘Cascade’, but possessed higher biomass, spring green-up, and seed production than ‘Cascade’. *Festuca valesiaca* accessions possessed similar trait performance, which was higher than ‘Cascade’ from principal component analysis. Given their morphological attributes, *Festuca valesiaca* accessions PI 659923, W6 30575, and W6 30588 should be considered for low-maintenance applications and use in plant improvement.
**INTRODUCTION**

*Festuca valesiaca* is a fine-leaved (average leaf width of 0.4 -0.5 mm) dense, perennial bunch grass with relatively good turf characteristics because of its resistance to drought, shade, and diseases or pests (Sărățeanu and Moisuc 2009). Thus, this is one several fine-leaved species which possesses potential for varied ornamental (bluish green color) and turf usages. It is a polyploid species ($2n = 2x, 4x, \text{ and } 6x$) and is native to grassy sub-continental or continental mountain slopes, subalpine meadows, grasslands, and roadsides between 1,000 to 3,700 m zones of China, Kazakhstan, Kyrgyzstan, Mongolia, Russia, Tajikistan, Turkmenistan, southwestern Asia, and Europe (Flora of China; Arndt 2008).

Recent collections of *F. valesiaca* were made in which the central region of the Caucasus Mountain area (Johnson 2010 expedition) includes alpine meadows and glacier ecosystems ranging in annual precipitation from 250 to 1,000 mm (http://geography.about.com/od/findmaps/u/maps.htm#s1). This region is dominated by the Tien-Shan and Pamir-Alai mountain systems whose elevations range from 132 to 7,439 m above sea level with about 25% of the land area above 3,500 m (Johnson 2010 expedition). These dry temperate mountainous rangelands with their saline soils (Lal 2002) are very similar to those of Great Basin area of the western United States, where annual precipitation and elevation ranges from 125 mm to 2,032 mm (http://www.wrcc.dri.edu/pcpn/westus_precip.gif) and 881 to 2,063 m (Behnke 2011), respectively.

*Festuca valesiaca* was reported as a dominant species in the plant communities residing in fairly acid rocky areas (Hroudová-Pučelíková 1972). Because of its dense tussocks, Zdenka (1972) indicated that *F. valesiaca* could tolerate more extreme dry and warm conditions when compared to its closely related sister species, *F. rupicola*. Field and experimental nursery observations indicated that young seedlings of *F. valesiaca* also can survive under relatively high soil temperatures (Zdenka 1972). Likewise, erect (10-60 cm), sod-forming, perennial *F. valesiaca* can
tolerate cold and drought conditions on heavily grazed barren rangelands (Firincioglu et al. 2010). Titlyanova et al. (1999) also demonstrated that this species becomes dominant after the initial stages of grassland degradation from overgrazing. *F. valesiaca*, in fact, is also a dominant grass on plateaus of 1,200 to 1,900 m in Turkmenistan (Habibulla et al. 1999) and in the arid rangeland region of central Anatolian Turkey (Firincioglu et al. 2007). In contrast, Montane grasslands are also frequently dominated by *F. valesiaca* (Taft 2011) and *F. valesiaca* is an important and abundant forage species in un-grazed rangeland environments (Firincioglu et al. 2009).

Takebayashi and Delph (2000) emphasized that the natural selection is the main driving force for diversification within and among plant species in degraded grasslands. *Festuca valesiaca* is one of the few plant species that survives after intensive grazing in arid Eurasian environments (e.g., after 27 years of grazing in Turkey) (Firincioglu et al. 2007). Moreover, due to its persistent nature, *F. valesiaca* has a dramatic effect on shaping the vegetation pattern in such arid environments (Firincioglu et al. 2009). Therefore, this species might be expected to have genetic potential for establishment and persistence in varied western U.S. environments where abiotic tolerance is necessary for survival.

**Turf Usages in United States**

Turfgrass acts as a vegetative ground cover that is mowed regularly in urban recreational lawn settings and roadsides. Apart from the direct recreational and economic benefits realized from turf grass (Johnson et al. 2006), it serves to prevent soil erosion by having extensive and dense root systems and abundant top growth that knits and holds the soil together (Emmons 2000). Turf grass also provides an aesthetic feature to landscapes because of its attractive green color and uniform appearance. Indeed, turfgrass provides an ideal surface for sports fields and other recreational facilities because of its ability to withstand sustained and rigorous use. Additionally, turf grass has a cooling effect on the environment via transpiration (Emmons 2000), and provides
important ecological benefits such as slower storm runoff, improved water infiltration, and soil holding capacity on sloping terrains (Milesi et al. 2005). Some *Festuca* species (e.g., *Festuca ovina*) have particular applications in low-input lawn operations and reclamation of degraded landscapes (Weibull et al. 1991).

Turf grass is the major vegetative ground cover in American landscape and is one of the fastest growing segments of the horticulture industry (Emmons 2000). The total surface under turf increased through the 1990’s in the United States because of residential construction, and recently it has been estimated that turf grasses occupies 1.9% of the surface of the continental United States (Milesi et al. 2005). It has been estimated that the surface cultivated with turf is three times larger than irrigated corn, making turf the largest irrigated crop in the United States since the early 1990s (Milesi et al. 2005). Individual regions in the arid western U.S. also maintain sizeable urban turfgrass areas for residential and sports purposes (e.g., 1,207 km² of turf grass in Utah).

**Problem Statement and Potential Solutions**

Irrigation of turfgrass in arid urban settings can account for as much as 50% to 75% of household water consumption (Mayer et al. 1999). If all turfgrass in arid U.S. environments are watered according to commonly recommended schedules found in more U.S. temperate regions, summer water would increase dramatically (Milesi et al. 2005).

Lawn watering restrictions during summer months, recycling of wastewater to replace drinking water for golf course and park sprinkling systems, and increased use of xeriscaping are increasingly being implemented in arid and semiarid regions of the U.S. to conserve portable water (Milesi et al. 2005; Mustafa 2010). However, these practices and continued public education of the importance of water resources may not suffice for future water conservation strategies (Milesi et al. 2005). Salinity, increasing traffic, and drought are major factors that reduce turfgrass establishment of persistence (Asay et al. 1999). In order to conserve resources,
reduce labor cost and water usages, there is a need to identify and breed for more salt and drought resistance, low-maintenance grasses to augment current and future conservation strategies. Homeowners, golf course managers, and park superintendents are, in fact, actively seeking alternatives to reduce water consumption in these areas.

Grass germplasm recently collected in Kyrgyzstan (Johnson 2007 expedition; Johnson 2010 expedition) may be a reservoir of genetic variation that could provide genes for the development of low-maintenance turf grasses for use in western U.S. If such genes are present (i.e., heat, drought, and salt tolerance) in grasses originating from Kyrgyzstan, then breeding strategies could be developed to develop germplasm to increase water conservation. The first step in this process is the identification of genetically diverse, agronomically superior grass genotypes that possess abiotic stress resistance. The phenotypic and genotypic characterization of Kyrgyzstan *F. valesiaca* accessions from requires field evaluation in areas of low annual precipitation and genetic structure analysis to determine their relatedness.

**MATERIALS AND METHODS**

**Plant Materials**

For morphological assessment, twelve *F. valesiaca* seeds of each accession (Table 1-6) were germinated in germination boxes with filter paper, and then seedlings were planted in nursery containers (“Container”, 164 ml, Stuewe and Sons Inc, Tangent, OR) containing a mixture of 3:1 pumice and peat moss (V/V) in a greenhouse in Logan, UT January 2008. Seedlings were grown at 21°C (daylight conditions)/15°C (dark conditions) with supplemental light supplied by high-pressure sodium lights [(average irradiance = 400 watts (1800 µmols/m²/sec.); Sun System III, Sunlight Supply, Inc. Vancouver, WA], at a relative humidity (RH) between 50 to 70%. Seedlings were fertigated daily with 20 mg/ml of Peters Professional water soluble 20-20-20 fertilizer (NPK) to provide 4,000 ppm N, 1,760 ppm P, and 3,280 ppm K (Scotts Horticultural
company, Marysville, OH).

Five plants of each accession were transplanted in May 2008 to a field nursery at the Utah State University Blue Creek Experimental Farm in Box Elder County, UT (42.4 N 114.6 W) approximately 80 km northwest of Logan, UT. At this location, an average of 307 mm of precipitation is received annually (20-year average), and average annual precipitation during experimentation (2009-2011) was about 388 mm. The soil type was a Parley’s deep silt loam (fine-silty, mixed, mesic, Calcic, Argixerolls) having a neutral to slightly acidic pH. Plants were arranged in a randomized complete block design (RCBD; table A-3) with six replications, where commercial cultivars ['Manhattan’ 4 (*Lolium perenne* L.), ‘Black sheep’ (*Festuca ovina* L.), ‘Coronado’ (*Festuca arundinacea* Schreb.), ‘Durar’ (*Festuca lemanii* T. Bastard), ‘Cascade’ (*Festuca rubra* L. subsp. *commutata* (Gaudin) Markgr.-Dann.), ‘Scaldis’ (*Festuca trachyphylla* Hackel Krajina), ‘Shademaster’ (*Festuca rubra* L. subsp. *rubra*), ‘Dawson E’ (*Festuca rubra* L. subsp. *litoralis* (G.F.W. Meyer) Auquier)] were used as controls. Plants were spaced 0.5 m within the row and 1 m between rows (~20,000 plants/ha), and PI 659984 plants (*Festuca rubra* L.; Qing hai, China) were used as end- and side-borders. No water or fertilizer was applied during experimentation and weed-free plots were achieved by hand weeding (May-August) and herbicide [Mecamine-D; Dimethylamine Salt of 2,4-D (30.56%), Dimethylamine Salt of R-2 propionic acid (8.17%), and Dimethylamine Salt of Dicamba (2.77%)] application once in April or May of each year at a rate of 5.7 liter/ha to control broad-leaved weeds.

**Morphological Trait Evaluation**

On April 26, 2010 and June 7, 2011, the relative plant vigor was assessed using a 0 to 5 visual rating scale, where plant spring green-up (size and color and transition from winter to spring growth) was defined as 0 = plant dead, 3 = plants possessing moderate biomass or leaf blade with light green (tussock evident), and 5 = dark green plants having the greatest above
ground biomass and/or longest leaf blades length of those examined. On June 30, 2010, and June 23, 2011, the height (cm) of each plant was measured as the distance (cm) from the plant base (soil surface) to the top of the highest panicle at full anthesis. At the same time, the width (cm) of each plant was measured at the harvest cutting height (~10 cm above ground). Above ground plant parts was harvested (leaves and seed stalks) and oven dried at 60°C to estimate biomass (g). Dried florets of each plant were mechanically threshed to separate seeds and chaff (poorly developed or aborted seeds), and seed weight, 100 seed weight (g) and number of seeds per plant were estimated. Given the consistent performance of ‘Cascade’ in NTEP trail (2004, 2005, 2009, and 2010) ratings for quality = 5/6.9, color = 6.5/6.9, spring green-up = 5.9/6.3, density = 6.7/8.3, percent living ground cover in Summer = 93.3/96.3, Winter color rating = 3.3/6, dollar spot disease = 5.3/8.7, red thread rating = 4.5/7.5, pythium blight rating = 4.7/9, pink patch rating = 5/7, fall color rating = 4/6.3, and Summer stress rating = 4.7/7 was used herein as a standard for comparison. AMOVA analysis and principal component analysis were used to analyze the data.

**Phenotypic Analysis**

Measurements of individual plants were taken over three years (2009-2011). After comparing the AIC (Akaike’s Information Criteria) value of each model (repeated measurement, strip plot, and split plot in time) to test the year and year by accession interaction effects, split plot in time was found to have the smallest AIC value (data not shown). Thus, morphological trait data (over three years) were analyzed using a split plot in time in which year was treated as the whole plot factor and accession was treated as the split plot to test the significance of year and year by accession interaction effects using SAS software (Oehlert 2000; http://www.ats.ucla.edu/stat/sas/notes2/; table A-1; table A-2; SAS version 9.3). The statistical model used for data analysis was:

\[ Y_{ijkl} = \mu + \alpha_i + \gamma_k + \delta_{l(ik)} + \beta_j + \alpha\beta_{ij} + \epsilon_{l(ijk)}; \]
where $Y_{ijkl}$ was the dependent traits variable measured at $i$th year, $j$th accession, and $k$th replication; $\mu$ was the average value of the accession on the traits measured; $\alpha_i$ was the effect of year on the traits measured; $\beta_j$ was the effect of accession on the traits measured; $\delta_k$ was whole plot error on the traits measured; $\alpha_i\beta_j$ was the effect of interaction between accession and year on the traits measured; $\gamma_{l(ik)}$ was the effect of block as random; $\varepsilon_{l(ijk)}$ indicated the error effect of residual for the traits measured. All random effects were assumed to be independent and normally distributed with mean zero and variance $\sigma^2_k$, $\sigma^2_{l(ik)}$, and $\sigma^2_{l(ijk)}$, respectively. If a significant ($p < 0.05$) effect of year by accession was detected for a trait, then that trait was analyzed separately by year, otherwise trait data were combined for over year’s analysis. Data from each year were analyzed separately based on the randomized complete block design (table A-3) to test the significant effect of genotype under Blue Creek, Utah. Therefore, the model used herein was: $Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$; $Y_{ij}$ was the dependent traits variable measured at $i$th accession at $j$th block (Replication); $\mu$ was the grand average value of the accession on the traits measured; $\alpha_i$ was the effect of accession on the traits measured; $\beta_j$ was the effect of block on the traits measured; $\varepsilon_{ij}$ indicated the error effect of residuals.

Initially, plant height and width, and vigor rating data were tested for their normality (F test) and homogeneity using SAS software (SAS 2011; Version 9.3). Biomass, seed weight, and seed number data were not normally distributed; they were transformed using square root (Biomass) and natural log functions (seed weight and seed number). However, data are reported in their original scales for biological relevance and interpretation.

Analyses were performed using PROC MIXED model of SAS to detect accession and year by accession interaction effects, and, then, significant accession effects ($p < 0.05$) were separated using Fisher’s least-significant difference (LSD) test in the lsmeans procedure resident in SAS (SAS 2011;version 9.3). Principal component analyses (PCA) were performed using morphological data taken collectively by reducing the variable numbers (traits evaluated) to
clarify accession relationships and to identify those traits that led most to accession discrimination (Kutner et al., 2004) through PROC FACTOR in SAS (SAS 2011; version 9.3). The final PCA data were plotted in Excel 2011 (Microsoft Office Excel 2007. Inkl) to show the discriminations among accessions (Oehlert 2000).

RESULTS

Climate at Blue Creek, Utah during Experimentation

The annual temperature, relative humidity, precipitation, and solar radiation over three experimental periods (2009-2011) at Blue Creek, UT were given in Fig. 1-3-panel A, B, C, and D, respectively. The annual precipitation over three years ranged from 216 (2011) mm to 389 (2009) mm with solar radiation of 65 W/m² (2010) to 333 W/m² (2010). During the same period, the temperature over three years ranged from -8°C (2009) to 23°C (2011) with relative humidity of 27% (2010) to 85% (2010), respectively. Although the annual temperature, the relative humidity, and solar radiation were similar over three years, annual precipitation varied considerably. From May to August, the average temp. = 17 °C, average humidity = 43%, and average precip. = 109 mm, respectively (Fig. 1-3).

Morphological Trait Description

The main effects of year, accessions, and the first order interaction (accession x year) of the twelve accessions evaluated over three years are presented in Table 2-3. There were year differences for all traits (P <0.0001), and accessions x year interactions were detected for plant height, plant width, total biomass, seed weight per plant, and seed number per plant. The accessions differed for all traits except for vigor rating.
**Total Biomass**

Overall, biomass production of the accessions examined in 2011 was greater than that of either 2009 or 2010 (Table 2-3). Biomass production of all accessions over three years ranged from 10.1 g (W6 30537; *F. valesiaca*) to 241.9 g (‘Coronado’; *F. arundinacea*), where the average biomass was 91.7 g per plant (Table 2-6). The mean biomass of *F. valesiaca* accessions over three years ranged from 0.1 g (W6 30537) to 126.9 g (PI 659923), where the average across all *F. valesiaca* accessions was 59.2 g (Table 2-6). The biomass of ‘Cascade’ and the *F. valesiaca* accessions examined was similar across all years, but less than ‘Coronado’ (p < 0.05). Biomass production of ‘Manhattan’ (*Lolium perenne*) and ‘Shademaker’ (*F. rubra*) was significantly higher than ‘Cascade’ and *F. valesiaca* accessions in 2010 and 2011, respectively. The biomass production of *F. valesiaca* PI 659923 and W6 30575 in 2011 was higher than that of ‘Cascade’, which was 50% higher than their production in 2009 and 2010 (Table 2-6). Even though the biomass of ‘Coronado’ was higher in all years than all other germplasm examined (Table 2-6), its biomass progressively decreased during examination (e.g. from 241.9 g in 2009 to 115.3 g in 2011) (Table 2-6).

In 2009, a significant difference (p < 0.0001) in biomass was detected among accessions (Table 2-5), which ranged between 10.1 (W6 30537) to 241.9 (‘Coronado’) g, where average biomass per plant was 72.8 g (Fig. 2-4, Table 2-6). The average biomass production of *F. valesiaca* accessions was 50.0 g, and values ranged from 10.1 (W6 30537) to 70.7 g (W6 30595) (Table 2-6). Commercial cultivars ‘Coronado’ (tall fescue) and ‘Manhattan’ (perennial ryegrass) produced significantly more biomass than the commercial cultivar fine-leaved ‘Cascade’ and *F. valesiaca* accessions (Fig. 2-4, Table 2-6).

In 2010, the average biomass production of *Festuca* accession was 42.3 g per plant and values ranged from 0.1 g (W6 30537) to 117.5 g (Coronado) (P < 0.0001; Fig. 2-4, Table 2-5, and Table 2-6). While the biomass production of W6 30588 (73.9 g) was higher than ‘Cascade’ (36.5
g), the biomass of *F. valesiaca* PI 659923 (64.6 g) and W6 30575 (62.7 g) were also higher than ‘Cascade’, but not significantly so (Fig. 2-4, Table 2-6).

In 2011, the average biomass production of *Festuca* accessions was 84.6 g per plant, and values ranged from 14.4 (W6 30537) to 154.5 (‘Shademaster’) g (Fig. 2-4, Table 2-6). The average biomass production of the *F. valesiaca* accessions examined was 95.6 g, and values ranged from 14.4 (W6 30537) to 123.1 (W6 30575) g (Table 2-6). Biomass production in accessions *F. valesiaca* PI 659923 (126.9 g) and W6 30575 (123.1 g) were higher than ‘Cascade’ (91.23 g), but not significantly so (Fig. 2-4, Table 2-6).

**Height**

Plant height among the germplasm examined was significantly different (P < 0.0001) over years and a significant accession x year interaction was detected (Table 2-5), thus, the data are presented by year. The mean plant height of all accessions examined was 54.6 cm over two years (2010-2011), where mean values for accessions ranged from 8.1 (W6 30537) to 78.7 (‘Durar’; *F. ovina*) cm (Table 2-6). The mean height of *F. valesiaca* accessions was 54.6 cm, where plants ranged from 8.1 (W6 30537) to 65.6 cm (W6 30575) (Table 2-6). In 2010 and 2011, ‘Durar’ (mean = 72.4 cm) and ‘Coronado’ (mean = 68.6 cm) were significantly (p < 0.001) taller than ‘Cascade’ (mean = 62 cm), which was similar to the *F. valesiaca* accessions examined.

In 2010, accessions differed significantly in plant height (P < 0.0001; Table 2-5). The mean plant height was 48.4 cm, where values among accessions ranged from 8.1 (W6 30537) to 75.8 (‘Coronado’) cm (Table 2-6). The average height of *F. valesiaca* accessions was 49.3 cm, and height values ranged from 8.1 (W6 30537) to 58.0 (W6 30575) cm in this species. The mean plant height of *F. valesiaca* W6 30575, W6 30506, W6 30588, and PI 659944 was similar to ‘Cascade’ (Fig. 2-4, Table 2-6). These fine-leaved *F. valesiaca* accessions were significantly shorter than ‘Coronado’ (Fig. 2-4, Table 2-6).

In 2011, the mean plant height of all accessions was 60.8, and mean values among the
accessions examined ranged from 46.6 (W6 30537) to 75.8 (‘Durar’) cm (Table 2-6). Mean height of *F. valesiaca* accessions was 59.9 cm, and values among these accessions ranged from 46.6 (W6 30537) to 66.1 (PI 659944) cm. ‘Coronado’ and ‘Shademaster’ were taller than ‘Cascade’ and the fine-leaved *F. valesiaca* accessions evaluated (p < 0.001; Fig. 2-4, Table 2-6).

**Width**

Accessions differed significantly with regards to plant width (P < 0.001) and, thus, data are presented herein by year. Over those years (2010-2011), the plant width was average of 39.7 cm, and mean values among the germplasm examined ranged from 7.7 (W6 30537) to 61.3 (‘Shademaster’) cm (Table 2-6). The mean plant width of the *F. valesiaca* accessions over the two years was 38.6 cm, and mean values for these accessions ranged from 7.7 (W6 30537) to 52.7 (PI 659944) cm. The plant width of *F. valesiaca* accessions and ‘Cascade’ were similar (Fig. 2-4, Table 2-6).

In 2010, significant differences were detected among accessions for plant width (P < 0.0001; Table 2-5). The mean plant width of all accessions in 2010 was 32.8 cm, where values ranged from 7.7 (W6 30537) to 47.5 (‘Coronado’) cm (Table 2-6). The mean plant width of *F. valesiaca* accessions was 32.1 cm, and width values for the accessions examined ranged from 7.7 (W6 30537) to 37.7 (W6 30588) cm (Table 2-6). The width of *F. valesiaca* W6 30588, W6 30506, PI 659944, and W6 30575 plants were similar to ‘Cascade’ (Fig. 2-4, Table 2-6). Likewise, the plant width of fine-leaved *F. valesiaca* accessions examined was similar to ‘Coronado’ (Fig. 2-4, Table 2-6).

In 2011, the average plant width of all accessions was 46.6 cm, and mean values among the germplasm examined ranged from 27.0 (W6 30537) to 61.3 (‘Shademaster’) cm (Table 2-6). The width of ‘Coronado’ and ‘Shademaster’ plants were significantly wider than ‘Cascade’, the mean width of *F. valesiaca* plants was 45.1 cm, ranging from 27.0 (W6 30537) to 52.7 (PI 659944) cm (Table 2-6). While the plant width of PI 659944 (52.7 cm) was wider than ‘Cascade’ (49.3 cm),
the width of *F. valesiaca* W6 30513 (51.7 cm) and W6 30588 (47.2 cm) plants did not differ from ‘Cascade’ (Fig. 2-4, Table 2-6). Likewise, the plant width of fine-leaved *F. valesiaca* accessions was similar to that of ‘Coronado’.

**Vigor Rating**

Significant differences (p < 0.001) in plant vigor were detected among the germplasm examined as well as the year by accession interactions (Table 2-3). Vigor rating was comparatively high in 2011. The average vigor rating among accessions was 2.1, where the vigor of plants ranged from 0.4 (poor vigor; W6 30537) to 3.5 (vigorous; ‘Shademaster’) (Table 2-6). While mean vigor rating of *F. valesiaca* accessions was 2.2, mean values for these accessions ranged from 0.4 (W6 30537) to 3.1 (W6 30588). *F. valesiaca* accessions PI 659923, PI 659932, W6 30575, and W6 30588 were rated significantly higher than ‘Cascade’ over two years (2010-2011; Fig. 2-4, Table 2-6). In contrast, ‘Durar’ and ‘Shademaster’ were more vigorous than ‘Cascade’ in 2011. The relative plant vigor of ‘Coronado’ and the fine-leaved *F. valesiaca* examined were similar (Fig. 2-4, Table 2-6).

**Seed Weight**

Significant (p < 0.001) effects of year and year by accession interactions were detected for seed weight (Table 2-3). While mean seed production was similar in 2009 and 2011, production in those years was significantly higher (p < 0.001) than production in 2010 (Fig. 2-4, Table 2-6). The mean seed weight of all accessions was 8.87 g per plant, and mean values for accessions ranged from 0 (W6 30537) to 32.4 (‘Coronado’) g per plant over three years (Table 2-6). Mean seed weight of *F. valesiaca* accessions was 9.83 g per plant, where values ranged from 0.8 (W6 30537) to 22.42 (W6 30595) g per plant over three years (Table 2-6). The average seed weight of *F. valesiaca* accessions was higher than ‘Cascade’ (7.71 g per plant) during experimentation (three years). For instance, the mean seed weight of *F. valesiaca* PI 659913 (13.2 g), PI 659923
(11.39 g), PI 659932 (12.9 g), W6 30575 (16.7 g), W6 30588 (18.32 g), and W6 30595 (15.19 g) were significantly higher (p < 0.001) than ‘Cascade’ (7.71 g) (Fig. 2-4, Table 2-6). Likewise, the mean seed weight of ‘Coronado’ (19.08) and ‘Manhattan’ (10.49 g) was however significantly (p < 0.001) higher than ‘Cascade’ (Fig. 2-4, Table 2-6).

There were significant differences detected in seed production among accessions (P<0.0001; Table 2-3). In 2009, mean seed weight of all accessions taken collectively was 10.42 g, and mean accession seed weights ranged from 0.79 (W6 30537) to 32.43 (‘Coronado’) g (Table 2-6). Mean seed weight of *F. valesiaca* accessions was 8.99 g per plant, and seed weight values among accession ranged from 0.79 (W6 30537) to 15.34 (W6 30595) g. The mean seed weight of *F. valesiaca* accessions PI 659913 (13.49 g), PI 659923 (10.9 g), PI 659932 (9.84 g), W6 30563 (8.87 g), W6 30575 (14.34 g), W6 30588 (15.10 g), and W6 30595 (15.34 g) were significantly higher than ‘Cascade’ (7.87 g) (Fig. 2-4, Table 2-6). In contrast, the mean seed weight of the fine-leaved *F. valesiaca* (8.28 to 12.22 g) accessions examined had significantly higher seed weight (p < 0.001) than ‘Coronado’ and ‘Manhattan’.

In 2010, the mean seed weight of the all germplasm examined was 6.1 g per plant, where mean weight ranged from 0.0 (W6 30537) to 18.38 (W6 30588) g (Table 2-6). The mean seed weight of *F. valesiaca* accessions was 8.28 g per plant, where seed weight values ranged from 0.00 (W6 30537) to 18.38 (W6 30588) g. The mean seed weight of PI 659913 (10.9 g), PI 659923 (14.68 g), W6 30563 (10.52 g), W6 30575 (8.72 g), W6 30575 (13.4 g), W6 30588 (18.38 g), and W6 30595 (7.8 g) was significantly (p < 0.001) higher than ‘Cascade’ (2.21 g) (Fig. 2-4, Table 2-6). The mean seed weight of the fine-leaved *F. valesiaca* accessions examined (8.28 g) was similar to ‘Coronado’ (Fig. 2-4, Table 2-6).

In 2011, the mean seed weight of all accessions was 10.1 g per plant, where seed weight values among accessions ranged from 0.2 (W6 30537) to 22.4 (W6 30595) g (Table 2-6). The mean seed weight of the *F. valesiaca* accessions was 10 g per plant, and seed weight values
among accessions ranged from 0.2 (W6 30537) to 22 (W6 30595) g among accessions (Table 2-6). The mean seed weight *F. valesiaca* of PI 659913 (15.19 g), PI 659932 (18.33 g), W6 30575 (22.38 g), W6 30588 (21.49 g), and W6 30595 (22.42 g) were significantly higher than ‘Cascade’ (13.06 g) (Fig. 2-4, Table 2-6). The mean seed weight of the fine-leaved *F. valesiaca* examined was also similar to ‘Coronado’ (Fig. 2-4, Table 2-6).

**Seed Number**

Even though the mean seed number per plant among accessions examined in 2009 and 2010 was similar (10,074 to 12,780), mean weight was significantly lower (p < 0.001) than those in 2011 (15,676) (Fig. 2-4, Table 2-6). Over three years, the mean seed number per plant in all accessions examined was 12,843, where the mean seed numbers ranged from 2 (Manhattan) to 47,963 (W6 30595) (Table 2-6). The mean seed number of the *F. valesiaca* accessions examined was 17,352 over three years, and mean values ranged from 9 (W6 30537) to 47,963 (W6 30595) (Table 2-6). The mean seed number per plant of *F. valesiaca* PI 659913 (22,822), PI 659923 (20,216), PI 659932 (22,748), W6 30563 (15,341), W6 30575 (27,173), W6 30588 (33,625), and W6 30595 (29,557) was significantly (p < 0.001) higher than ‘Cascade’ over three years (Fig. 2-4, Table 2-6). Likewise, the mean seed number of ‘Coronado’ and ‘Manhattan’ was higher than ‘Cascade’ in 2009.

In 2009, 2010, and 2011, significant differences (p < 0.001) were detected for mean seed number among the accessions examined (Table 2-5). In 2009, the mean seed number of all accessions taken collectively was 12,779 per plant, where the mean seed number ranged from 1,253 (W6 30537) to 24,731 (W6 30588) (Table 2-6). The mean seed number of *F. valesiaca* PI 659913 (21,649), PI 659923 (19,255), PI 659932 (15,733), W6 30563 (16,372), W6 30575 (22,388), W6 30588 (24,731), and W6 30595 (24,263) were significantly (p < 0.001) higher than ‘Cascade’ (6,905) (Fig. 2-4, Table 2-6). The mean seed number of *F. valesiaca* accessions examined was similar to ‘Coronado’.
In 2010, the mean seed number of all accessions taken collectively was 10,073 per plant, and mean seed number among accessions ranged from 2 (W6 30537) to 33,606 (W6 30588) (Table 2-6). The mean seed number of *F. valesiaca* PI 659913 (18,474), PI 659923 (27,222), PI 659932 (20,204), W6 30563 (16,744), W6 30575 (16,865), W6 30588 (33,606), and W6 30595 (16,445) was significantly (p < 0.001) higher than ‘Cascade’ (3,212) (Fig. 2-4, Table 2-6). The mean number of seeds produced by the *F. valesiaca* accessions examined was significantly (p < 0.001) higher than ‘Coronado’.

In 2011, the mean seed number of all accessions taken collectively was 10,073, and mean seed number among accessions ranged from 67 (W6 30537) to 47,962 (W6 30595) (Table 2-6). Compared with the control ‘Cascade’, accessions of the mean seed number of *F. valesiaca* PI 659913 (28,344), PI 659923 (14,171), PI 659932 (32,308), W6 30563 (12,906), W6 30575 (42,276), W6 30588 (42,539), and W6 30595 (47,963) was significantly (p < 0.001) higher than ‘Cascade’ (12,875) (Fig. 2-4, Table 2-6). The mean number of seeds produced by the *F. valesiaca* accessions examined was significantly (p < 0.001) higher than ‘Coronado’.

**Principal Component Analyses**

**Principal Component Analysis of 2009 Data**

Trait values for some of species examined were comparatively high (Table 2-6). The outcome of principal component analysis is dramatically affected disproportionate values of loading factors. Therefore, in order to obtain realistic appraisal of the relative genetic relationships among the fine-leaved fescue species examined data from ‘Manhattan’ (*Lolium perenne*), ‘Coronado’ (*F. arundinacea*), and W6 30537 (*F. valesiaca*) were not used in PCA. The average values of three traits from 17 accessions measured in 2009 were subjected to a principal component analysis (Fig. 2-5-Panel A). The PCA loading plot (Fig. 2-5-Panel A) displayed the relationships of three traits (total biomass, seed weight, and seed number) among 17 accessions at the same time which...
indicated similar information were contributed and clustered together with correlations.

The first two components explained largest portions of the observed variation (97%) and, thus, they were retained for graphic rotation of accessions to depict species relationships (Hatcher and Stepanski 1994). *Festuca valesiaca* accessions PI 659913, PI 659932, W6 30575, W6 30588, and W6 30595 were positioned in Quadrant IV after PCA and possessed similar and/or comparatively high values for the biomass, seed weight, and seed number, but lower height and width values examined (Table 2-6). PI 659944 (*F. valesiaca*) and ‘Scaldis’ (*F. trachyphylla*), and ‘Cascade’ (*F. rubra*) were positioned in the Quadrant III and possessed similar and moderate values for all traits examined. The commercial cultivar ‘Shademaster’ (*F. rubra*) contributes unique (high) values for the six traits used as loading factors examined and was positioned in quadrant I after principal component analysis.

**Principal Component Analysis of 2010 Data**

The average values of six traits (plant height, plant width, vigor rating, total biomass, seed weight, and seed number) from 17 accessions measured in 2010 were subjected to PCA (Fig. 2-5-Panel B). The first two components explained large portions of the observed variation (90%) and, thus, were retained for varimax rotation to elucidate accession relationships. *Festuca valesiaca* accessions PI 659944 and W6 30506 were positioned in Quadrant I due to their comparatively high biomass, vigor rating, seed weight, and seed number (Table 2-6). Likewise, *F. valesiaca* accessions W6 30563, W6 30575, W6 30588, W6 30595, PI 659913, PI 659923, and PI 659932 were positioned in Quadrant IV after PCA because of their comparatively similar (high) values for vigor rating, biomass, seed weight, and seed number. Commercial cultivar ‘Cascade’ (*F. rubra*) was placed into Quadrant II based on moderate values for the traits examined. The remaining accessions were positioned Quadrant III after PCA because of their comparatively low trait values.
Principal Component Analysis of 2011 Data

The average values for six traits of 17 accessions measured in 2011 were subjected to PCA (Fig. 2-5-Panel C). The first two components explained large portions of the observed variation (86%) and, thus, they were used to determine accession relationships. *Festuca valesiaca* accessions W6 30595, W6 30588, and W6 30575 were positioned in Quadrant I because of comparatively higher values for vigor rating, biomass, seed weight, and seed number examined in 2011. Accessions PI 659944 and W6 30563 along with ‘Cascade’, ‘Shademaster’ and ‘Durar’ were positioned in Quadrant IV due to comparatively high values for vigor rating, seed weight, and seed number. The commercial cultivars ‘Scaldis’ (*F. trachyphylla*) and ‘Black sheep’ (*F. rubra*) were positioned into Quadrant III after PCA because their similar and moderate trait values.

Principal Component Analysis of Combined 2009-2011 Data

Average values of six traits from 2009 to 2011 were used in PCA to characterize genetic relationships among the array of 17 *Festuca* accessions (Fig. 2-6). The first two components explained large portions of the observed variation (88%) and allows for a determination of accession relationships. Commercial cultivars ‘Dawson E’ (*F. rubra*) and ‘Scaldis’ (*F. trachyphylla*) contributed comparatively low values of all traits examined in each of the three years of observation and were positioned in Quadrant III. Similarly, commercial cultivars ‘Shademaster’ (*F. rubra*), ‘Durar’ (*F. ovina*) and ‘Cascade’ contributed similar and comparatively high values of all traits examined over three years and were positioned in Quadrant I. *F. valesiaca* accessions PI 659923, PI 659913, W6 30563, W6 30575, PI 659932, and W6 30588 were positioned in Quadrant IV after PCA since they contributed similar and moderate values for all of the traits evaluated.
DISCUSSION

Breeding of *Festuca valesiaca*

Low-maintenance turfgrass refers to use of grass in reduced fertilizer, irrigation, pesticide, and mowing (McKernan et al. 2001). Examples of cultivars that may have applicably in reduced input application are ‘Fairway’, ‘Ruff’, and ‘RoadCrest’ (Asay et al. 1999). Fine-leaved fescue species (*Festuca ovina* and *Festuca rubra*) have been considered for use as a low-maintenance turfgrass because of their salinity and drought tolerance (Diesburg et al. 1997; Meyer and Pedersen 1999). However, fine fescue in combination with other species as mixture (Meyer and Pedersen 1999) has succeeded its use as a candidate in turfgrass a mixtures. Similarly, sheep fescue (*Festuca ovina*) may have potential for use as a turfgrass on low-input golf course fairways (Watkins et al. 2010).

Suitable parental plants for plant improvement should be selected based on phenotypic characteristics relating to agronomic performance (e.g., biomass, seed production, and vigor) (Amini et al. 2011). The performance of *F. valesiaca* accessions PI 659923, W6 30575, and W6 30588 was consistent (over years) and comparatively high (with respect to ‘Cascade’), and, thus, should receive further consideration for inclusion into breeding programs whose goals include the development of low-maintenance turf and rangeland grasses in the western U.S.. Although leaf color is influenced by growing environment, it is genetically determined and coloration can be genetically manipulated (Sărățeanu and Moisuc 2009). The *F. valesiaca* accessions examined herein possess green to bluish-green coloration which can be genetically manipulated for specific applications. For instance, blue to bluish-green coloration may be attractive for ornamental usage and as unique color of other urban landscapes (e.g., golf courses) under arid growing conditions. Likewise, abiototic stress tolerant *F. valesiaca* accessions which originated from over grazed areas in Asian may have potential as rangeland grasses in the western U.S. Therefore, these
Morphological Traits in *Festuca* Species

Plant height and biomass are the traits that enhance competitive ability and, thus, determine vegetation structure in grasslands (Noy-Meir 1995). Likewise, in tropical Africa, *Festuca* species provide relatively high biomass as forage grasses for support of wild and domesticated animals (Namaganda et al. 2006). Moreover, they act to control soil erosion in tropical mountainous regions. The wide-leafed tall fescue (*Festuca arundinacea*) commercial cultivar ‘Coronado’ performed better than all other accessions and cultivars in an arid environment in the western U.S. (Fig. 2–4). However, its biomass production decreased from 241.9 g in 2009 to 115.3 g in 2011. This was not the case for many of the fine-leafed *F. valesiaca* accessions examined herein (Table 2–6). Moreover, preliminary data from short-term (45 days) heat stress experiments in a greenhouse (40°C day/32 °C night) suggests that *F. valesiaca* accessions W6 30588 and W6 30595 possess higher heat stress and drought tolerance than ‘Durar’ and ‘Coronado’ (USDA-ARS, Forage and Range Research Laboratory, unpublished). Given the persistence and putative heat tolerance of these *F. valesiaca* accessions, they should be considered for further physiological and genetic evaluation in breeding programs.

Drought, low humidity, and cold temperature are factors that cause plants stress. The climate in Blue Creek, Utah where the research project was carried out is a harsh during May to August (average temp. = 17 °C, average humidity = 43%, average precip. = 109 mm; Fig. 2-3) environment which is stressful to the plants. Therefore, the plants that survive and thrive under these environmental conditions should be considered for low-maintenance turf and rangeland application. The *F. valesiaca* accessions PI 659913, PI 659923, PI 659932, W6 30575, W6 30588, and W6 30595 were found to be better than the commercial control ‘Cascade’ for seed production.
and biomass which are extremely important considerations for the seed industry.

The *Festuca valesiaca* accessions examined also did not exhibit winter injury symptoms during the years evaluated. In contrast, the biomass production of the tall fescue (‘Coronado’) decreased dramatically over during this period which might due to winter injury and no irrigation. Assuming that have harvesting is similar to mowing, harvesting two times of mowing was carried out each year and it was suggested that two times each year under the stressful environment experimented at Blue Creek Utah may be indicated of their potential value for low mowing frequency. Also, two times of mowing is considered as acceptable low-maintenance turf (Meyer and Pedersen 1999).

**Plant Height in *Festuca* Species**

Plant height was considered as a trait for evaluation of mowing frequency in low-maintenance turfgrass applications (McKernan et al. 2001). Plant height in this research was measured during flowering, which is appreciably higher than plant height in advanced vegetative stages (pre-flowering), however, the plant height of all the *Festuca valesiaca* were lower than commercial control ‘Cascade’, which is indicative of its comparatively less mowing frequency under turfgrass application. The overall plant height of the *F. valesiaca* accessions examined was lower than ‘Cascade’ (Fig. 2-4; Table 2-6). Grass leaves begin expanding horizontally rather than vertically after certain height is reached, and, thus, initial plant height alone is not very indicative of eventual plant canopy (Payero et al. 2004). So, it is recommended that plant height should be measured at the pre-flowering stage for turf applications. Plant height, however, is also associated with plant competitiveness under low-maintenance applications (i.e., road side and re-vegetation on rangelands). Although plant height was measured at the flowering stage, the *Festuca species* were still slower in stature when compared the commercial ‘Cascade’. Making this criterion is still an indicator for competitiveness at flowering stage. Thus, since the *Festuca valesiaca* accessions examined were not as tall as ‘Cascade’, they should be considered further for their
applications as low-maintenance turf grass.

**Plant Width in *Festuca* Species**

Percent ground cover of perennial grasses is an important characteristic to land managers as a measure of vigor, competitive ability, and productivity on rangelands (Afolayan 1979; Grime 1977; Riney 1963). Plant width is another way of demonstrating grass ground cover which is associated with the plant width. Thus, rhizomatous and bunch grasses that possess considerable plant width are sought after for their competitive ability (i.e., spread) especially under harsh arid conditions. The *F. valesiaca* accessions examined in this study are intended for use as a low-maintenance turfgrass or in rangeland settings where stand establishment and persistence care necessary for reclamation under arid conditions. The plant width of all of the *F. valesiaca* accessions evaluated was as wide as ‘Cascade’ (Table 2-6, Fig. 2-4), and thus, from this standpoint has potential in low-maintenance plant improvement programs. Since the significant interactions between year and accessions were detected, growing environment (year effects) is an important factor affecting plant growth and persistence under arid conditions.

**Vigor Rating in *Festuca* Species**

Under minimum management, aesthetic visual attributes (i.e. greenness) and plaut uniformity are essential characteristics of low-maintenance turfgrasses (Diesburg et. al. 1997; Wang and Zhang 2011). Substantial plant vigor in the early spring (i.e., transition from winter dormancy) is desirable in rangeland and turf settings (Bertin et al. 2009). A quality rating of 6 or above is generally considered acceptable during NTEP evaluation [1 = poorest (straw color) or dead and 9 = vigorous and green without biotic or abiotic stress damage; NTEP, 2008]. The visual rating (color and biomass) showed that these accessions of PI 659923, PI 659932, W6 30575, and W6 30588 were rated significantly (P<0.001) higher (i.e., more intense deeper green coloration) than the commercial control ‘Cascade’, which is indicative of their potential for low-maintenance turf
or range applications. These accessions should be further considered for incorporation into plant improvements, where spring green-up and vigor in the arid environments are important objectives. Spring vigor ratings were consistent over years and mean vigor rating (2010 and 2011) was correlated with plant height ($P = 0.01; r^2 = 0.70$), total biomass ($P = 0.001; r^2 = 0.80$) (Table 2-1). Thus, use plant vigor as a selection criterion during breeding may allow for the concomitant way.

**Seed Production in *Festuca* Species**

Seed production is an important component of commercial grass cultivars marketability (Fang et al. 2004). However, seed yield is a complex trait and is dependent on species, agricultural practices, environment, and their interaction with growing environment (Elgersma 1990; Elgersma and van Wijk 1997; Fang et al. 2004). Both seed number and seed weight are important components of seed quality at harvest (Chastain et al. 2011). Other agronomic character, such as plant height, leaf area, dry-matter yield, flowering date, lodging resistance and proneness to seed shattering (Griffiths 1965), 1000-seed weight, and number of florets per panicle (Fang et al. 2004) also influence seed yield. Moreover, seed yield of rhizomatous cool-season perennial grass species declines as stands age (CaCluster and Law 1975; Chastain et al. 2011).

The seed production of the *F. rubra* accessions ‘Shademaster’ and ‘Dawson E’ examined herein declined over years (Table 2-6; Fig. 2-4) even though the performance of the other traits examined was relatively high. Thus, it is important to understand the correlates responses to selection of seed yield traits and other economically important traits when attempting to improve seed yield in grasses (Fang et al. 2004). For instance, fertile tiller number (Griffiths 1965; Hill and Watkin 1975; Lewis 1966) and panicle fertility (Fang et al. 2004) are major factor contributing to seed yield. Variation in flowering date in open-pollinated grasses can lead to progeny with reduced fertility and seed quality, and enhanced seed shattering (Fang et al. 2004; Griffiths 1965). Chastain et al. (2011), in fact, suggested that improvement of panicle characteristics could be important for increasing seed weight in strong creeping red fescue (*F.*
rubra). Likewise, the number of panicles bearing tillers, size of panicles, the number of fertile florets (Makela and Kousa 2009), and seed weight per panicle (Bruno et al. 2008) are considered important characteristics that can increase seed yield in meadow fescue and perennial ryegrass.

There are positive correlations between reproductive and vegetative traits in grass species (Dujardin et al. 2011). For instance, in meadow fescue plants, reduced seed yields and lighter seed weights are associated with taller plants with relatively early heading dates (Fang et al. 2004). In this research, seed production (i.e., seed weight) was significantly correlated with plant total biomass ($P = 0.05; r^2 = 0.56$), and seed number ($P = 0.001; r^2 = 0.88$) in 2009 (Table 2-7). In 2010, seed production was significantly correlated with total biomass ($P = 0.001; r^2 = 0.84$), height ($P = 0.05; r^2 = 0.58$), vigor rating ($P = 0.001; r^2 = 0.83$), and seed number ($P = 0.001; r^2 = 0.98$) (Table 2-8). Given these associations, seed production could be assessed and selected by evaluating less time-consuming traits, thereby increasing breeding efficiency.

As observed in this research, maturity date is associated with flowering date (preliminary data, not presented). Thus, relative maturity of these $F. valesiaca$ accessions should be considered during breeding for making crosses. The maturity date of the grass species studied herein was 51 days (i.e., middle of June) after first date of harvesting which started in late June (June 23, 2009) and ending in early August (Aug 5, 2010) depending on species and growing environment (year). Compare to the other commercial cultivars, $F. valesiaca$ accessions are relatively early flowering (data not shown) and early maturing species having comparatively high seed production.

**Interpretation of Principal Component Analyses**

Principal component analysis is a variable reduction procedure that allows for the identification of a relatively small number of components that account for a certain amount of the variance that is associated with in a set of observed variables (e.g., biomass, seed weight, seed number) (Hatcher and Stepanski 1994). The first component extracted in a PCA accounts for
explanation of the maximum amount of total variance associated with the variables used (Hatcher and Stepanski 1994). The remaining components account for a lesser portion of the observed variation, each of which is not correlated with the preceding components. The first and second principal component explained 73% / 24%, 70% / 20%, and 54% / 32% of the observed variation in 2009, 2010, and 2011, respectively. Thus, the first two principal components explained a substantial proportion of the variance associated with the data and were used to graphically display accessions according to their morphological similarities (Fig. 2-5 and Fig. 2-6). Over three years, *F. valesiaca* accessions PI 659913, PI 659932, W6 30575, W6 30588, and W6 30595 (Quadrant IV) clustered together based on higher value on the traits (Fig. 2-6). So, these accessions had similar performance with relatively on higher value on the traits (i.e., total biomass, seed weight, and seed number). These accessions, therefore, have potential for the breeding of germplasm having relatively high biomass and seed production. In contrast, ‘Dawson E’ (*F. rubra*) and ‘Scaldis’ (*F. trachyphylla*) showed morphological similarities over three years in Quadrant III (Fig. 2-6) and did not perform as well as the other accessions evaluated. Similarly, ‘Shademaster’ (*F. rubra*), and ‘Durar’ (*F. ovina*) possessed morphological similarities (Quadrant I) and performed well for the traits evaluated.

**CONCLUSIONS**

In conclusion, *F. valesiaca* species produce relative large quantities of small seeds. Seed production trait was significantly correlated with the total biomass, plant height, and plant vigor rating. Although *F. valesiaca* species were smaller than the control ‘Cascade’ but they possessed comparatively higher biomass, spring green-up, and seed production. These differences were also demonstrated after PCA. Given their morphological attributes, *F. valesiaca* accessions PI 659923, W6 30575, and W6 30588 should be considered for low-maintenance applications and use in plant improvement. This project is also a scientific report on the values of the *Festuca valesiaca*
collections in Kyrgyzstan.

REFERENCES

Afolayan TA (1979) Change in percentage ground cover of perennial grasses under different burning regimes. Vegetatio 39(1):35-41


of dominant plant species to local conditions in herbaceous successional stages of a
calcereous hillside. Flora 206: 1030-1039

Diesburg KL, Christians NE, Moore R, Branham B, Banneberger TK, Reicher ZJ, Voight T,
Midwest. Agron J 89: 690-694

Elgersma A (1990) Genetic variation for seed yield in perennial ryegrass (Lolium perenne L.).
Plant Breed 105:117-125

Elgersma A, van Wijk A (1997) Breeding for higher seed yield in grasses and forage legumes. In:
Fairey DT, Hampton JG (eds) Forage seed production. Temperate species. CAB International,
Oxon, pp 243-272

Albany, New York, pp 1-58

production traits within a full-sib family of meadow fescue. Plant Breed 123: 241-246

125(1):1-15

Fırıncıoğlu HK, Seefeldt SS, Sahin B (2007) The Effects of Long-Term Grazing Exclusions on
Range Plants in the Central Anatolian Region of Turkey. Environ Manage 39: 326-337

Fırıncıoğlu HK, Seefeldt SS, Sahin B, Vural M (2009) Assessment of grazing effect on sheep
fescue (Festuca valesiaca) dominated steppe rangelands, in the semi-arid Central Anatolian
region of Turkey. Journal of Arid Environments 73: 1149-1157

Fırıncıoğlu HK, Adıgüzel N, Bani B, Sahin B (2010) Assessment of Grazing Effect on Two Sub-
Shrubs (Astragalus schottianus and Thymus sipyleus) Dominated Mountain Bozoglan
Grasslands in the Semi-Arid Central-Southern Anatolian Region of Turkey. Arid Land
Research and Management 24:282-300
Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance
to ecological and evolutionary theory. Am Nat 111:1169-1194
Griffiths DJ (1965) Breeding for higher seed yields from herbage varieties. J Nat Inst Agric Bot
10: 320-331
Habibulla IA, Fet NG, Fet V, Valdez R, Feldman RW (1999) Biodiversity, Genetic Diversity, and
Protected Areas in Turkmenistan, Journal of Sustainable Forestry 9(1-2): 73-88
Hill MJ, Watkin BR (1975) Seed production studies on perennial ryegrass, timothy and prairie
grass: Effect of tiller age on tiller survival, ear emergence and seed head components. J. Br.
Grassld Soc. 30: 63-71
Hroudová-Pučelíková ZA (1972) Comparative Study of the Ecology of Festuca valesiaca Gaudin
and Festuca rupicola Heuff. Folia Geobotanica & Phytotaxonomica 7(1): 53-79
Johnson GA, Davis JG, Qian YL, Doesken KC (2006) Topdressing turf with composted manure
212
362
Lewis J (1966) The relationship between seed yield and associated characters in meadow fescue
Makela P, Kousa M (2009) Seed production of two meadow fescue cultivars differing in growth
habit. Agricultural and food science 18: 91-99


Riney T (1963) A rapid field technique and its application in describing conservation status and trends in semi-arid pastoral areas. African Soils 8: 159-258


2-1 Probability values of type III error tests of fixed effects in a split plot in time design of plant traits used to assess *Festuca* species examined over multiple years (2009-2011).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
<td>Height</td>
<td>Width</td>
<td>Vigor rating</td>
<td>Total biomass</td>
<td>Seed weight</td>
<td>Seed number</td>
</tr>
<tr>
<td>Entry</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Year</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.4982</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Entry*Year</td>
<td>&lt;.0001</td>
<td>0.0127</td>
<td>0.0143</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
2-2 Probability values of type III error tests of fixed effects (accession) evaluated using a randomized block design for plant traits of *Festuca* species accessions examined over multiple years (2009-2011) at Blue Creek, Utah.

<table>
<thead>
<tr>
<th>Year</th>
<th>Height</th>
<th>Width</th>
<th>Vigor rating</th>
<th>Total biomass</th>
<th>Seed weight</th>
<th>Seed number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>2010</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>2011</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
2-3 Mean values of phenotypic traits of *Festuca* species accessions evaluated over three years at Blue Creek, Utah.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Species</th>
<th>Total biomass (g)</th>
<th>Height (cm)</th>
<th>Width (cm)</th>
<th>Vigor rating (0-5)</th>
<th>Seed weight (g)</th>
<th>Seed No</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronado</td>
<td><em>F. arundinacea</em></td>
<td>241.89</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32.43</td>
<td>18,352</td>
</tr>
<tr>
<td>Durar</td>
<td><em>F. ovina</em></td>
<td>85.96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.26</td>
<td>9,232</td>
</tr>
<tr>
<td>Black Sheep</td>
<td><em>F. ovina</em></td>
<td>74.55</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.23</td>
<td>10,084</td>
</tr>
<tr>
<td>Cascade</td>
<td><em>F. rubra</em></td>
<td>59.89</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.87</td>
<td>6,905</td>
</tr>
<tr>
<td>Dawson E</td>
<td><em>F. rubra</em></td>
<td>73.78</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.95</td>
<td>8,546</td>
</tr>
<tr>
<td>Shademaster</td>
<td><em>F. rubra</em></td>
<td>66.31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.14</td>
<td>4,454</td>
</tr>
<tr>
<td>Scaldis</td>
<td><em>F. trachyphylla</em></td>
<td>49.87</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.15</td>
<td>2,182</td>
</tr>
<tr>
<td>Manhattan</td>
<td><em>L. perenne</em></td>
<td>204.78</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27.48</td>
<td>18,234</td>
</tr>
<tr>
<td>PI 659913</td>
<td><em>F. valesiaca</em></td>
<td>58.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.49</td>
<td>21,694</td>
</tr>
<tr>
<td>W6 30438</td>
<td><em>F. valesiaca</em></td>
<td>43.97</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.68</td>
<td>11,985</td>
</tr>
<tr>
<td>PI 659923</td>
<td><em>F. valesiaca</em></td>
<td>49.83</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.84</td>
<td>15,733</td>
</tr>
<tr>
<td>PI 659932</td>
<td><em>F. valesiaca</em></td>
<td>43.53</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.09</td>
<td>9,601</td>
</tr>
<tr>
<td>W6 30506</td>
<td><em>F. valesiaca</em></td>
<td>43.97</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.28</td>
<td>5,807</td>
</tr>
<tr>
<td>W6 30513</td>
<td><em>F. valesiaca</em></td>
<td>49.83</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.84</td>
<td>15,733</td>
</tr>
<tr>
<td>W6 30537</td>
<td><em>F. valesiaca</em></td>
<td>43.97</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.28</td>
<td>5,807</td>
</tr>
<tr>
<td>W6 30558</td>
<td><em>F. valesiaca</em></td>
<td>49.83</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.84</td>
<td>15,733</td>
</tr>
<tr>
<td>W6 30595</td>
<td><em>F. valesiaca</em></td>
<td>43.97</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.28</td>
<td>5,807</td>
</tr>
<tr>
<td>F. valesiaca mean</td>
<td></td>
<td>49.96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.99</td>
<td>14,800</td>
</tr>
<tr>
<td>Grand mean</td>
<td></td>
<td>72.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.42</td>
<td>12,780</td>
</tr>
<tr>
<td>LSD (P&lt;0.05)</td>
<td></td>
<td>24.76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.27</td>
<td>6,094</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>15%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>34%</td>
<td>62%</td>
</tr>
</tbody>
</table>

2010

<table>
<thead>
<tr>
<th>Accession</th>
<th>Species</th>
<th>Total biomass (g)</th>
<th>Height (cm)</th>
<th>Width (cm)</th>
<th>Vigor rating (0-5)</th>
<th>Seed weight (g)</th>
<th>Seed No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronado</td>
<td><em>F. arundinacea</em></td>
<td>117.53</td>
<td>75.8</td>
<td>47.5</td>
<td>2.3</td>
<td>12.09</td>
<td>6,031</td>
</tr>
<tr>
<td>Durar</td>
<td><em>F. ovina</em></td>
<td>45.60</td>
<td>66.1</td>
<td>42.2</td>
<td>2.5</td>
<td>3.74</td>
<td>5,807</td>
</tr>
<tr>
<td>Black Sheep</td>
<td><em>F. ovina</em></td>
<td>33.63</td>
<td>49.1</td>
<td>37.5</td>
<td>2.3</td>
<td>1.28</td>
<td>1,935</td>
</tr>
<tr>
<td>Cascade</td>
<td><em>F. rubra</em></td>
<td>36.47</td>
<td>54.5</td>
<td>36.6</td>
<td>1.6</td>
<td>2.21</td>
<td>3,212</td>
</tr>
<tr>
<td>Dawson E</td>
<td><em>F. rubra</em></td>
<td>14.31</td>
<td>29.6</td>
<td>27.8</td>
<td>0.9</td>
<td>0.07</td>
<td>90</td>
</tr>
<tr>
<td>Shademaster</td>
<td><em>F. rubra</em></td>
<td>58.54</td>
<td>52.4</td>
<td>40.7</td>
<td>1.7</td>
<td>3.51</td>
<td>3,484</td>
</tr>
<tr>
<td>Scaldis</td>
<td><em>F. trachyphylla</em></td>
<td>24.79</td>
<td>34.3</td>
<td>27.6</td>
<td>1.5</td>
<td>0.20</td>
<td>360</td>
</tr>
<tr>
<td>Manhattan</td>
<td><em>L. perenne</em></td>
<td>9.14</td>
<td>14.7</td>
<td>11.1</td>
<td>0.3</td>
<td>0.01</td>
<td>2</td>
</tr>
<tr>
<td>PI 659913</td>
<td><em>F. valesiaca</em></td>
<td>41.73</td>
<td>48.5</td>
<td>31.3</td>
<td>2.2</td>
<td>10.90</td>
<td>18,474</td>
</tr>
<tr>
<td>W6 30438</td>
<td><em>F. valesiaca</em></td>
<td>19.94</td>
<td>50.1</td>
<td>33.9</td>
<td>2.1</td>
<td>1.86</td>
<td>4,443</td>
</tr>
<tr>
<td>PI 659923</td>
<td><em>F. valesiaca</em></td>
<td>64.57</td>
<td>51.7</td>
<td>34.5</td>
<td>2.8</td>
<td>14.68</td>
<td>27,222</td>
</tr>
<tr>
<td>PI 659932</td>
<td><em>F. valesiaca</em></td>
<td>44.77</td>
<td>51.7</td>
<td>30.1</td>
<td>2.6</td>
<td>10.52</td>
<td>20,204</td>
</tr>
<tr>
<td>W6 30506</td>
<td><em>F. valesiaca</em></td>
<td>34.81</td>
<td>56.3</td>
<td>36.1</td>
<td>2.5</td>
<td>6.47</td>
<td>15,104</td>
</tr>
<tr>
<td>W6 30513</td>
<td><em>F. valesiaca</em></td>
<td>29.65</td>
<td>52.2</td>
<td>35.4</td>
<td>2.2</td>
<td>3.02</td>
<td>6,547</td>
</tr>
<tr>
<td>W6 30537</td>
<td><em>F. valesiaca</em></td>
<td>0.11</td>
<td>8.1</td>
<td>7.7</td>
<td>0.5</td>
<td>0.00</td>
<td>9</td>
</tr>
<tr>
<td>PI 659944</td>
<td><em>F. valesiaca</em></td>
<td>42.72</td>
<td>55.0</td>
<td>36.3</td>
<td>2.5</td>
<td>3.66</td>
<td>4,902</td>
</tr>
<tr>
<td>W6 30563</td>
<td><em>F. valesiaca</em></td>
<td>44.95</td>
<td>52.8</td>
<td>35.5</td>
<td>2.5</td>
<td>8.72</td>
<td>16,744</td>
</tr>
<tr>
<td>Accession</td>
<td>Species</td>
<td>Total biomass (g)</td>
<td>Height (cm)</td>
<td>Width (cm)</td>
<td>Vigor rating (0-5)</td>
<td>Seed weight (g)</td>
<td>Seed No</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
<td>------------------</td>
<td>-------------</td>
<td>------------</td>
<td>-------------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>W6 30575</td>
<td><em>F. valesiaca</em></td>
<td>62.65</td>
<td>58.0</td>
<td>35.7</td>
<td>2.7</td>
<td>13.40</td>
<td>16,856</td>
</tr>
<tr>
<td>W6 30588</td>
<td><em>F. valesiaca</em></td>
<td>73.88</td>
<td>56.1</td>
<td>37.7</td>
<td>3.1</td>
<td>18.38</td>
<td>33,606</td>
</tr>
<tr>
<td>W6 30595</td>
<td><em>F. valesiaca</em></td>
<td>45.37</td>
<td>51.8</td>
<td>30.9</td>
<td>2.3</td>
<td>7.80</td>
<td>16,445</td>
</tr>
<tr>
<td></td>
<td><em>F. valesiaca</em> mean</td>
<td>42.09</td>
<td>49.3</td>
<td>32.1</td>
<td>2.3</td>
<td>8.28</td>
<td>15,046</td>
</tr>
<tr>
<td></td>
<td>Grand mean</td>
<td>42.26</td>
<td>48.4</td>
<td>32.8</td>
<td>2.1</td>
<td>6.12</td>
<td>10,074</td>
</tr>
<tr>
<td></td>
<td>LSD (P&lt;0.05)</td>
<td>18.36</td>
<td>10.2</td>
<td>7.4</td>
<td>0.6</td>
<td>4.47</td>
<td>9,805</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>67%</td>
<td>21%</td>
<td>31%</td>
<td>40%</td>
<td>59%</td>
<td>59%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Accession</th>
<th>Species</th>
<th>Height (cm)</th>
<th>Width (cm)</th>
<th>Vigor rating (0-5)</th>
<th>Seed weight (g)</th>
<th>Seed No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronado</td>
<td><em>F. arundinacea</em></td>
<td>117.53</td>
<td>75.8</td>
<td>47.5</td>
<td>2.3</td>
<td>12.09</td>
</tr>
<tr>
<td>Durar</td>
<td><em>F. ovina</em></td>
<td>45.60</td>
<td>66.1</td>
<td>42.2</td>
<td>2.5</td>
<td>3.74</td>
</tr>
<tr>
<td>Black Sheep</td>
<td><em>F. ovina</em></td>
<td>33.63</td>
<td>49.1</td>
<td>37.5</td>
<td>2.3</td>
<td>1.28</td>
</tr>
<tr>
<td>Cascade</td>
<td><em>F. rubra</em></td>
<td>36.47</td>
<td>54.5</td>
<td>36.6</td>
<td>1.6</td>
<td>2.21</td>
</tr>
<tr>
<td>Dawson E</td>
<td><em>F. rubra</em></td>
<td>14.31</td>
<td>29.6</td>
<td>27.8</td>
<td>0.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Shademaster</td>
<td><em>F. rubra</em></td>
<td>58.54</td>
<td>52.4</td>
<td>40.7</td>
<td>1.7</td>
<td>3.51</td>
</tr>
<tr>
<td>Scaldis</td>
<td><em>F. trachyphylla</em></td>
<td>24.79</td>
<td>34.3</td>
<td>27.6</td>
<td>1.5</td>
<td>0.20</td>
</tr>
<tr>
<td>Manhattan</td>
<td><em>L. perenne</em></td>
<td>9.14</td>
<td>14.7</td>
<td>11.1</td>
<td>0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>PI 659913</td>
<td><em>F. valesiaca</em></td>
<td>41.73</td>
<td>48.5</td>
<td>31.3</td>
<td>2.2</td>
<td>10.90</td>
</tr>
<tr>
<td>W6 30438</td>
<td><em>F. valesiaca</em></td>
<td>19.94</td>
<td>50.1</td>
<td>33.9</td>
<td>2.1</td>
<td>1.86</td>
</tr>
<tr>
<td>PI 659923</td>
<td><em>F. valesiaca</em></td>
<td>64.57</td>
<td>51.7</td>
<td>34.5</td>
<td>2.8</td>
<td>14.68</td>
</tr>
<tr>
<td>PI 659932</td>
<td><em>F. valesiaca</em></td>
<td>44.77</td>
<td>51.7</td>
<td>30.1</td>
<td>2.6</td>
<td>10.52</td>
</tr>
<tr>
<td>W6 30506</td>
<td><em>F. valesiaca</em></td>
<td>34.81</td>
<td>56.3</td>
<td>36.1</td>
<td>2.5</td>
<td>6.47</td>
</tr>
<tr>
<td>W6 30513</td>
<td><em>F. valesiaca</em></td>
<td>29.65</td>
<td>52.2</td>
<td>35.4</td>
<td>2.2</td>
<td>3.02</td>
</tr>
<tr>
<td>W6 30537</td>
<td><em>F. valesiaca</em></td>
<td>0.11</td>
<td>8.1</td>
<td>7.7</td>
<td>0.5</td>
<td>0.00</td>
</tr>
<tr>
<td>PI 659944</td>
<td><em>F. valesiaca</em></td>
<td>42.72</td>
<td>55.0</td>
<td>36.3</td>
<td>2.5</td>
<td>3.66</td>
</tr>
<tr>
<td>W6 30563</td>
<td><em>F. valesiaca</em></td>
<td>44.95</td>
<td>52.8</td>
<td>35.5</td>
<td>2.5</td>
<td>8.72</td>
</tr>
<tr>
<td>W6 30575</td>
<td><em>F. valesiaca</em></td>
<td>62.65</td>
<td>58.0</td>
<td>35.7</td>
<td>2.7</td>
<td>13.40</td>
</tr>
<tr>
<td>W6 30588</td>
<td><em>F. valesiaca</em></td>
<td>73.88</td>
<td>56.1</td>
<td>37.7</td>
<td>3.1</td>
<td>18.38</td>
</tr>
<tr>
<td>W6 30595</td>
<td><em>F. valesiaca</em></td>
<td>45.37</td>
<td>51.8</td>
<td>30.9</td>
<td>2.3</td>
<td>7.80</td>
</tr>
<tr>
<td></td>
<td><em>F. valesiaca</em> mean</td>
<td>42.09</td>
<td>49.3</td>
<td>32.1</td>
<td>2.3</td>
<td>8.28</td>
</tr>
<tr>
<td></td>
<td>Grand mean</td>
<td>42.26</td>
<td>48.4</td>
<td>32.8</td>
<td>2.1</td>
<td>6.12</td>
</tr>
<tr>
<td></td>
<td>LSD (P&lt;0.05)</td>
<td>18.36</td>
<td>10.2</td>
<td>7.4</td>
<td>0.6</td>
<td>4.47</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>67%</td>
<td>21%</td>
<td>31%</td>
<td>40%</td>
<td>59%</td>
</tr>
</tbody>
</table>

PI and W6 = Plant introduction (PI) number and pre-PI designation, especially, according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN).
2-4 Pearson correlation coefficients ($r^2$) with associated significance (P) value as superscript between traits of *Festuca* species accessions evaluated in randomized block design in 2009.

<table>
<thead>
<tr>
<th></th>
<th>Seed weight</th>
<th>Seed number</th>
<th>Total biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed weight</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed number</td>
<td>0.88***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total biomass</td>
<td>0.56*</td>
<td>0.31NS</td>
<td>1</td>
</tr>
</tbody>
</table>

Significant differences at P< 0.05, 0.01, 0.001, and nonsignificant designated as *, **, ***, and NS, respectively.
2-5 Pearson correlation coefficients ($r^2$) with associated significance (P) value as superscript between traits of *Festuca* species accessions evaluated in randomized complete block design in 2010 (below diagonal) and 2011 (above diagonal).

<table>
<thead>
<tr>
<th></th>
<th>Total biomass</th>
<th>Height</th>
<th>Vigor rating</th>
<th>Seed number</th>
<th>Seed weight</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total biomass</td>
<td>1</td>
<td>0.61**</td>
<td>0.88***</td>
<td>0.36NS</td>
<td>0.44NS</td>
<td>0.63**</td>
</tr>
<tr>
<td>Height</td>
<td>0.59*</td>
<td>1</td>
<td>0.64**</td>
<td>-0.06NS</td>
<td>-0.00NS</td>
<td>0.61**</td>
</tr>
<tr>
<td>Vigor rating</td>
<td>0.71**</td>
<td>0.75***</td>
<td>1</td>
<td>0.15NS</td>
<td>0.28NS</td>
<td>0.76***</td>
</tr>
<tr>
<td>Seed number</td>
<td>0.74***</td>
<td>0.54*</td>
<td>0.84***</td>
<td>1</td>
<td>0.95***</td>
<td>-0.07NS</td>
</tr>
<tr>
<td>Seed weight</td>
<td>0.84***</td>
<td>0.58*</td>
<td>0.83***</td>
<td>0.98***</td>
<td>1</td>
<td>0.09NS</td>
</tr>
<tr>
<td>Width</td>
<td>0.46NS</td>
<td>0.78***</td>
<td>0.42NS</td>
<td>0.12NS</td>
<td>0.20NS</td>
<td>1</td>
</tr>
</tbody>
</table>

*Significant differences at P< 0.05, 0.01, 0.001, and nonsignificant designated as *, **, ***, and NS, respectively.*
2-6 Pearson correlation coefficient ($r^2$) with significance (P) value as superscript between traits of *Festuca* species accessions evaluated in randomized complete block design over three years (2009-2011).

<table>
<thead>
<tr>
<th></th>
<th>Width</th>
<th>Vigor rating</th>
<th>Total biomass</th>
<th>Seed number</th>
<th>Seed weight</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vigor rating</td>
<td>0.63***</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total biomass</td>
<td>0.58**</td>
<td>0.79***</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed number</td>
<td>0.15&lt;sub&gt;NS&lt;/sub&gt;</td>
<td>0.77***</td>
<td>0.56*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed weight</td>
<td>0.12&lt;sub&gt;NS&lt;/sub&gt;</td>
<td>0.7***</td>
<td>0.69***</td>
<td>0.91***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.73***</td>
<td>0.7***</td>
<td>0.63***</td>
<td>0.44&lt;sub&gt;NS&lt;/sub&gt;</td>
<td>0.32&lt;sub&gt;NS&lt;/sub&gt;</td>
<td>1</td>
</tr>
</tbody>
</table>

Significant differences at P < 0.05, 0.01, 0.001, and nonsignificant designated as *, **, ***, and NS, respectively.
2-1 Monthly mean of temperature (panel A), relative humidity (panel B), precipitation (panel C), and solar radiation (panel D) over three years (2009-2011) at Blue creek, UT. (Data from Mesco West at the University of Utah, Salt Lake City, Utah).
2-2 Morphological trait means of *Festuca valesiaca* accessions and *Festuca* species commercial cultivars (Controls) assessed over multiple years at Blue Creek, UT. Asterisks indicate significant (P<0.05) difference when compared to ‘Cascade’, and horizontal lines signify the average value of ‘Cascade’ by trait (Plant height (Panel A), plant width (Panel B), vigor rating (Panel C), total biomass (Panel D), seed weight (Panel E), seed number (Panel F); F.V. = *Festuca valesiaca*; F.R. = *Festuca rubra*; F.O. = *Festuca ovina*; F.A. = *Festuca arundinacea*; F.T. = *Festuca trachyphylla*; L. P. = *Lolium perenne*); PI and W6 = Plant introduction number according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN).
Panel C

Plant Vigor Rating Over Two Years

![Graph showing plant vigor ratings over two years.](image)

Panel D

Total Biomass Over Three Years

![Graph showing total biomass over three years.](image)

(Fig. 2-2 continued)
Seed Weight Over Three Years

2009  2010  2011

Panel E

Seed Number Over Three Years

2009  2010  2011

Panel F

(Fig. 2-2 continued)
2-3 Principal component analysis plot of the first two principal components assessing *Festuca valesiaca* accessions and *Festuca* species commercial cultivars (Controls) based on eight morphological traits examined in 2009 (Panel A), 2010 (Panel B), and 2011 (Panel C) at Blue Creek, UT; PI and W6 = Plant introduction and collection number according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN); Principal component 1: percentage of variation explained; Principal component 2: percentage of variation explained.
2-4 Principal component analysis plot of the first two principal components assessing *Festuca valesiaca* accessions and *Festuca* species commercial cultivars (Control) based on eight traits examined over three years (2009-2011) at Blue Creek, UT; PI and W6 = Plant introduction and collection number according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN); Principal component 1: percentage of variation was explained; Principal component 2: percentage of variation was explained.
3. GENETIC RELATIONSHIP BETWEEN FESTUCA VALESIACA AND FESTUCA OVINA COMPLEX BASED ON AFLP MARKER

ABSTRACT

Morphological, anatomical and ploidy level characteristics have been previously used to examine the genetic relationships between *Festuca valesiaca* and members of the *Festuca ovina* complex. Nevertheless, open pollination under natural hybridization have caused difficulties in species characterization using these traditional method. Therefore, multi-locus AFLP genotyping was used to determine the relationships among a diverse array of *Festuca valesiaca* accessions and species of the *Festuca ovina* complex. An AFLP-based neighbor-joining analysis partitioned the accessions examined into five distinct clusters consisting of diverse *Festuca* species (Cluster 1); *Festuca idahoensis* with *Festuca roemeri* (Cluster 3), *Festuca rubra* (Cluster 2), and the fourth cluster contained two *Festuca valesiaca* accessions W6 30506 and W6 30513 (Cluster 4). *Festuca ovina* and *Festuca valesiaca* (Cluster 6), and *Festuca trachyphylla* with *Festuca filiformis* (Cluster 7). These species relationships were further confirmed by a Bayesian cluster analysis. Analysis of molecular variance detected nonsignificant differences within species in the cluster but significant difference among all species examined. Also, a significant (P = 0.0001) but low admixture (2%) between *Festuca valesiaca* and *Festuca ovina* was identified. The broad-leaved species (*Festuca arundinacea*, *Festuca pratensis*, and *Lolium perenne*) were different from fine-leaved *Festuca* species. Based on neighbor-joining tree and Bayesian cluster analysis, *Festuca valesiaca* is closely related to the *Festuca ovina* accessions examined and, thus, should be considered as one species. The *Festuca trachyphylla* possessed genetic affinities with the *Festuca ovina* and *Festuca valesiaca* accessions examined. *Festuca idahoensis* accessions had genetic affinities with *Festuca roemeri*. Genetic admixture in *Festuca rubra* and *Festuca trachyphylla* accessions was relatively low (5%) while the admixture level detected among the commercial
cultivars was considerable (40%).

INTRODUCTION

The genus *Festuca* is high polymorphic (Jenkin 1959) and is cross-compatible with species in the genera *Lolium* and *Bromus* (Clayton and Renvoize 1986). Taxonomic classification within *Festuca* has historically been based on morphology and anatomy (Bhandari et al. 2004; Fjellheim and Rognli 2005a). However, such assessments of *Festuca* have led to difficulties in distinguishing morphologically similar taxa having different ploidy levels and geographic distribution. Molecular technologies (e.g., genomic markers and flow cytometry) offer powerful tools for genetic diversity and ploidy level analyses that can augment traditional morphological analyses (Cresswell et al. 2001; Amini et al. 2011). Molecular markers such as restriction fragment length polymorphism (RFLP; Charmet et al. 1997; Xu and Sleper 1994), internal transcribed spacer (ITS; Catalan et al. 2004; Gaut et al. 2000; Torrecilla et al. 2003a) and chloroplast DNA (cpDNA; Darbyshire and Warwick 1992) have been used to elucidate the systematics and phylogeny of *Festuca* species. In addition, amplified fragment length polymorphism (AFLP) marker technology has been successfully used for diversity assessment in wheat (*Triticum aestivum*; Lage et al. 2003) and fescue to describe population structure differences (Jones et al. 2008).

*Festuca filiformis* Pourret (2n = 2x = 14), *F. idahoensis* Elmer (2n = 2x = 14), *F. ovina* L. ssp. *hirtula* (Hackel ex Travis) M. Wilkinson (2n = 2x = 14), *F. trachyphylla* (Hackel) Krajina (2n = 2x = 14), and *F. valesiaca* Schleich. ex Gaudin (2n = 2x = 14) form the *Festuca ovina* aggregate that is often called the “*ovina* complex” (Ruemmele et al. 2003). Report based on morphological, anatomical, and ploidy characterization have demonstrated that *F. ovina* var. *guinochetii* (2n = 10x = 70) belongs to the *F. valesiaca* cluster (Arndt 2008). Arndt (2008) also suggested treating individuals of each ploidy level within the *F. valesiaca* cluster as a single species due to different
genetic constitutions and reproductive isolation. However, given the relatively small morphological and anatomical differences and cross-compatibility with other species (i.e., *Festuca laevigata*) among accessions in *F. valesiaca* cluster, individuals within the cluster are often distinguished by their geographical distribution (Arndt 2008). He, thus, recommended that molecular tools be used to another way of distinguishing the small differences in *F. valesiaca* cluster.

*F. trachyphylla* is a Eurasian native species that has received genetic improvement initially under the designation as a “hard”, “sheep”, or “ovina” fescue in Netherlands, and was then introduced to North America for land stabilization on pipelines, mine investigations, and roadside protection (Chen et al. 2003). *Festuca ovina* has been sold under the name of *F. trachyphylla* which is not native to North America (Wilson 2007). Dabrowska (2012) also mentioned that morphological and anatomical traits of the leaf blade width in this species were considerably unstable and modified by the environmental effect. Thus, a molecular genetic relatedness investigation is necessary to clarify the relationship between them.

**MATERIALS AND METHOD**

**DNA Extraction and PCR Amplification**

Seed of 30 *F. valesiaca* accessions, 42 *F. ovina* complex species representatives, 19 broad-leaved controls (*F. arundinacea, F. pratensis*, and *Lolium perenne*) and 15 narrow-leaved controls (*Festuca rubra*) were planted in the greenhouse Logan, Utah for DNA analysis. Leaf samples of each accession were collected from actively growing plants and lyophilized and, then ground into fine powder using a Retsch model MM 300 shaker (F. Kurt Retsch GmbH and Co., Hann, Germany). Nuclear DNA was extracted from samples using a DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer’s instructions, and quantified using a Nanodrop Spectrophotometer (ND-1000, NanoDrop Technologies, Inc., Wilmington, DE, USA).
Restriction digestion for the AFLP procedure was performed according to Vos et al. (1995), in which 50 ng/µL of genomic DNA was digested with 3 Units of EcoRI (New England Biolabs Inc.) and 6.5 Units of Msel (New England Biolabs Inc.) for 4 h at 37 °C, and then heat inactivated for 15 min at 70 °C. Subsequently, 0.02 µM of the EcoRI and Msel adapters were ligated with 5 Units T4 DNA Ligase (Fermentas Life Science, U.S.A.) prior to DNA fragments digestion by incubation at 16 °C for 24 h. The diluted (1:5) restriction/ligation products were then pre-amplified using the polymerase chain reaction (PCR) with two selective nucleotides, AC and CT, which were added to the EcoRI and Msel preamplification primers, respectively. After pre-amplification, the products were visualized on 2% agarose gel to verify amplification. The buffer (10 mM Tris pH 7.5, 0.1 mM EDTA) was used to dilute pre-amplification by 20-fold and stored at 4 °C prior to the selective PCR amplification. The primer combinations of E-ACAC/M-CTAC, E-ACAG/M-CTCA, E-ACAC/M-CTAG, E-ACAC/M-CTTC, E-ACCT/M-CTCT, E-ACTC/M-CTTG, E-ACT/M-CTA, E-ACT/M-CTG, E-ATA/M-CAA, and E-AGG/M-CGC were used for selective amplification at the temperature and time profiles (Jones et al. 2008). In order to visualize AFLP fragments, the EcoRI selective amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein) label on the 5’ nucleotide end. Finally, GeneScan 500 LIZ Size Standard (Applied Biosystems, Carlsbad, California, U.S.A.) was added to the PCR products according to the manufacturer’s instructions, and then denatured in 80% formamide solution at 95 °C for 5 minutes. Samples were subsequently size-fractionated by the Utah State University Center for Integrated Biosystems (USU, CIB) using an ABI 3100 Capillary Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) possessing 50 cm capillaries using 8 s for on injection run and a 28 min run. The data was initially converted by a converter and then analyzed using Genescan software (PE Applied Biosystems, Foster City, CA), and finally visually scored for the presence and absence of DNA fragments between 50 and 400 bp using Genographer version 1.5 (Benham et al. 1999; Fig. 3-1, Fig. 3-2). Some samples (4%) were
replicated to identify reproducible marker bands and determine their marker error rates.

**AFLP-based Clustering Analyses**

Accession relationships were characterized by multivariate analysis using AFLP fragment data. Data matrices [AFLP band present (1) or absent (0)] were constructed and used to create a neighbor-joining dendrogram using PAUP computer software version 4.0b10 (Swofford 1998). The dendrogram was based on the Nei and Li distance method (Nei 1979) which uses the pair-wise genetic distance values between each pair of accessions. Internal support for cluster groupings was assessed using 1,000 bootstrap replications (Felsenstein 1985). The genetic similarity (GS) between accessions was calculated using the formula: $GS = 2N_{ij}/(N_i + N_j)$, where $N_{ij}$ is the number of AFLP bands shared between genotype $i$ and $j$, $N_i$ and $N_j$ are the total number of AFLP bands observed for genotypes $i$ or $j$, respectively (Nei 1979). Pair-wise genetic distance matrices were used for analysis of molecular variance (AMOVA) which is a method of estimating population differentiation indirectly by comparing genetic diversity within and among populations using ARLEQUIN 3.1 software (Excoffier et al. 1992) to partition the variation within- and among-population variances. Bayesian cluster analysis of population genetic structure was conducted according to Pritchard et al. (2000) and Falush et al. (2003) using structure program (Version 2.3.1, February, 2009) using the Utah State University Center for High Performance Computing. Three runs under recessive model by using 10,000 lengths of Burn-in period plus 100,000 MCMC interactions (Replication) for computing the population structure. The number of populations postulated by structure computing was estimated based on natural log probability of K of ancestry coefficients which having the highest variance on natural log probability between two K values as K is approaching a true number of ancestry coefficients.
The ploidy level of each accession was determined using a Partec PA I flow cytometer (Partec GmbH Munster, Germany) based on cytological examination of mitotic chromosomes at metaphase (Table 3-1). Rapidly growing, immature leaves from each accession were harvested and macerated to extract nuclei using a Partec CyStain UV precise P extraction buffer (reagent kit; Partec GmbH Munster, Germany). After incubation of 30 to 60 seconds by staining with the staining buffer, the sample solution was filtered through a CellTrics filter according to the manufacturer’s protocol (Partec GmbH, Munster, Germany). The resulting cell suspension was passed through a Partec PA ploidy analyzer for comparisons of relative fluorescence of sample nuclei. Samples of known ploidy (F. valesiaca W6 30588, 2n = 2x = 14) whose chromosome number has been previously determined by cytogenetic analysis was used as an internal standard for comparative analysis. About 5,000 to 8,000 cells per sample were analyzed and each sample was measured once, comparisons of peak positions were used to calculate the sample/standard ratio, which provide a determination of the relative ploidy level. If the sample/standard ratio equaled 1, 1.5, 2, 3, 4, or 5, the sample was declared as a diploid, triploid, tetraploid, hexaploid, octoploid, or decaploid, respectively. Data on standard/sample ratios of the accessions examined are given in Table 3-1. However, a sub-sample of 16 randomly chosen accessions was measured two times to access its methodological repeatability.

RESULTS

AFLP-based Neighbor-joining Tree Analyses

AFLP marker bands (1,689) originating from ten primer combinations were polymorphic (95%) and were used to genotype for 102 Festuca accessions (Table 3-1). The average number of markers recovered per primer combination was 175. A midpoint rooted Neighbor-joining tree was
constructed based on NeiLi’s pair wise distance comparisons between plants with 1000 bootstrap permutations (Fig. 3-3) (Nei 1979). Accessions of the *F. ovina* complex and *F. rubra* (Cluster 2) were distinct and separated from the other reference taxon used (Cluster 1) which included *F. arundinacea*, *F. pratensis*, and *Lolium perenne* (Fig. 3-3; Table 3-1). The reference taxon were further partitioned into a cluster containing tall fescue and meadow fescue cluster (*F. arundinacea* and *F. pratensis*), and perennial ryegrass (*Lolium perenne*). *F. idahoensis* species accessions (8) and two *F. roemeri* accessions (BFI-10-101495481 and BFI-10-101496621) clustered together into one cluster (Cluster 3). Likewise, *F. rubra* species accessions (14) clustered into one cluster (Cluster 2), along with one *F. valesiaca* accession (W6 30537) and *F. roemeri* accession (W6 32677). The fourth cluster contained two *F. valesiaca* accessions W6 30506 and W6 30513 (Cluster 4). The fifth large cluster consisted of *F. valesiaca*, *F. ovina*, *F. filiformis*, and *F. trachyphylla* accessions, where *F. valesiaca* and *F. ovina* accessions (Clusters 5) were similar but distinct from *F. trachyphylla* and *F. filiformis* accessions, except for *F. ovina* cultivars ‘MX86’, ‘Bighorn’, ‘Blacksheep’, and ‘Marco Polo’ and *F. idahoensis* cultivar ‘Siskiyou Blue’ which clustered together with *F. trachyphylla* and *F. filiformis* accessions (Cluster 7). The large *F. ovina* (15) and *F. valesiaca* (30) cluster (Cluster 6) also included the closely-related *F. rupicola* (PI 440387) accession.

**AFLP-based Population Structure**

Bayesian cluster analysis is a quantitative clustering method that uses markow chain Monte Carlo to detect the underlying genetic structure among a set of individuals genotyped at multiple markers. Based on the Ln probability assessment of AFLP-based genetic structure of seven the *Festuca* species examined (*F. valesiaca*, *F. ovina*, *F. rubra*, *F. idahoensis*, *F. trachyphylla*, *F. filiformis*, and *F. roemeri*), the K value of 4 derived from three replications (Ln P = -69806) was determined to provide the “best-fit” for inferring population structure differences (Fig. 3-4). This
allowed for the use of ancestry coefficients from the simplest (K = 4) model not the more complex (K = 5 and 6) to compare individual plant.

The use of the K = 4 model (Fig. 3-4) allowed for the discrimination of four unique populations, which included *F. valesiaca* and *F. ovina* (light green), *F. trachyphylla* and *F. filiformis* (purple), *F. rubra* (light blue), *F. idahoensis* and *F. roemeri* (aqua), and *F. ovina* (orange) (Fig 3-5). Based on AFLP banding similarities of K value from 3 to 6, the ancestry coefficient of *F. trachyphylla* and *F. rubra* accessions were relatively homogeneous, where only 5% of their inferred ancestry coefficient possessed DNA introgressed from the other species examined (hereafter designated as introgression DNA). Likewise, the inferred ancestry coefficient of the *F. idahoensis* and *F. roemeri* accessions examined was relatively uniform (95%), except for the *F. idahoensis* cultivar ‘Siskiyou Blue’ which possessed substantial amounts of introgression DNA from *F. trachyphylla*. Similarly, the genomic constitution of two *F. roemeri* accessions (BFI-10-101495481 and BFI-10-101496621) was similar (99%) to that of the majority of the *F. idahoensis* species examined. The inferred ancestry coefficient of one *F. roemeri* accession (W6 32677) also possessed substantial similarities to the *F. rubra* (60%) and *F. idahoensis* (40%) accessions examined. Moreover, the genome of the *F. rupicola* accession possessed substantial similarities to the *F. valesiaca* (75%) and *F. ovina* (25%) accessions examined, and the genomic constitution of two *F. filiformis* accessions (‘Barok’ and PI 255361) was similar (60%) to that of *F. trachyphylla* accessions inspected. In fact, the genome of four *F. ovina* cultivars (‘MX-86’, ‘Black sheep’, ‘Bighorn’, and ‘Marco Polo’) possessed substantial amounts of DNA (60%) introgressed from *F. trachyphylla*. Five *F. ovina* accessions (PIs 634304, 618975, 595178, and 595160) and ‘Covar’ held substantial genetic affinities (95%) with the *F. valesiaca* accessions studied, and the genomic constitution of one *F. ovina* accession (PI 659944) possessed sizeable amounts of introgression DNA (at least 55%) from *F. rubra, F. valesiaca*, and *F. trachyphylla*. In contrast, the genome of the *F. valesiaca* accession W6 30537 was similar (60%) to the genomes of the *F. rubra* accessions
examined.

**Genetic Distance-based AMOVA Analyses**

Hierarchical AMOVA apportioned 15.7% of the genetic variations among species clusters (F. rubra, F. idahoensis, F. ovina, F. valesiaca, and F. trachyphylla) and 4.6% among accessions within clusters (within one population) (Table 3-2).

All species pair-wise genetic variance comparisons were significant (P < 0.01), except for the comparison between F. roemeri and Festuca idahoensis (Table 3-4). The general detection of such variation based on pair-wise contrasts demonstrated that distinct population structures existed for each of the species examined (Table 3-4). Although, in the case of the F. roemeri and F. filiformis accession examined, the nonsignificant contrast defined a comparatively close genetic relationship, this result may be influenced by the relatively small sample size of both species (Table 3-4). Significant (P < 0.01) pair-wise differences were detected between all the species examined, except for the relationship between F. roemeri and F. idahonesis (7.81) and the relationship between F. trachyphylla and F. filiformis (53.22) (Table 3-4).

**Ploidy Estimation for the Festuca Species**

Six levels of ploidy [2n = 2x, 4x, 4x (varied), 6x, 8x, and 9x (varied)] were detected from the accessions examined which were presented in Table 3-1 based on flow cytometry. Within those six ploidy levels, the Festuca arundinacea was octoploid except for one accession (PI 318987 = 4x), Festuca pratensis possessed three types of ploid (2n = 2x, 4x, and 6x), Lolium perene was tetraploid, Festuca idahoensis was tetroploid except for two accessions (‘Siskiyou Blue’ = 6x and ‘Nezpurs’ = 4x (varied)), Festuca filiformis was diploid, Festuca roemerii possessed two types of poidy levels (2n = 4x (varied) and 6x), Festuca rubra was hexploid except for one diploid (‘Merlin’ = 2x), two octoploid accessions (PI 659984 and PI 578735 = 8x), and one nanoploid
(‘Boreal’ = 9x (varied)), *Festuca trachyphylla* was hexploid except for one cultivar (‘Quatro’ = 4x), *Festuca valesiaca* possessed two types of ploidy levels (2n = 2x and 4x), and *Festuca ovina* possessed four types of ploidy levels (2n = 2x, 4x, 4x (varied) and 6x).

**DISCUSSION**

**Genetic Clustering of Fine-leaved Festuca Species**

Based on relatively few taxonomic characters, *F. ovina* and *F. valesiaca* were classified to be different species by Markgraf-Dannenberg (1980). Nevertheless, in this research, all accessions with different ploidy level were clustered together (Table 3-1; Fig. 3-3).

Two clusters were identified by Smarda (2008) which included *F. valesiaca* cluster with *F. pseudodalmatica* Krajina, *F. pseudovina* Wiesb., and *F. rupicola* Heuff. while *F. ovina* cluster with *F. filiformis* Pourr., *F. lemanii* Bastard, *F. ovina* L. subsp. *ovina*. In contrast to the separate clustering of *F. valesiaca* and *F. ovina* by Smarda (2008), Bayesian cluster analysis of these species indicated that *F. valesiaca* and *F. ovina* share close genetic affinities (Table 3-1; Fig. 3-5). Moreover, an AFLP-based neighbor-joining analysis of an array of diverse *Festuca* species conducted herein identified four fine-leaved *Festuca* clusters (*F. rubra*, *F. valesiaca* and *F. ovina*, *F. trachyphylla* and *F. filiformis*, and *F. idahoensis* and *Froemerii*) (Fig. 3-3). Interestingly, two *F. pseudodalmatica* accessions (W6 30438 and PI 283321), one *F. rupicola* (PI 440387), and *F. lemanii* accession (PI 578732/ ‘Durar’) were clustered with *F. valesiaca* accessions. Similarly, two *F. filiformis* accessions (‘Barok’ and PI 255361) shared considerable genetic affinities with the *F. trachyphylla* accessions examined which themselves showed relatively strong genetic affinities to *F. valesiaca* and *F. ovina*. The close genetic relationship between *F. rupicola* and *F. valesiaca* accessions defined herein confirms earlier work by Chen et al. (2003). *F. rupicola* typically grows in many types of dry grasslands on sandy soil (Smarda 2008), and the *F. valesiaca* species examined herein originated from dry and heavily grazed regions in Asia, such
as mountainous areas in Kyrgyzstan (Table 3-1; Johnson 2006).

**Genetic Clustering of Festuca trachyphylla**

Based on neighbor-joining tree analyses, the *F. trachyphylla* accessions examined possessed genetic affinities with the cluster *F. ovina* accessions examined herein (Fig. 3-3). Nevertheless, Bayesian cluster analysis indicated that *F. trachyphylla* accessions held unique differences from *F. ovina* accessions (Fig. 3-5). Therefore, *F. trachyphylla* should be considered a distinct species but closely related to the *F. ovina* and *F. valesiaca*. Several *F. ovina* commercial cultivars (‘MX-86’, ‘Big horn’, ‘Black sheep’, and ‘Marco polo’) were tested herein, and they shared genetic affinities with *F. trachyphylla*. These results were further confirmed by Bayesian cluster analysis which demonstrated that those commercial cultivars contained most of the genome from the *F. trachyphylla*. Similarly, *F. valesiaca* and *F. roemeri* accessions were clustered in the *F. rubra* cluster based on AFLP neighbor-joining tree. In the Bayesian cluster analysis, most of the genome proportion of those accessions was from *F. rubra*.

**Genetic Clustering of Festuca valesiaca and Festuca ovina**

Based on taxonomical trait differences (spikelet, lemma, and leaf blade width; Table A-4), Sheidai and Bagheri-Shabestarei (2007) identified six subspecies within *F. valesiaca* Schleich. ex Gaudin cluster. These include: 1) *F. valesiaca* subsp. *pseudovina* (Hack. ex Wiesb.) Hegi from central Asia; 2) *F. valesiaca* subsp. *sulcata* (Hack.) Schinz and R. Keller from western and southern Tien Shan; 3) *F. valesiaca* subsp. *hypsofila* (Saint-Yves) Tzvel from central Tien Shan; 4) *F. valesiaca* subsp. *kirghisorum* (Katsch. ex Tzvel.) Tzvel from Tien Shan, Pamir, Mongolia, Altai, and southwestern China; 5) *F. valesiaca* subsp. *valesiaca* from Altai, Tien Shan, Pamir, and western China; and 6) *F. valesiaca* subsp. *pseudodalmatica* (Kraj.) Soo from northern and western Tien Shan, and southwestern China. Three subspecies of *F. valesiaca* subsp. *kirghisorum*
(Katsch. ex Tzvel.) Tzvel, *F. valesiaca* subsp. *valesiaca*, and *F. valesiaca* subsp. *pseudodalmatica* (Kraj.) were compared in this study (Table 3-1). AFLP-based genotyping indicated that *F. valesiaca* subsp. *valesiaca* accessions were genetically similar, except for accessions PI 659944, W6 30506, and W6 30513 (Fig. 3-3), which indicated that they were genetically similar. However, two *F. valesiaca* subsp. *pseudodalmatica* (Kraj.) accessions (W6 30438 and PI 283321) were genetically distinct from other *F. valesiaca* accessions examined (Fig. 3-3). The *F. valesiaca* and *F. ovina* accessions examined herein are genetically similar both in morphology and in DNA-based assessments, but often possessed alleles shared with other species (Fig. 3-3, Fig. 3-5). The European accessions and Asian accessions introduced to North America, however, shared considerable genetic affinities based on their AFLP profile (Fig. 3-3, Fig. 3-5). Thus, although *F. valesiaca* and *F. ovina* possess introgression DNA, they likely possess common evolutionary origins and should be considered as one species in the *F. ovina* complex.

**Ploidy Level Differences Among Fine-leaved Festuca Species**

The characterization of ploidy level is considered important and often essential information for differentiating closely related central polyploid European fescue complexes (Smarda 2008). Flow cytometry is a frequently used method for assessing ploidy in plants (Doležel 1991; Doležel et al. 2005). Šmarda et al. (2005) reported that five ploidy levels were present in *F. valesiaca* cluster, which included diploids, hexaploids, heptaploids, octoploids, and decaploids. Although only two ploidy levels (diploids and tetraploids) were detected in the *F. valesiaca* cluster accessions examined herein (Table 3-1).

The characterization of ploidy level using this method, however, did not detect appreciable differences among the closely related *Festuca* species in the *ovina* complex examined herein (Table 3-1) due to the high degree of introgression of *F. valesiaca* and *F. ovina* (Fig 3-5). Therefore, both ploidy characterization and genetic structure analysis will be essential in
developing breeding strategies for those *F. valesiaca* germplasm that having prerequisite agronomic potential as identified herein. This is especially true if other uncharacterized *F. valesiaca* accessions are considered for breeding.

Šmarda (2008) reported, with some deviations, the ploidy level of the closely related fine-leaved fescues, *F. filiformis* \((2n = 2x)\), *F. ovina* subsp. *ovina* \((2n = 2x)\), *F. rupicola* \((2n = 6x)\), and *F. valesiaca* \((2n = 2x, 4x, \text{and} 6x)\). The data presented herein confirmed these ploidy designations (Table 3-1). For instance, Šmarda and Kočí (2003) reported two ploidy levels existed in *F. ovina*; diploid *F. ovina* subsp. *ovina* and tetraploid *F. ovina* subsp. *guestfalica* from the Czech Republic. While, in the main, similar results were found herein, some *F. ovina* (i.e., \(2n = 2x, 4x, \text{and} 6x\)) accessions examined possessed higher ploidy levels than have been reported (Table 3-1). However, the ploidy level detected in this research differed from the *F. rupicola* accession \((2n = 2x)\) examined by Šmarda (2008) (Table 3-1).

Tetraploid forms of *F. pseudodalmatica* originating from Austria, the Czech Republic, Hungary, and Slovakia have been reported (Aiken et al. 1996). Additionally, Šmarda (2008) indicated that tetraploid *F. pseudodalmatica* was morphologically similar to diploid *F. valesiaca*, and then concluded that these taxa were genetically similar, except for differences in their ploidy level. Predictably, the two *F. pseudodalmatica* accessions W6 30438 = 2\(x\) from Ysyk-Kol, Kyrgyzstan and PI 283321 = 4\(x\) from Czechoslovakia examined herein differed in ploidy level and in genomic structure as well (Fig. 3-3; Table 3-1).

CONCLUSIONS

Results from AFLP-based neighbor joining tree, structure analysis, and analysis of molecular variance analysis are congruent. The broad-leaved species differed genetically were different from fine-leaved *Festuca* species examined. It was determined that *F. valesiaca* is closely related to *F. ovina* and that these species should be considered as one species. Data indicated that *F.
idahoensis also is closely related to F. roemeri which was considered a subspecies of F. idahoensis (F. idahoensis subsp. roemeri (Pavlick) S. Aiken) by Aiken in 1998. Likewise, F. trachyphylla possesses considerable genetic affinities with F. ovina and F. valesiaca. Although low admixtures were detected among the F. rubra and F. trachyphylla accessions examined and higher DNA admixture (introgression) is detected among commercial cultivars which likely a consequence of their cross breeding during plant improvement. The Bayesian cluster analysis also provided a report on the purities of the designated cultivars examined.

REFERENCES


Gaut BS, Tredway LP, Kubik C, Gaut RL, Meyer W (2000) Phylogenetic relationships and
genetic diversity among members of the *Festuca-Lolium* complex (*Poaceae*) based on ITS sequence data. Plant Syst Evol 224: 33-53


Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endo-nucleases. Proc Natl Acad Sci USA 76:5269-5273


Sheidai M, Bagheri-Shabestarei E (2007) Cytotaxonomy of some *Festuca* species and
populations in Iran. Acta Bot Croat 66 (2):143-151

Šmarda P (2008) DNA ploidy level variability of some fescues (Festuca subg. Festuca, Poaceae) from Central and Southern Europe measured in fresh plants and herbarium specimens. Biologia Section Botany 63(3): 349-367


### Festuca species germplasm used for morphological traits evaluation and genetic relationship analyses

<table>
<thead>
<tr>
<th>Identification</th>
<th>Cultivar</th>
<th>Species name</th>
<th>Ploidy</th>
<th>Origin</th>
<th>Seed Source</th>
<th>NJ-tree analysis Cluster</th>
<th>Structure analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>R08-148</td>
<td>Matador</td>
<td><em>Dactylis glomerata</em></td>
<td>4x</td>
<td>Russian Federation</td>
<td>FRRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 318987</td>
<td></td>
<td><em>F. arundinacea</em> Schreb.</td>
<td>8x</td>
<td>Oregon, USA</td>
<td>GSC</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 357184</td>
<td>Coronado</td>
<td><em>F. arundinacea</em> Schreb.</td>
<td>4x</td>
<td>Ciudad Real, Spain</td>
<td>GRIN</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 577096</td>
<td></td>
<td><em>F. arundinacea</em> Schreb.</td>
<td>8x</td>
<td>Oregon, USA</td>
<td>GSC</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 595048</td>
<td>Barok</td>
<td><em>F. arundinacea</em> Schreb.</td>
<td>8x</td>
<td>Wales, United Kingdom</td>
<td>FRRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 255361</td>
<td></td>
<td><em>F. arundinacea</em> Schreb.subsp.</td>
<td>4x</td>
<td>France</td>
<td>FRRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 601054</td>
<td>Siskiyou Blue</td>
<td><em>F. idahoensis</em> Elmer</td>
<td>6x</td>
<td>Tennessee</td>
<td>WPN</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>PI 578731</td>
<td>Joseph</td>
<td><em>F. idahoensis</em> Elmer</td>
<td>4x</td>
<td>Idaho, USA</td>
<td>GRIN</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>PI 232288</td>
<td>Nezpus</td>
<td><em>F. idahoensis</em> Elmer</td>
<td>4x</td>
<td>Idaho, USA</td>
<td>GRIN</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>PI 232293</td>
<td></td>
<td><em>F. idahoensis</em> Elmer</td>
<td>4x</td>
<td>Oregon, USA</td>
<td>GRIN</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>PI 344616</td>
<td></td>
<td><em>F. idahoensis</em> Elmer</td>
<td>4x</td>
<td>Wyoming, USA</td>
<td>FRRRL</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>PI 344631</td>
<td></td>
<td><em>F. idahoensis</em> Elmer</td>
<td>4x</td>
<td>Montana, USA</td>
<td>FRRRL</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>PI 504313</td>
<td></td>
<td><em>F. idahoensis</em> Elmer</td>
<td>4x</td>
<td>Oregon, USA</td>
<td>GRIN</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>W6 27177</td>
<td></td>
<td><em>F. idahoensis</em> Elmer</td>
<td>4x</td>
<td>Oregon, USA</td>
<td>FRRRL</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>PI 193151</td>
<td>Black Sheep</td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Washington, USA</td>
<td>GSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>PI 193151</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Idaho, USA</td>
<td>GRIN</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>PI 229453</td>
<td>Marco Polo</td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Badakhshan, Afghanistan</td>
<td>GSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>PI 229503</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>4x</td>
<td>Iran</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 229533</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Iran</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 251125</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>4x</td>
<td>Former Serbia and Montenegro</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 268234</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Iran</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 380846</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Iran</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 383652</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>2x</td>
<td>Turkey</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 383654</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>4x</td>
<td>Turkey</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 384861</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Iran</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 549274</td>
<td>Big horn</td>
<td><em>F. ovina</em> L.</td>
<td>NA</td>
<td>Oregon, USA</td>
<td>GSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>PI 578732</td>
<td>Durar</td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Washington, USA</td>
<td>GSC</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 578733</td>
<td>Covar</td>
<td><em>F. ovina</em> L.</td>
<td>6x</td>
<td>Turkey</td>
<td>GSC</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 595160</td>
<td></td>
<td><em>F. ovina</em> L.</td>
<td>4x</td>
<td>Xinjiang, China</td>
<td>FRRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>Identification</td>
<td>Cultivar</td>
<td>Species name</td>
<td>Ploidy</td>
<td>Origin</td>
<td>Seed Source</td>
<td>NJ-tree analysis Cluster</td>
<td>Structure analysis</td>
</tr>
<tr>
<td>------------------</td>
<td>----------</td>
<td>-----------------------------------</td>
<td>--------</td>
<td>--------------</td>
<td>-------------</td>
<td>--------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>PI 595178</td>
<td>F. ovina</td>
<td>L.</td>
<td>2x</td>
<td>Xinjiang, China</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 618975</td>
<td>F. ovina</td>
<td>L.</td>
<td>2x</td>
<td>Xinjiang, China</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 634304</td>
<td>F. ovina</td>
<td>L.</td>
<td>2x</td>
<td>Xinjiang, China</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 221918</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>2x</td>
<td>Afghanistan</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 311046</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>2x</td>
<td>Romania</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 502378</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>4x</td>
<td>Uzbekistan</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 380858</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>6x</td>
<td>Iran</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 577106</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>2x</td>
<td>Norway</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 595018</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>2x</td>
<td>Switzerland</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 595021</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>2x</td>
<td>Italy</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>PI 636667</td>
<td>F. pratensis Huds.</td>
<td></td>
<td>6x</td>
<td>Kazakhstan</td>
<td>FRRL</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>BFI-10-101495481</td>
<td>F. roemeri (Pavlick) E. B. Alexeev</td>
<td></td>
<td>4x</td>
<td>Puget Sound, Washington, USA</td>
<td>BFI</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>BFI-10-101496621</td>
<td>F. roemeri (Pavlick) E. B. Alexeev</td>
<td></td>
<td>4x</td>
<td>Washington, USA</td>
<td>BFI</td>
<td>3</td>
<td>F. I.</td>
</tr>
<tr>
<td>W6 32677</td>
<td>F. roemeri (Pavlick) E. B. Alexeev</td>
<td></td>
<td>6x</td>
<td>Oregon, USA</td>
<td>GRIN</td>
<td>4</td>
<td>F. R.</td>
</tr>
<tr>
<td>PI 578735</td>
<td>F. rubra L.</td>
<td></td>
<td>8x</td>
<td>Oregon, USA</td>
<td>GRIN</td>
<td>4</td>
<td>F. R.</td>
</tr>
<tr>
<td>PI 659899</td>
<td>KGZ-036</td>
<td>F. rubra L.</td>
<td></td>
<td>6x</td>
<td>Ysyk-Kol, Kyrgyzstan</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td>PI 659946</td>
<td>KGZ-203</td>
<td>F. rubra L.</td>
<td></td>
<td>6x</td>
<td>Naryn, Kyrgyzstan</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td>PI 659950</td>
<td>KGZ-231</td>
<td>F. rubra L.</td>
<td></td>
<td>6x</td>
<td>Kyrgyzstan</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td>PI 659954</td>
<td>KGZ-266</td>
<td>F. rubra L.</td>
<td></td>
<td>6x</td>
<td>Chuy, Kyrgyzstan</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td>PI 659965</td>
<td>PRC-069</td>
<td>F. rubra L.</td>
<td></td>
<td>6x</td>
<td>Nei menggu, China</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td>PI 659981</td>
<td>PRC-391</td>
<td>F. rubra L.</td>
<td></td>
<td>6x</td>
<td>Nei menggu, China</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td>PI 659966</td>
<td>PRC-072</td>
<td>F. rubra L.</td>
<td></td>
<td>6x</td>
<td>Nei menggu, China</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td>PI 659984</td>
<td>PRC-1574</td>
<td>F. rubra L.</td>
<td></td>
<td>8x</td>
<td>Qing hai, China</td>
<td>FRRL</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Gilfrood</td>
<td>F. rubra L. subsp. commutata</td>
<td>6x</td>
<td>Gilfrood, Netherlands</td>
<td>GRIN</td>
<td>4</td>
<td>F. R.</td>
</tr>
<tr>
<td>PI 158376</td>
<td>Cascade</td>
<td>F. rubra L. subsp. commutata</td>
<td>6x</td>
<td>Cascade, Oregon, USA</td>
<td>USU</td>
<td>4</td>
<td>F. R.</td>
</tr>
<tr>
<td></td>
<td>Merlin</td>
<td>F. rubra L. subsp. rubra</td>
<td>2x</td>
<td>Merlin, United Kingdom</td>
<td>GRIN</td>
<td>4</td>
<td>F. R.</td>
</tr>
<tr>
<td>W6 31031</td>
<td>Boreal</td>
<td>F. rubra L. subsp. rubra</td>
<td>9x</td>
<td>USA Cultivar</td>
<td>USU</td>
<td>4</td>
<td>F. R.</td>
</tr>
<tr>
<td></td>
<td>Seabreeze</td>
<td>F. rubra L. subsp. trichophylla</td>
<td>6x</td>
<td>North Carolina, USA</td>
<td>USU</td>
<td>4</td>
<td>F. R.</td>
</tr>
<tr>
<td>PI 440387</td>
<td>F. rupicola Heuff.</td>
<td></td>
<td>2x</td>
<td>Former Soviet Union</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td></td>
<td>Azay</td>
<td>F. trachyphylla (Hackel) Krajina</td>
<td>6x</td>
<td>USA Cultivar</td>
<td>GSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td></td>
<td>Ecostar</td>
<td>F. trachyphylla (Hackel) Krajina</td>
<td>6x</td>
<td>USA Cultivar</td>
<td>GSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td></td>
<td>Granite</td>
<td>F. trachyphylla (Hackel) Krajina</td>
<td>6x</td>
<td>USA Cultivar</td>
<td>OSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td></td>
<td>Hardtop</td>
<td>F. trachyphylla (Hackel) Krajina</td>
<td>6x</td>
<td>USA Cultivar</td>
<td>OSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td></td>
<td>Little big born</td>
<td>F. trachyphylla (Hackel) Krajina</td>
<td>6x</td>
<td>USA Cultivar</td>
<td>GSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>PI 614892</td>
<td>Scaldis</td>
<td>F. trachyphylla (Hackel) Krajina</td>
<td>6x</td>
<td>Netherlands</td>
<td>USU</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>Identification</td>
<td>Cultivar</td>
<td>Species name</td>
<td>Ploidy</td>
<td>Origin</td>
<td>Seed Source</td>
<td>NJ-tree analysis Cluster</td>
<td>Structure analysis</td>
</tr>
<tr>
<td>----------------</td>
<td>---------</td>
<td>--------------</td>
<td>--------</td>
<td>--------</td>
<td>-------------</td>
<td>--------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>PI 633849</td>
<td>Berkshire</td>
<td><em>F. trachyphylla</em> (Hackel) Krajina</td>
<td>6x</td>
<td>USA Cultivar</td>
<td>USU</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>-</td>
<td>Quatro</td>
<td><em>F. trachyphylla</em> (Hackel) Krajina</td>
<td>4x</td>
<td>USA Cultivar</td>
<td>USU</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>-</td>
<td>SR3000</td>
<td><em>F. trachyphylla</em> (Hackel) Krajina</td>
<td>6x</td>
<td>Oregon, USA</td>
<td>USU</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>-</td>
<td>Warick</td>
<td><em>F. trachyphylla</em> (Hackel) Krajina</td>
<td>6x</td>
<td>USA Cultivar</td>
<td>OSC</td>
<td>7</td>
<td>F. T.</td>
</tr>
<tr>
<td>PI 380863</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>2x</td>
<td>Iran</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 440388</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Russian Federation</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 494701</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Romania</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 502380</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>2x</td>
<td>Russian Federation</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 502381</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Stavropol, Russian Federation</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 502382</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Stavropol, Russian Federation</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 502383</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>2x</td>
<td>Stavropol, Russian Federation</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 632505</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Ankara, Turkey</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 634225</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>2x</td>
<td>Krym, Ukraine</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>R10-05-020</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Russian Federation</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>R10-07-027</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>2x</td>
<td>Russian Federation</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>R10-16-064</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Russian Federation</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>R10-22-094</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>2x</td>
<td>Russian Federation</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>R10-25-116</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>4x</td>
<td>Russian Federation</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>R10-34-144</td>
<td>F. valesiaca Schleih, ex Gaudin</td>
<td>2x</td>
<td>Russian Federation</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>W6 30537</td>
<td>KGZ-189</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. kirghizorum</td>
<td>4x</td>
<td>Naryn, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F. R.</td>
</tr>
<tr>
<td>W6 30438</td>
<td>KGZ-082</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. pseudodalmatica</td>
<td>2x</td>
<td>Ysk-Yol, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 283321</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. pseudodalmatica</td>
<td>4x</td>
<td>Czechoslovakia</td>
<td>GRIN</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
<td></td>
</tr>
<tr>
<td>PI 659913</td>
<td>KGZ-068</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Ysk-Yol, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 659923</td>
<td>KGZ-094</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Ysk-Yol, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 659932</td>
<td>KGZ-119</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Naryn, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>PI 659494</td>
<td>KGZ-198</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>4x</td>
<td>Naryn, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>W6 30506</td>
<td>KGZ-155</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Naryn, Kyrgyzstan</td>
<td>FRRL</td>
<td>5</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>W6 30513</td>
<td>KGZ-162</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Naryn, Kyrgyzstan</td>
<td>FRRL</td>
<td>5</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>W6 30563</td>
<td>KGZ-217</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Naryn, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>W6 30575</td>
<td>KGZ-229</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Chu, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>W6 30588</td>
<td>KGZ-242</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Chu, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>W6 30595</td>
<td>KGZ-249</td>
<td>F. valesiaca Schleih, ex Gaudin subsp. valesiaca</td>
<td>2x</td>
<td>Chu, Kyrgyzstan</td>
<td>FRRL</td>
<td>6</td>
<td>F.O. &amp; F.V.</td>
</tr>
<tr>
<td>-</td>
<td>Manhattan</td>
<td><em>L. perenne</em> L.</td>
<td>4x</td>
<td>Oregon, USA</td>
<td>GSC</td>
<td>2</td>
<td>NT*</td>
</tr>
<tr>
<td>-</td>
<td>Paragon GLR</td>
<td><em>L. perenne</em> L.</td>
<td>4x</td>
<td>Oregon, USA</td>
<td>GSC</td>
<td>2</td>
<td>NT*</td>
</tr>
</tbody>
</table>
$2x = 14; 4x = 28; 4x^5 = 4x^5$ (varied) = 28–35; $6x = 42; 8x = 56; 9x = 63.$  

$^1$ FRRL = USDA-ARS Forage Range Research Laboratory, D. Johnson Logan Utah; GRIN = Germplasm Resource Information Network, USDA; BSC = Barenbrug Seed Company, Tangent, Oregon; GSC = Granite seed company, Tremonton, Utah; USU = Utah State University, Paul G. Johnson, 2010 expedition, Logan Utah; BFI = BFI native seeds company, Moses Lake, Wyoming; WPN = Plant was obtained as a whole from White pine Nursery, North Logan Utah; OSC = Oregro seed company, Albany, Oregon.  

$^3$ See figure 8 (Swofford 1998).  

$^4$ See figure 9 (Pritchard et al 2000); NT* = Accessions not tested to simplify the analysis; PI, R, and W6 = Plant introduction number according to the US Department of Agriculture Germplasm Resources Information Network (GRIN); PRC = People’s Republic of China; KGZ = Kyrgyzstan; F. O. & F. V. = Festuca ovina and Festuca valesiaca; F. T. = Festuca trachyphylla; F. R. = Festuca rubra; F. I. = Festuca idahoensis.
3-2 Analysis of molecular variance of seven fine-leaved *Festuca* species (*F. valesiaca*, *F. ovina*, *F. rubra*, *F. idahoensis*, *F. trachyphylla*, *F. roemerii*, and *F. filiformis*) partitioned into five clusters (*Festuca ovina*, *Festuca idahoensis*, *Festuca trachyphylla*, *Festuca Festuca rubra*, and *Festuca valesiaca*) based on pair wise genetic distance matrix constructed using 1,689 AFLP markers (*EcoR I* and *Mse I*).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance of components</th>
<th>Percentage of variation</th>
<th>P value</th>
<th>Fixation index (F_{st})*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups</td>
<td>4</td>
<td>3957.9</td>
<td>37.7</td>
<td>15.7</td>
<td>0.0665</td>
<td>0.16</td>
</tr>
<tr>
<td>Among populations within groups</td>
<td>2</td>
<td>469.9</td>
<td>10.9</td>
<td>4.6</td>
<td>0.0108</td>
<td>0.05</td>
</tr>
<tr>
<td>Within populations</td>
<td>80</td>
<td>15358.8</td>
<td>191.9</td>
<td>79.8</td>
<td>0.0001</td>
<td>0.20</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>19786.6</td>
<td>240.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A measure of population differentiation and genetic distance based on genetic polymorphic data = Hartl et al. 2007.
3-3 Pair wise matrix of percentage the genetic variation among accessions of seven fine-leaved *Festuca* species as assessed with 1,689 AFLP markers (*EcoR I* and *Mse I*).

<table>
<thead>
<tr>
<th></th>
<th><em>F. trachyphylla</em></th>
<th><em>F. filiformis</em></th>
<th><em>F. ovina</em></th>
<th><em>F. valesiaca</em></th>
<th><em>F. idahoensis</em></th>
<th><em>F. roemer</em></th>
<th><em>F. rubra</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. trachyphylla</em></td>
<td>0.12*</td>
<td>0</td>
<td>0.17**</td>
<td>0.22***</td>
<td>0.28***</td>
<td>0.29**</td>
<td>0.27**</td>
</tr>
<tr>
<td><em>F. filiformis</em></td>
<td>0</td>
<td>0</td>
<td>0.13**</td>
<td>0.16**</td>
<td>0.20*</td>
<td>0.19**</td>
<td>0.21**</td>
</tr>
<tr>
<td><em>F. ovina</em></td>
<td>0.17**</td>
<td>0.13**</td>
<td>0</td>
<td>0.02***</td>
<td>0.22***</td>
<td>0.23**</td>
<td>0.23**</td>
</tr>
<tr>
<td><em>F. valesiaca</em></td>
<td>0.22***</td>
<td>0.16**</td>
<td>0.13**</td>
<td>0</td>
<td>0.28***</td>
<td>0.24**</td>
<td>0.26**</td>
</tr>
<tr>
<td><em>F. idahoensis</em></td>
<td>0.28***</td>
<td>0.20*</td>
<td>0.22***</td>
<td>0.23**</td>
<td>0.30**</td>
<td>0.29**</td>
<td>0.21**</td>
</tr>
<tr>
<td><em>F. roemer</em></td>
<td>0.29**</td>
<td>0.19**</td>
<td>0.22***</td>
<td>0.24**</td>
<td>0.03**</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>F. rubra</em></td>
<td>0.27**</td>
<td>0.21**</td>
<td>0.23**</td>
<td>0.26**</td>
<td>0.29**</td>
<td>0.29**</td>
<td>0</td>
</tr>
</tbody>
</table>

Significant differences at P< 0.05, 0.01, 0.001, and nonsignificant designated as *, **, ***, and NS, respectively.
3-4 Matrix of corrected (below diagonal) average number of pairwise differences between seven *Festuca* species (populations), within species (diagonal), and total difference between species (above diagonal) as assessed by 1,689 AFLP markers.

<table>
<thead>
<tr>
<th></th>
<th><em>F. trachyphylla</em></th>
<th><em>F. filiformis</em></th>
<th><em>F. ovina</em></th>
<th><em>F. valesiaca</em></th>
<th><em>F. idahoensis</em></th>
<th><em>F. roemeri</em></th>
<th><em>F. rubra</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. trachyphylla</em></td>
<td>346.07(^{NS})</td>
<td>385.75</td>
<td>453.37</td>
<td>469.10</td>
<td>500.50</td>
<td>518.60</td>
<td>513.90</td>
</tr>
<tr>
<td><em>F. filiformis</em></td>
<td>53.22(^{NS})</td>
<td>319.00(^{NS})</td>
<td>438.56</td>
<td>435.17</td>
<td>453.11</td>
<td>466.50</td>
<td>482.43</td>
</tr>
<tr>
<td><em>F. ovina</em></td>
<td>79.92(^{***})</td>
<td>78.64(^{*})</td>
<td>400.82(^{NS})</td>
<td>399.24</td>
<td>498.24</td>
<td>519.89</td>
<td>524.19</td>
</tr>
<tr>
<td><em>F. valesiaca</em></td>
<td>106.49(^{***})</td>
<td>86.09(^{*})</td>
<td>9.25(^{*})</td>
<td>379.15(^{NS})</td>
<td>489.83</td>
<td>509.64</td>
<td>522.53</td>
</tr>
<tr>
<td><em>F. idahoensis</em></td>
<td>139.09(^{***})</td>
<td>105.24(^{*})</td>
<td>109.45(^{***})</td>
<td>111.88(^{***})</td>
<td>376.75(^{NS})</td>
<td>402.52</td>
<td>548.70</td>
</tr>
<tr>
<td><em>F. roemeri</em></td>
<td>139.23(^{**})</td>
<td>100.67(^{NS})</td>
<td>113.14(^{***})</td>
<td>113.74(^{***})</td>
<td>7.81(^{NS})</td>
<td>412.67(^{NS})</td>
<td>512.00</td>
</tr>
<tr>
<td><em>F. rubra</em></td>
<td>139.61(^{***})</td>
<td>121.68(^{*})</td>
<td>122.52(^{***})</td>
<td>131.69(^{***})</td>
<td>159.06(^{***})</td>
<td>104.41(^{**})</td>
<td>402.51(^{NS})</td>
</tr>
</tbody>
</table>

Significant differences at P< 0.05, 0.01, 0.001, and nonsignificant designated as *, **, ***, and NS, respectively.
3-1 A genographer gel showing amplification of DNA fragments of 48 Festuca species using AFLP markers (Derived from EcoRI and MseI restriction digestion) from 50 bp to 500 bp at 100% resolution. Each blue band in the row indicates one marker and each column demonstrates every marker for one sample.
3-2 Thumbnail of 48 *Festuca* species and each of them represents the signal intensity (peak) for a specific individual’s AFLP (*EcoR I* and *Mse I*) marker as present (A) or absent (B).
3-3 Genetic relationships among *Festuca valesiaca* (va), *Festuca rubra* (ru), *Festuca idahoensis* (id), *Festuca ovina* (ov), *Festuca trachyphylla* (tr), *Festuca roemeri* (ro), and *Festuca filiformis* (fi) accessions based on NeiLi’s genetic distance among AFLP profiles after 1000 bootstrap permutations (Swofford 1998).
3-4 Natural log (Ln) probability of clustering seven fine-leaved *Festuca* species (*Festuca valesiaca*, *Festuca ovina*, *Festuca rubra*, *Festuca idahoensis*, *Festuca trachyphylla*, *Festuca filiformis*, and *Festuca roemeri*) over K values as inferred by genetic AFLP-based Bayesian cluster analysis with three replications according to Pritchard et al (2000).
3-5 Colored bar graphs depicting the inferred ancestry coefficients from Bayesian cluster analysis of AFLP genotypes from *Festuca valesiaca* (va), *Festuca rubra* (ru), *Festuca idahoensis* (id), *Festuca ovina* (ov), *Festuca trachyphylla* (tr), *Festuca roemeri* (ro), and *Festuca filiformis* (fi) accessions with 100000 burn-in where Pop 1, Pop 2, Pop 3, Pop 4, Pop 5, and Pop 6 are population numbers as defined within each K value according to Pritchard et al (2000).
4. GENETIC RELATEDNESS OF FESTUCA OVINA COMPLEX TO THE REFERENCE TAXA BY CHLOROPLAST DNA SEQUENCES

ABSTRACT

*Festuca* species have considerable agronomic and horticultural importance and are often clustered broad species complex. The *Festuca ovina* complex includes *Festuca idahoensis*, *Festuca ovina*, *Festuca trachyphylla*, *Festuca filiformis*, and *Festuca valesiaca*. There are few relatively morphological differences between *Festuca ovina* and *Festuca valesiaca* and these are assorted with glume length, anther, and glume. However, the genetic relationships among the *Festuca ovina* complex and the other reference taxa have not been investigated. This project was designed to determine the phylogenetic relationship among *Festuca ovina* complex and the other reference taxa using chloroplast DNA sequence analysis. Three chloroplast intergenic spacers (800 bp) were selected to sequence three genetic sub regions using the universal primer combinations designed for plant chloroplast gene sequences. Phylogenetic relationships were determined by heuristic parsimony and genetic distance analysis of these three regions defined three distinct clusters. These three clusters were reference taxon (*Festuca pratensis* and *Dactylis glomerata*), *Festuca rubra* cluster, and *Festuca ovina* complex accession examined. Within the *Festuca ovina* complex accessions examined which showed considerable affinities, genetic comparison detected a difference between *Festuca ovina* and *Festuca valesiaca* cluster and other *Festuca ovina* complex taxon in distance method based tree while no significant divergence was detected in the parsimony method based tree. Similarly, *Festuca ovina* complex species differed from the *Festuca rubra* and other reference taxon. The *Festuca ovina* and *Festuca valesiaca* were clustered together.
INTRODUCTION

The chloroplast genome (cpDNA) is a comparatively small portion of the plant genome (Clegg et al. 1994), however, phylogenetically informative variations exist among closely related plants species in noncoding regions of the chloroplast genome, which can be useful in determining evolutionary relationships (Olmstead and Palmer 1994). Direct sequencing of DNA is considered to be a reliable tool for phylogenetic analysis (Jude 1999) and, thus, has been used widely in determining evolutionary relationships (Demere et al. 1995; Downie and Palmer 1992; Olmstead et al. 1990; Palmer et al. 1988). Since the circular chloroplast DNA has been shown to be highly conserved in structure (Palmer and Stein 1986), cpDNA sequence analysis at intergenic spacers has been widely used to investigate the intraspecific relationships (Clegg et al. 1991; Palmer et al. 1988). The noncoding intergenic spacer of \textit{trnT} (UAA)-\textit{trnF} (GAA) has been the most frequently used cpDNA sequencing region for phylogenetic studies (Bohle et al. 1994; Gielly and Taberlet 1994; Ham et al. 1994; Mes and Hart 1994). Nevertheless, other intergenic spacer regions such as \textit{psbA-trnH}-2 have also been used to assess interspecific relationships in \textit{Paeonia} (Aldrich et al. 1988; Sang et al. 1997), barcode flowering plants, and phylogenetic relationship assessment at the species level (Kress et al. 2005).

SDS-PAGE on seed protein analysis had been used to define the relationships among fine-leaved \textit{Festuca} species (i.e., \textit{F. campestris}, \textit{F. altaica}, \textit{F. hallii}, \textit{F. calijornica}, \textit{F. brachyphylla}, \textit{F. idahoensis}, and \textit{F. trachyphylla}) (Aiken and Gardiners1991). Genetic relationships among North American \textit{Festuca} and related genera have also been described using chloroplast DNA based restriction site variation analysis (Darbyshire and Warwick 1992). Phylogenetic relationships among species of the \textit{Festuca-Lolium} complex have been assessed using ITS sequence data (Gaut et al. 2000). Likewise, Catalan et al. (2004) used ITS and \textit{TrnL-F} chloroplast sequences to define the genetic relationships among broad-leaved \textit{Festuca} species (\textit{F.pratensis} and the \textit{F. arundinacea} complex) and fine-leaved \textit{F.} species (\textit{F. ovina} and \textit{F. rubra}), as well as orchard
grass (*Dactylis*). Nevertheless, species relationships ancestral lineages in fine-leaved *F*. species were not completely resolved using both ITS and chloroplast sequence analysis (Catalan et al. 2004). Given that hybridization among open pollinated *Festuca* species is considered as the main source of driving evolution (Soreng and Davis 2000). Difficulty in defining species relationships among fine-leaved *Festuca* species is predictable. Therefore, a project was designed to define the phylogenetic relationships among the fine-leaved *Festuca* species using three intergenic chloroplast spacers regions.

**MATERIALS AND METHODS**

**DNA Extraction, PCR Amplification, and DNA Sequencing**

A subsample of species representatives (Table 3-1) were used and their genomic DNA was extracted from lyophilized tissue using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA) following the manufacturer’s instructions, which was then used as template DNA for PCR amplification. The universal chloroplast primer combinations of a/b (*trn*T-L) and c/f (*trn*L-F) as described by Taberlet et al. (1991), as well as *psbA*-F (Sang et al. 1997), and *trnH*-2 (Tate and Simpson 2003) were used to amplify the *trn*T-L, *trn*L-F, and *psbA*- *trnH*-2 regions of the species examined, respectively. A total volume of 25µL containing 10 ng of genomic DNA, 1x buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 2-5 mM MgCl₂), 0.4 µM of each primer (Table 4-1), 10 µM of each dNTP (New England Biolabs Inc.), and 0.5 U of Taq polymerase (Life technologies) was subjected to polymerase chain reaction (PCR) of 1 min at 94 °C, 35 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C, and 90 seconds at 72 °C, followed by an extension step of 5 min at 72°C according to Taberlet et al. (1991). PCR reaction fragments were visualized on a 1.6% agarose gel to verify amplification and for quantification (Taberlet et al. 1991). Amplified products were purified for sequencing by adding Exonuclease I to get rid of the left over primers (0.0075U/µL of PCR product) and Shrimp Alkaline Phosphatase to remove the dNTPs (0.15U/µL of PCR
product) and incubated for 20 min at 37°C, then inactivating for 15 min at 80°C in a thermocycler according to the ExoSAP-IT kit protocol (Affymetrix, Santa Clara, CA). Thereafter, 50 ng of purified amplicons were combined with 10 μM forward or reverse primer, 1X PCR buffer (Applied Biosystems), 0.25 μL of Big Dye (Applied Biosystems) at a total volume of 10 μL for sequencing reaction. This sequencing reactions were carried out as 1 min at 94 °C followed by 55 cycles of 10 seconds at 94 °C, 5 seconds at 50 °C, and 4 min at 60 °C. Sequencing reaction products were purified with the BigDye X Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems) according to the manufacturer’s protocol, and subsequently sequenced on an ABI PRISM 310 automated sequencer (Applied Biosystems) at the Utah State University Center for Integrated Biosystems Logan, UT.

**Phylogenetic Analysis**

Sequence alignments (Fig. 4-1) of overlapping, forward, and reverse reads for each of three chloroplast sub regions \[trn\text{-}T\text{-}L\ (a/b), \text{trn}\text{-}L\text{-}F\ (c/f), \text{psbA\text{-}trnH}\text{-}2\] were compared using software resident in the Staden Package, version 1.5 (Staden 1977; 1978). Suspect base calls were visually inspected from chromatograms (Fig. 4-2) and corrected by comparing forward and reverse sequences manually. The sequence information on insertions/deletions (indels) was converted into binary matrix data and added to the end of the sequence of each individual accession (Simmons and Ochoterna 2000). If both components (forward and reverse) of the sequences could not be joined into one single sequence, then the sequences between forward and reverse were designated as missing data. The subsequent sequences (1977) alignments information from all three regions was used to perform a phylogenetic analysis by heuristic parsimony and distance method using PAUP version 4.0b10 (Swofford 1998). The neighbor-joining trees were constructed after 1,000 bootstrap replications using the heuristic search option with TBR (tree bisection and reconnection) branch swapping and random sequence addition to
assess the reliability of the tree for both types of analyses (Felsenstein 1985). The *D. glomerata* (R08-148) and *P. pratensis* (R08-105) accessions examined were used to root the cladograms for comparative species analysis.

**RESULTS**

**Chloroplast DNA Sequence**

In total, 1,977 aligned cpDNA nucleotide positions were identified from intergenic spacer regions between *trn*T and *trn*L 5’ exon (970 bp), *trn*L 3’ exon and *trn*F (832 bp), as well as *psb*A-F and *trn*H-2 (653 bp). The region sequenced by *psb*A-F and *trn*H-2 was less variable than the other two regions (*trn*T and *trn*L 5’ exon and *trn*L 3’ exon and *trn*F). Totally, 40 insertions/deletions (indels) with at least one nucleotide were identified in these three intergenic spacers regions. The longest indels had eight-nucleotides (position 128-136 from *trn*L 3’ exon to *trn*F) from *F. arundinacea* (‘Coronado’), *F. pratensis* (PI380858), and *F. rubra* accessions of PI 659950, PI 659954, PI 659981, and W6 30537 (position 704-711, 539-546). The 1,977 sites (236; 12%) defined polymorphisms in at least one of the taxon plus indels and these were used for accessions differentiations.

**Chloroplast DNA Sequence-based Cluster Analyses**

Fifty percent majority rule consensus trees from 218 trees were constructed based on heuristic parsimony method (Fig. 4-3) genetic distance appraisal method (Fig. 4-4). However, the cladogram based on heuristic parsimony was not significantly different from the distance method based cladogram (Fig. 4-3; Fig. 4-4). Given the phylogenetic trees from heuristic parsimony analysis, two clusters ond consisting of *F. rubra* cluster and *F. ovina* complex cluster (*F. ovina*, *F. valesiaca*, *F. trachyphylla*, *F. filiformis*, and *F. idahoensis*) were defined in the fine-leaved fescue besides the root which were *D. glomerata* cluster, *F. arundinacea* (‘Coronado’ and ‘Matador’),
and *F. pratensis* (PI 380858) (Fig. 4-3). Species accessions of *F. ovina* complex, shared considerable genic affinities (Fig. 4-3). *F. rubra* commercial cultivar (‘Boreal’) was, however, similar to the *F. ovina* complex accessions (Fig. 4-3).

Fine-leaved fescue accessions were partitioned into three clades by (Fig. 4-4) distance based phylogenetic analysis. Two clades of *F. rubra* species and the *F. ovina* complex were partitioned from both methods (Fig. 4-4). One *F. valesiaca* accession (W6 30537), however, was placed with the *F. rubra* accessions based on sequences similarities (Fig. 4-4). Within the *F. ovina* complex, one large cluster including *F. ovina* and *F. valesiaca* accessions were defined in a single cluster (Fig. 4-4). However, one *F. idahoensis* accession (‘Siskiyou Blue’) possessed genetic affinities with the large *F. ovina* accessions (Fig. 4-4). No significant differences were detected among *F. trachyphylla*, *F. filiformis* lineage, *F. idahoensis*, and two *F. ovina* accessions (‘Marcopolo’ and PI 383652) based on cpDNA genetic distance analysis (Fig. 4-4).

**DISCUSSION**

**Over-arching Genetic Relationships among *Festuca* Species**

The genetic size of the three chloroplast intergenic spacer regions of *Festuca* species studied herein were similar to other related grasses which are similar to other species (Taberlet et al. 1991). Studies based on RFLP and ITS phylogenetic indicated that the genus *Festuca* was paraphyly (Charmet et al. 1997; Darbyshire and Warwick 1992; Gaut et al. 2000). However, phylogenetic reconstructions of diverse fine fescue species defined them as a monophyletic cluster (Torrecilla and Catalan 2002). More recently, both separate and combined ITS and *trnL*-F sequences analyses confirmed this assertion (Catalan et al. 2004) and is also supported by the data presented herein (Fig 4-4, Fig 4-4). This research achieved similar results of monophyly for fine-leaved *Festuca* species from the most parsimonious tree based on the cpDNA sequences with strong bootstrap values (Fig. 4-3). Seeds protein analysis by SDS-PAGE (Aiken and Gardiners
1991) proved for differentiation of *F. rubra* and *F. ovina* complex species which was further confirmed by the chloroplast DNA sequence analysis conducted herein (Fig. 4-3, Fig. 4-4).

Several studies (Catalan et al. 2004; Charmet et al. 1997; Darbyshire and Warwick 1992) have demonstrated that the broad-leaved (*F. arundinacea* and *F. pratensis*) *Festuca* species were differed genotypically from fine-leaved *Festuca* species while this research further confirmed the significant differences between these two based on the heuristic parsimony based and distance based phylogenetic trees (Fig. 4-3, Fig. 4-4). The chloroplast DNA sequence analysis in this study differentiated the broad-leaved *Festuca* species (‘Coronado’, ‘Matador’, and PI 380858) and the fine-leaved *Festuca* species accessions examined (Fig. 4-3, Fig. 4-4). In addition, data indicated that meadow fescue (*F. pratensis*) and tall fescue (*F. arundinacea*) from North America and European countries shared considerable genetic affinities (Fig. 4-3, Fig. 4-4).

Darbyshire and Warwick (1992) indicated that while phylogenies of the maternally inherited fescue chloroplast genome may not completely reflect evolutionary relationships of species due to hybridization, maternal information can be used to reveal their maternal origins of species. The research conducted herein focused mainly on genetic relationships among fine-leaved species in the genus Festuca (Fig. 4-3; Fig. 4-4). As depicted in the heuristic parsimony and distance based trees, two clusters were identified at species level (Fig. 4-3; Fig. 4-4). As such, these data confirmed the results of Gaut et al. (2000) which assessed that *F. ovina* complex differed genetically from *F. rubra* (Fig. 4-3, Fig. 4-4). The *F. ovina* complex species accessions species; however, possess considerable genetic affinities based on the chloroplast DNA sequences analysis conducted herein. The maternal gene (cytoplasm) of the *F. ovina* complex was from *F. ovina* species while the pollen was from each designated species defined based on the heuristic parsimony phylogenetic tree (Fig. 4-3, Fig. 4-4). For example, the *F. rubra* commercial cultivar ‘Boreal’ was defined different from *F. rubra* (Fig. 4-3, Fig. 4-4) because the pollen was from *F. rubra* while female parent was from the *F. ovina* plants during pollination. Similarly, three *F.
commercial cultivars (‘Siskiyou Blue’, ‘Blacksheep’, and ‘Marcopolo’) were defined in the

*F. trachyphylla* clade based on AFLP profile while ‘Siskiyou Blue’ and ‘Blacksheep’ were closer
to *F. ovina* and ‘Marcopolo’ was closer to *F. trachyphylla* based on cpDNA sequence (Fig. 3-3; Fig. 4-3; Fig. 4-4). Additionally, cultivar (‘Boreal’) clustered in *F. trachyphylla* clade can be postulated to be the hybridizations between *F. trachyphylla* and *Festuca rubra*. Likewise, the *F. ovina* cultivar (‘Marcopolo’) could be the hybrid from *F. trachyphylla* and *F. ovina* based on the tree from cpDNA sequence (Fig. 4-3; Fig. 4-4).

**Genetic Relationships among *Festuca rubra* and *Festuca trachyphylla***

Based on the similarities in the early stages of sheath development between *F. rubra* and *F. trachyphylla*, they were clustered together (Liu and Dengler 1992). In contrast, the cpDNA analysis conducted herein indicated that these two species do not possess strong genetic affinities (Fig. 4-3, Fig. 4-4). Catalán et al. (2004) concluded the *F. rubra* differed apparently from *F. ovina* and *F. idahoensis* based on the majority rule consensus tree which confirmed the AFLP and cpDNA analysis conducted herein (Fig. 4-3, Fig. 4-4). Likewise, AFLP profiles indicated that *F. idahoensis* and *F. roemeri* are genetically similar (Fig. 4-3; Fig. 4-4) which confirmed the assertion of Jones et al. (2008). This will help to clarify the genetic profiles of commercial cultivars and provide scientific report of introduced *F. valesiaca* under the environment of western United State.

**CONCLUSIONS**

Both cpDNA sequences (Fig. 4-3, Fig. 4-4) and AFLP profiles demonstrated that *F. valesiaca* is a sister taxa of *F. ovina*. Likewise, *F. trachyphylla* and *F. filifromis* share considerable genetic affinities. However, four *F. ovina* accessions (PI 618975, PI 634304, PI 595178, and PI 252125) examined were genetically similar to *F. valesiaca* and the *F. valesiaca* accession (W6 30537) should be reclassified as *F. rubra*. The *F. ovina* complex species had the similar maternal
inheritance. In the same vein, three previously classified *F. ovina* commercial cultivars (‘Siskiyou Blue’, ‘Blacksheep’, and ‘Marcopolo’) possess genetic affinities with *F. trachyphylla*, however, based on AFLP profiles, ‘Fsiskiyou Blue’ and ‘Blacksheep’ are genetically similar to *F. ovina* and ‘Marcopolo’ is closely related to *F. trachyphylla* based on cpDNA sequence analysis (Fig. 4-3, Fig. 4-4). Additionally, the *F. rubra* cultivar (‘Boreal’) which clustered with *F. trachyphylla* accessions can be postulated to be the result of hybridization between *F. trachyphylla* and *F. rubra* germplasm. Likewise, the *F. ovina* cultivar (‘Marocpolo’) is likely to be the result of hybridization between *F. trachyphylla* and *F. ovina* (Fig. 4-4).

REFERENCES


Clegg MT, Learn GH, Golenberg EM (1991) Molecular evolution of chloroplast DNA. In:
Selander RK, Clark AG, Whittam TS (eds) Evolution at the molecular level, Sinauer Associates, Sunderland, Massachusetts, pp 135-149


Liu Y, Dengler NG (1992) Development of open and closed leaf sheaths in Festuca trachyphylla
and *Festuca rubra* (*Poaceae*). Can J Bot 70: 1417-1428


Tate JA, Simpson BB (2003) Paraphyly of Tarasa (Malvaceae) and diverse origins of the polyploidy species. Syst Bot 28: 723-737

4-1 Chloroplast DNA sequence order of six primers used for amplification of three cpDNA intergenic spacer regions in fine-leaved *Festuca* species

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sequence (5’-3’)</th>
<th>size (bp)</th>
<th>Annealing temperature</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrnT-L</td>
<td>CGAAATCGGTAGACGCTACG</td>
<td>1050</td>
<td>55 °C</td>
<td>Catalan 2004; Torrecilla et al. 2003; Taberlet et al. 1991</td>
</tr>
<tr>
<td></td>
<td>ATTTGAACTGTGACACGAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trnL-F</td>
<td>CATTACAAATCGATGCTCT</td>
<td>900</td>
<td>55 °C</td>
<td>Teberlet et al. 1991</td>
</tr>
<tr>
<td></td>
<td>TCTACCGATTTCCCATATC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CGCGCATGGTGATTCCAAATC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4-1 Graphic depiction of sequence chromatogram showing evenly-spaced peaks presented of a baseline ('noise') from six *Festuca* species accessions.
4.2 Sequences alignment from *Festuca* species as depicted using software from Staden Package v1.50 by optimally aligning with polymorphic sites (indicated with blue highlights) and manually adjusted nucleotide (indicated by lowercase letter).
4-3 Chloroplast DNA sequence strict consensus tree obtained from 1,000 heuristic parsimony bootstrap search for 36 *Festuca* (*Festuca valesiaca* (va), *Festuca rubra* (ru), *Festuca idahoensis* (id), *Festuca ovina* (ov), *Festuca trachyphylla* (tr), and *Festuca filiformis* (fi)) taxon.
4-4 Neighbor-joining tree constituted from pairwise comparisons of NeiLi’s distance for 36 *Festuca* (*Festuca valesiaca* (va), *Festuca rubra* (ru), *Festuca idahoensis* (id), *Festuca ovina* (ov), *Festuca trachyphylla* (tr), and *Festuca filiformis* (fi)) taxon based on chloroplast DNA sequences analysis.
5. SUMMARY

Morphological trait evaluation indicated that the plant height, plant width, and total biomass of the *F. valesiaca* accessions examined were equal to the control ‘Cascade’ (*F. rubra*). The plant vigor and seed weight of accessions PI 659923, PI 659932, W6 30575, and W6 30588 were, however, significantly higher than ‘Cascade’. Principal component analysis using all traits as loading factors suggested that these accessions were distinct from the majority of the accessions examined. In conclusion, the *F. valesiaca* accessions examined produced abundance of small seeds. Seed production was significantly (\(P = 0.001\)) correlated (\(r^2 = 0.84\)) with the total biomass, plant height, and plant vigor rating. The *F. valesiaca* accessions examined possessed lower height than the control ‘Cascade’ but higher biomass, spring green-up, and seed production. *F. valesiaca* accessions possess similar trait performance, which was higher than ‘Cascade’ in principal component analysis. Given their morphological attributes, *F. valesiaca* accessions PI 659923, W6 30575, and W6 30588 should be considered for low-maintenance applications and use in plant improvement.

The AFLP-based neighbor-joining analysis partitioned *F. valesiaca* accessions and closely related taxa into five distinct clusters consisting of reference *F.* species (Cluster 1), *F. idahoensis* (Cluster 3), *F. rubra* (Cluster 2), *F. ovina* and *F. valesiaca* (Cluster 6), and *F. trachyphylla* (Cluster 7). These species relationships were further confirmed by a Bayesian cluster analysis. Analysis of molecular variance detected low admixture but significant between the *F. valesiaca* and *F. ovina* accessions examined predictably. In conclusion, broad-leaf species (*F. arundinacea*, *F. pratensis*, and *Lolium perenne*) were different from fine-leaved *Festuca* species. *F. valesiaca* is a closely related subcluster of *F. ovina* and should be considered as one species. *Festuca trachyphylla* is a subcluster under *F. ovina* and *F. valesiaca*. *F. idahoensis* has close relationship with *F. roemeri* but not with *F. ovina*. Low admixture was detected between the *F. rubra* and *F. trachyphylla* accessions examined, while a comparative high admixture was detected among the commercial cultivars examined.

Three 800 bp polymorphic chloroplast intergenic spacer subregions were identified to evaluate the genetic differences among the *Festuca* species examined. Phylogenetic relationships were determined
by heuristic parsimony and distance analyses of genetic variation within these regions. Three clusters containing reference taxa (*Festuca pratensis* and *D. glomerata*), *F. rubra* cluster, and *F. ovina* complex cluster were defined by both analyses. Within the *F. ovina* complex, there was a divergence between *F. ovina* with *F. valesiaca* cluster and other *F. ovina* complex accessions in distance based tree while no significant divergence existed in the parsimony based tree. Data reconfirmed that the *F. ovina* complex genetically differed from *F. rubra* and the other reference taxa examined. *F. valesiaca* and *F. ovina* possessed the same maternal lineage based on chloroplast DNA sequence analysis. One *F. valesiaca* accession, W6 30537, was genetically similar to the *F. rubra* examined and should be putatively reclassified as *F. rubra* pending further taxonomic analysis.
A statistical model summary of a split plot in time design used to assess differences among *Festuca* species accessions over multiple years (2009-2011) at Blue Creek, Utah.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Factor levels</th>
<th>Factors fixed/ random</th>
<th>Factors crossed/ nested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year (Y) _α_i</td>
<td>3</td>
<td>Fixed</td>
<td>Crossed with Entry</td>
</tr>
<tr>
<td>Accession (E) _β_j</td>
<td>20</td>
<td>Fixed</td>
<td>Crossed with Year</td>
</tr>
<tr>
<td>Block (B) _γ_k</td>
<td>6</td>
<td>Random</td>
<td>Crossed with Year</td>
</tr>
</tbody>
</table>
A statistical model summary of a split plot in time design where year is the whole plot factor for the assessment of morphological traits of *Festuca* species accessions examined over multiple years (2009-2011) at Blue Creek, Utah.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Fixed i</th>
<th>Fixed j</th>
<th>Random k</th>
<th>Random l</th>
<th>Product</th>
<th>Variance</th>
<th>Expected Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_i(Y)$</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>18</td>
<td>$Q(Y, E^*Y)$</td>
<td>$\sigma^2_{SPE} + 3\sigma^2_{WPE} + 18Q(Y, E^*Y)$</td>
</tr>
<tr>
<td>$\beta_j(E)$</td>
<td>20</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>120</td>
<td>$Q(E, E^*Y)$</td>
<td>$\sigma^2_{SPE} + 120Q(E, E^*Y)$</td>
</tr>
<tr>
<td>$\alpha\beta_{ij}(EY)$</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>$Q(E^*Y)$</td>
<td>$\sigma^2_{SPE} + 6Q(E^*Y)$</td>
</tr>
<tr>
<td>$\gamma_k(B)$</td>
<td>20</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>60</td>
<td>$\sigma^2_{BLK}$</td>
<td>$\sigma^2_{SPE} + 3\sigma^2_{WPE} + 60\sigma^2_{BLK}$</td>
</tr>
<tr>
<td>$\delta_{ijk}(YB)$</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>$\sigma^2_{WPE}$</td>
<td>$\sigma^2_{SPE} + 3\sigma^2_{WPE}$</td>
</tr>
<tr>
<td>$\epsilon_{ijkl}(EBY)$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>$\sigma^2_{SPE}$</td>
<td>$\sigma^2_{SPE}$</td>
</tr>
</tbody>
</table>

i, j, k, l are the subscript for factor of year, accession, block, and error, respectively.
A-3 A statistical model summary of a randomized complete block design and data analysis of morphological traits (plant height, width, vigor rating, total biomass, seed weight, and seed number) per plant of *Festuca* species accessions examined over multiple years (2009-2011) at Blue Creek, Utah.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Factor levels</th>
<th>Factor fixed/random</th>
<th>Factor crossed/ nested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accession (E_\alpha_i)</td>
<td>20</td>
<td>Fixed</td>
<td>Crossed with Block</td>
</tr>
<tr>
<td>Block (B_\beta_j)</td>
<td>6</td>
<td>Random</td>
<td>Crossed with Accession</td>
</tr>
</tbody>
</table>
A-4 Taxonomical traits descriptions of five *Festuca ovina* complex species as Ruemmele et al. (2003) complied.

<table>
<thead>
<tr>
<th>Taxonomical traits</th>
<th><em>F. filiformis</em></th>
<th><em>F. idahoensis</em></th>
<th><em>F. trachyphylla</em></th>
<th><em>F. ovina</em></th>
<th><em>F. valesiaca</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clums (cm)</td>
<td>15-55</td>
<td>30-100</td>
<td>20-75</td>
<td>10-45</td>
<td>20-50</td>
</tr>
<tr>
<td>Ligules (mm)</td>
<td>0.1-0.3</td>
<td>0.3-0.6</td>
<td>short</td>
<td>short</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Blades length (mm)</td>
<td>5-30</td>
<td>15-30</td>
<td>3.5-19</td>
<td>2-10</td>
<td>NA</td>
</tr>
<tr>
<td>Blades width (mm)</td>
<td>0.2-0.4</td>
<td>0.6-1</td>
<td>0.4-1</td>
<td>0.2-0.7</td>
<td>0.2-0.6</td>
</tr>
<tr>
<td>Inflorescence length (cm)</td>
<td>1-7</td>
<td>7-16</td>
<td>3-9.5</td>
<td>5-10</td>
<td>3-10</td>
</tr>
<tr>
<td>Spikelet length (mm)</td>
<td>3-6.5</td>
<td>7.5-13.5</td>
<td>5.5-9</td>
<td>4-6</td>
<td>6-6.7</td>
</tr>
<tr>
<td>Lower Glume length (mm)</td>
<td>1.2-2.5</td>
<td>2.4-4.5</td>
<td>2-3.5</td>
<td>1.7-2.5</td>
<td>2-2.5</td>
</tr>
<tr>
<td>lemma length (mm)</td>
<td>2.3-4.4</td>
<td>5-8.5</td>
<td>3-5.5</td>
<td>2.2-4</td>
<td>2.6-3.9</td>
</tr>
<tr>
<td>Awns length (mm)</td>
<td>0-0.4</td>
<td>3-6</td>
<td>0.5-2.5</td>
<td>0.7-2</td>
<td>1.2</td>
</tr>
<tr>
<td>Anthers (mm)</td>
<td>1.5-2.2</td>
<td>2.5-4</td>
<td>2.5-3.4</td>
<td>1.6-2.5</td>
<td>2.2-2.6</td>
</tr>
<tr>
<td>Large veins</td>
<td>3</td>
<td>3-5</td>
<td>5-7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Small veins</td>
<td>0-4</td>
<td>2-5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ribs</td>
<td>1</td>
<td>3-5</td>
<td>5-7</td>
<td>1</td>
<td>1.5</td>
</tr>
</tbody>
</table>