

Phenotypic and genetic characterization of Kyrgyz fine-leaved *Festuca valesiaca* germplasm for use in semi-arid, low-maintenance turf applications

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Abstract Fine-leaved *Festuca valesiaca* Schleich. ex Gaudin ($2n = 2x-4x$) is native to heavily-grazed, cold, semi-arid, Asian rangelands. However, its potential for low-maintenance turf applications in the semi-arid western United States and its relatedness to other agriculturally important *Festuca* species have not been investigated. Therefore, a project was designed to identify *F. valesiaca* accessions that possess horticultural potential when grown under semi-arid growing conditions and to characterize their relatedness to other *Festuca* species. In 2008, 12 *F. valesiaca* accessions originating from Kyrgyzstan and eight US *Festuca* and one *Lolium* cultivar were transplanted as replicated, spaced plants to a field nursery at Blue Creek, Utah.

Relative vigor, height, width, total biomass (above-ground dry matter yield), seed weight, and seed number were evaluated between 2009 and 2011. Plant height, width, and total biomass of the *F. valesiaca* accessions examined were approximately equal to the commercial control, ‘Cascade’ (*F. rubra* L. subsp. *commutata* Gaudin; 6x; chewings fescue). Plant vigor and seed weight of *F. valesiaca* accessions PI 659923, PI 659932, W6 30575, and W6 30588 under semi-arid conditions (~ 300 mm annual precipitation) were significantly ($P < 0.05$) greater than ‘Cascade’. Moreover, principal component analysis based on all traits as loading factors indicated that these 12 *F. valesiaca* accessions were distinct from a majority of the other *Festuca* accessions examined. These *F. valesiaca* accessions produced abundant amounts of small seed, and this seed yield was significantly correlated with total aboveground biomass (dry weight; $r^2 = 0.84$, $P < 0.001$), plant height ($r^2 = 0.58$, $P < 0.05$), and plant vigor ($r^2 = 0.83$, $P < 0.001$). Amplified fragment length polymorphism (AFLP) analysis (1,454 polymorphic bands) was used to characterize *F. valesiaca* relatedness to other economically important *Festuca* species. The AFLP-based, neighbor-joining analysis differentiated *F. valesiaca* accessions from US *Festuca* cultivars examined, except for ‘Durar’ (*F. ovina* L.; 6x; sheep fescue), to which they had strong genetic affinities. Given their morphological attributes, *F. valesiaca* PI 659923, W6 30575, PI 659932, and W6 30588 should be considered for use in low-maintenance, semi-arid turf improvement programs in the western US.

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Introduction

About 300 perennial, turfgrass species are members of the genus *Festuca* L. (Ruemmele et al. 2003). *Festuca* is a genetically diverse taxon that is comprised of both wide- and narrow-leaved phenotypes that have various commercial uses. There have been several taxonomic classifications of fine-leaved *Festuca* species that are congruent in many regards (e.g., Hultén and Fries 1986; Tutin et al. 1993; Barkworth et al. 2007). The taxonomic treatment of Barkworth et al. (2007) is used herein because of its historical context to North American flora and its recognition of introduced *Festuca* species worldwide to the North American continent. Where possible, other species designations are also cited to provide continuity with other taxonomic treatments.

Some *Festuca* species are used worldwide as turfgrasses {e.g., *F. rubra* L.; *F. ovina* L.; *F. arundinacea* Schreb. [synonym *Schedonorus arundinaceus* (Schreb.) Dumort.; Soreng et al. 2001]}, and others as roadside plantings [e.g., *F. ovina*; *F. trachyphylla* (Hack.) Krajina], and rangeland reclamation [e.g., *F. ovina*; *F. scabrella* Torr. ex Hooker (synonym *F. altaica* Trin.); Pavlick and Looman 1984] (Johnson 2003; Ruemmele et al. 1995). Turfgrass is the most widely planted irrigated crop in the United States (Milesi et al. 2005) and has multi-billion dollar economic impacts (Haydu et al. 2006). Both native and introduced fine-leaved fescues (such as *F. rubra*, *F. ovina*, and *F. trachyphylla*) are important species used in low-maintenance turf applications throughout much of North America, including parts of the western US (Bertin et al. 2009; Ruemmele et al. 2003) because of their drought and shade tolerance (Beard 1997; Ruemmele et al. 1995).

Arndt (2008) has classified diploid *F. valesiaca* Schleich. ex Gaudin subspecies [subsp. *valesiaca* (Tzvelev), *parviflora* (Hack.) Tracey, and *hypsophila* (St.-Yves) Tzvelev] into a polyploid series [$2n = 14$ (diploid) – 70 (decaploid)] consisting of at least 20 *Festuca* species, which are distributed in the sub-

continental and continental regions of the meridional and temperate zones in Eurasia, from Germany and France eastwards to northwest China. These and other North American *Festuca* and closely related species form a polyploid aggregate often called the Ovina Complex with a basic chromosome number of $2n = 2x = 14$ (Darbyshire and Warwick 1992; Jones et al. 2008; Ma 2012; Pavlick 1984). The Ovina Complex is composed of *F. ovina* (sheep fescue; $2x$), *F. valesiaca*, *F. filiformis* Pourr. [hair fescue; $2x$], *F. idahoensis* Elmer (Idaho fescue; $4x$), *F. roemerii* (Pavlick) E. B. Alexeev (Roemer's fescue; $4x-6x$), *F. trachyphylla* (hard fescue; $6x$), and *F. viviparoidea* Krajina ex Pavlick subsp. *viviparoidea* ($4x-6x$)]. While fine-leaved *F. rubra* [$2n = 42$ (hexaploid) or 56 (heptaploid)] is not considered part of the Ovina Complex, morphological comparisons suggest that an Asian fine-leaved fescue, *F. valesiaca* (Volga fescue; $2x-4x$), resembles members of the Ovina Complex (Arndt 2008; Ma 2012; Pils 1984; Pavlick 1984). The distribution of *F. ovina* (North America, Scotland, Europe, and Asia), *F. filiformis* (North, Central, and South America, and Western Europe), *F. idahoensis* (western North America with the exception of Mexico), and *F. trachyphylla* (North America with the exception of Mexico and Western Europe) is geographically diverse and includes a wide range of growing environments (Jones et al. 2008; Pavlick 1984). Likewise, *F. valesiaca* has a broad distribution and extends from near sea level in central Europe, up to about 2,000 m in the European Alps and 3,000 m in Xinjiang, Northwestern China (Arndt 2008).

Historically, *Festuca* germplasm has been collected from their areas of origin to provide needed genetic resources for germplasm preservation and use in plant improvement programs. Several plant exploration expeditions have been made to Eurasia since 1898 by the U.S. Department of Agriculture (USDA; initial collections by Frank N. Meyer and David Fairchild), which collected various *Festuca* species. Information about these collections can be accessed on the National Plant Germplasm System (NPGS) website (www.ars-grin.gov/npgs). Most recently, collections of *F. valesiaca* have been made in Kyrgyzstan (2006), Inner Mongolia (2006), and Russia (2010) where annual precipitation ranges from 250 to 1,000 mm and elevation ranges from 132 to 3,100 m above sea level (Johnson 2006, 2010).

Thirty of these *F. valesiaca* collections had not been evaluated for their horticultural or agronomic potential for low-maintenance applications (www.ars-grin.gov/npgs). Moreover, the genetic relationships among these accessions and other important North American *Festuca* turfgrass species of the Ovina Complex had not been defined. Adaptations of *F. valesiaca* to cold and drought conditions on heavily-grazed, barren arid Asian rangelands (Fırıncıoğlu et al. 2007, 2009, 2010) may be useful for the development of low-maintenance turfgrasses to increase water conservation in the western US. Therefore, a project was designed to evaluate the phenotypic variation of *F. valesiaca* accessions under semi-arid conditions of the western US. Great Basin (125–2,032 mm annual precipitation on landscapes ranging from 881 to 2,063 m; Behnke 2011; Comstock and Ehleringer 1992) and to characterize genotypic variation among these accessions and some closely related North American *Festuca* species. Since some *F. valesiaca* accessions may possess commercially acceptable turf characteristics for low-maintenance applications (Sărățeanu and Moiscu 2009), this initial identification and characterization may provide information for their effective use as parental stocks in plant improvement programs.

Materials and methods

Germplasm

Seeds of 12 *F. valesiaca* ($2n = 2x = 14$; Ma 2012) accessions (Table 1), chosen based on their native adaptation to overgrazed arid sites from Naryn (41°43'59"N 75°27'2"E; W6 30537, PI 659932, PI 659944, W6 30506, W6 30513, W6 30563), Ysyk-Kol (42°10'58"N 78°0'28"E; W6 30438, PI 659913, PI 659923), and Chu (42°11'34"N 73°45'22"E; W6 30575, W6 30588, W6 30595) Kyrgyzstan were received from the NPGS (Johnson 2006). All *F. valesiaca* accessions were diploid, except for W6 30537 and PI 659944, which were tetraploid (Ma 2012). Commercial varieties of several turf species were used as controls for field comparisons. These included 'Manhattan 4' [4x; *Lolium perenne* L.; perennial ryegrass, pasture; Granite Seed Company (GSC), Lehi, Utah], 'Black sheep' (6x; *F. ovina*; sheep fescue, rangeland; GSC), 'Coronado' (8x; *F. arundinacea*; tall

fescue, pasture, GSC), 'Durar' (6x; *F. ovina*; sheep fescue, rangeland; GSC), 'Cascade' [6x; *F. rubra* subsp. *commutata* Gaudin; chewings fescue, turf; US National Turfgrass Evaluation Program (NTEP)], 'Scaldis' (6x; *F. trachyphylla*; hard fescue, turf; NTEP), 'Shademaster' (8x; *F. rubra* L. subsp. *rubra*; creeping red fescue, turf; NTEP), and 'Dawson E' [6x; *F. rubra* var. *littoralis* Vasey ex Beal; creeping red fescue, turf; NTEP]. Although 'Durar' is sometimes classified under several species names (e.g., *F. trachyphylla*, *F. lemanii*, *F. ovina*, *F. brevipila*), herein it will be referred to as *F. ovina* sensu lato (sheep fescue in the broad sense) following Jones et al. (2008) since it is closely related to *F. ovina* (Ma 2012). Since no commercial varieties of *F. valesiaca* were available, these other fine-leaved fescue varieties and one ryegrass accession were used for comparison. Given the consistent and exceptional multi-location performance of 'Cascade' in the US National Turfgrass Evaluation Program (NTEP; www.ntep.org/contents2.shtml) trial (2004–2010), this turfgrass cultivar was used herein as a standard for comparison. All ploidy levels were confirmed by flow cytometry and/or karyotype analysis (Ma 2012).

The taxa chosen for study were based on morphological and genomic differences. Taxa of the fine-leaved Red Fescue Complex [e.g., *F. rubra* subspecies *arctica* (Hack.) Govor., *arenaria* (Osbeck) F. Aresch., *aucta* (V. I. Krecz. and Bobrov) Hultén, *fallax* (Thuill.) Nyman, *littoralis*, *mediana* (Pavlick) Pavlick, *pruinosa* (Hack.) Piper, *rubra*, *secunda* (J. Presl) Pavlick, *vallicola* (Rydb.) Pavlick] are closely related but distinct from those of the Ovina Complex (Catalán et al. 2004; Torrecilla et al. 2004; Jones et al. 2008). Moreover, the fine-leaved fescues, including taxa of the Ovina and Red Fescue Complexes, are genetically distinct from broad-leaved fescues, including tall fescue and other well-known turfgrasses such as perennial ryegrass (Catalán et al. 2004; Gaut et al. 2000; Torrecilla and Catalán 2002; Torrecilla et al. 2004). Based on these relationships, the choice of commercial varieties used allowed for comparisons to define the genetic relationships of the *F. valesiaca* accessions examined.

Plot establishment and maintenance

In January 2008, seeds of each entry were germinated on blotter paper, and then seedlings were planted in

Table 1 Mean values of phenotypic traits of *Festuca* accessions and checks evaluated over three years at Blue Creek, Utah

Accession	Species	2009						2010						2011									
		Total dry weight (g)	Seed weight (g)	Seed no.	Total dry weight (g)	Height (cm)	Width (cm)	Vigor rating (0–5)	Seed weight (g)	Seed no.	Total dry weight (g)	Height (cm)	Width (cm)	Vigor rating (0–5)	Seed weight (g)	Seed no.	Total dry weight (g)	Height (cm)	Width (cm)	Vigor rating (0–5)	Seed weight (g)	Seed no.	
Coronado	<i>F. arundinacea</i>	241.89	32.43	18,352	117.53	75.8	47.5	2.3	12.09	6,031	115.28	61.5	56.1	2.1	12.71	7,209							
Duraz	<i>F. ovina</i>	85.96	7.26	9,232	45.60	66.1	42.2	2.5	3.74	5,807	113.55	78.7	52.3	2.8	4.87	5,974							
Black Sheep	<i>F. ovina</i>	74.55	9.23	10,084	33.63	49.1	37.5	2.3	1.28	1,935	46.96	51.6	45.8	2.1	4.67	3,154							
Cascade	<i>F. rubra</i>	59.89	7.87	6,905	36.47	54.5	36.6	1.6	2.21	3,212	91.23	69.4	49.3	2.6	13.06	12,875							
Dawson E	<i>F. rubra</i>	73.78	6.95	8,546	14.31	29.6	27.8	0.9	0.07	90	70.23	60.4	44.7	1.8	6.50	6,716							
Shadmaster	<i>F. rubra</i>	66.31	7.14	4,454	58.54	52.4	40.7	1.7	3.51	3,484	154.49	72.0	61.3	3.5	6.47	4,920							
Scaldis	<i>F. trachyphylla</i>	49.87	2.15	2,182	24.79	34.3	27.6	1.5	0.20	360	36.42	53.1	36.3	1.3	2.28	3,298							
Manhattan	<i>L. perenne</i>	204.78	27.48	18,234	9.14	14.7	11.1	0.3	0.01	2	36.46	49.8	46.0	0.7	3.97	2,877							
PI 659913	<i>F. valesiaca</i>	58.05	13.49	21,649	41.73	48.5	31.3	2.2	10.90	18,474	82.21	55.2	42.9	2.1	15.19	28,344							
W6 30438	<i>F. valesiaca</i>	43.97	6.68	11,985	19.94	50.1	33.9	2.1	1.86	4,443	67.14	62.9	44.2	2.3	5.39	9,932							
PI 659923	<i>F. valesiaca</i>	56.65	10.90	19,255	64.57	51.7	34.5	2.8	14.68	27,222	126.92	63.2	48.9	2.5	8.60	14,171							
PI 659932	<i>F. valesiaca</i>	49.83	9.84	15,733	44.77	51.7	30.1	2.6	10.52	20,204	84.60	56.2	42.7	2.3	18.33	32,308							
W6 30506	<i>F. valesiaca</i>	43.53	5.09	9,601	34.81	56.3	36.1	2.5	6.47	15,104	67.14	60.1	42.7	2.1	5.60	8,343							
W6 30513	<i>F. valesiaca</i>	39.71	5.11	7,114	29.65	52.2	35.4	2.2	3.02	6,547	56.39	60.5	51.7	1.8	10.31	17,193							
W6 30537	<i>F. valesiaca</i>	10.13	0.79	1,254	0.11	8.1	7.7	0.5	0.00	9	14.40	46.6	27.0	0.4	0.29	68							
PI 659944	<i>F. valesiaca</i>	39.88	2.28	3,260	42.72	55.0	36.3	2.5	3.66	4,902	77.84	66.1	52.7	2.3	8.66	10,455							
W6 30563	<i>F. valesiaca</i>	49.71	8.87	16,372	44.95	52.8	35.5	2.5	8.72	16,744	104.57	62.0	47.3	2.7	8.05	12,906							
W6 30575	<i>F. valesiaca</i>	70.40	14.34	22,388	62.65	58.0	35.7	2.7	13.40	16,856	123.11	65.6	47.2	2.6	22.38	42,276							
W6 30588	<i>F. valesiaca</i>	66.94	15.10	24,731	73.88	56.1	37.7	3.1	18.38	33,606	114.94	55.6	51.5	2.8	21.49	42,539							
W6 30595	<i>F. valesiaca</i>	70.67	15.34	24,263	45.37	51.8	30.9	2.3	7.80	16,445	108.12	64.6	41.9	2.2	22.42	47,963							
<i>F. valesiaca</i> mean		49.96	8.99	14,800	42.09	49.3	32.1	2.3	8.28	15,046	85.62	59.9	45.1	2.2	12.22	22,208							
Grand mean		72.82	10.42	12,780	42.26	48.4	32.8	2.1	6.12	10,074	84.60	60.8	46.6	2.1	10.06	15,676							
LSD ($\alpha = 0.05$)		24.76	5.27	6,094	18.36	10.2	7.4	0.6	4.47	9,805	36.99	7.9	8.3	0.8	4.99	9,097							
CV (%)		15	34	62	67	21	31	40	59	59	49	14	20	40	21	21							

PI and W6 = Plant introduction (PI) number and pre-PI designation (W6) according to the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN)

nursery containers (“conetainer”, 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. 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 (1,800 $\mu\text{mol}/\text{m}^2/\text{s}$); Sun System III, Sunlight Supply, Inc. Vancouver, WA], at a relative humidity (RH) between 50 and 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).

Seedlings 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 (41°56'3.14"N 112°26'20.01"W) approximately 80 km northwest of Logan, UT (elevation = 1,433 m), where the average annual precipitation during the experiment (2009–2011) was about 388 mm (average 20-year precipitation is 307 mm). The soil type was a Parley's deep silt loam (fine-silty, mixed, mesic, Calcic, Argixerolls) having a neutral to slightly acidic pH (<http://websoilsurvey.nrcs.usda.gov/>). Plants were arranged in a randomized complete block design (RCBD) with five plants per plot in six replications spaced at 0.5 m within the rows and 1 m between rows ($\sim 20,000$ plants/ha) with plants of PI 659984 (*F. rubra*; Qinghai, China) used as end- and side-borders. Although plants were given water at transplanting, no water or fertilizer was applied during the experiment, and plots were hand-weeded from May to August. Broadleaf weeds were also controlled with 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 %); EPA Reg. No. 34704-239] application once in April or May of each year at a rate of 4.7 l/ha.

Phenotypic trait evaluation

On April 26, 2010 and June 7, 2011, the relative plant vigor of all accessions in the field plot was assessed using a 0–5 visual rating scale, where plant spring green-up (size, color, and transition from winter to spring growth) was defined as 0 = plant dead, 3 = plants possessing moderate biomass or leaf blade length with green (light to dark) foliage (tussock

evident), and 5 = green plants having comparatively abundant above ground biomass and/or long leaf blade length. Because of variation in leaf color among entries, light and dark green were given equal value, and color hue (intensity) was considered in the determination of relative plant vigor. On June 30, 2010, and June 23, 2011, the height (cm) of each plant was measured as the distance from the plant base (soil surface) to the top of the highest panicle at full anthesis. The width (cm) of each plant was measured at the harvest (cutting) height (~ 10 cm above ground) at the same time. Leaves and seed stalks were harvested at seed maturity and oven dried at 60 °C to estimate dry weight (g). Dried florets of each plant were mechanically threshed to separate seeds and chaff (poorly developed or aborted seeds), and seeds were weighed. One hundred seeds were counted and weighed in at least three replicates for each entry to convert seed weights to an estimate of the number of seeds per plant.

Phenotypic trait analysis

Data for all traits were tested for normality (F test) and homogeneity of variance with the UNIVARIATE procedure of SAS software (version 9.3 for Windows, SAS Institute Inc., Cary, NC, USA). Deviations from normality for data of plant dry matter, seed weight, and seed number were corrected by standard data transformation procedures using square root (dry weight) or natural log functions (seed weight and seed number) to comply with the assumptions necessary for analysis of variance (ANOVA) (Sokal and Rohlf 1995). Nevertheless, data are reported herein in their original scales for biological relevance and interpretation (Draper and Cox 1969). Data distributions for all other traits approached normality and could be used directly for ANOVA. Pearson product-moment correlation was used to measure the strength of linear associations between the traits, a test that assesses a “best fit” line between any two traits (Székely et al. 2007). Thus, tests of putative linear relationships provided assessment of the strength of associations among the traits examined herein.

Morphological trait data (over 3 years) were analyzed as a repeated measure by using a split-plot in time approach, in which year was treated as the whole-plot factor and accession was treated as

the split-plot factor in a RCBD. Year and accession main effects, and the year-by-accession interaction effects were tested by using PROC MIXED in SAS software (Oehlert 2000). Accession means were separated using Fisher's protected least-significant difference (LSD) test by applying the lsmeans statement in SAS. Multivariate principal component analyses (PCA) were performed on lsmean values of three traits in 2009 and six traits in 2010 and 2011 using PROC FACTOR in SAS to clarify accession relationships and to identify those traits that led most to accession discrimination (Kutner et al. 2004). Principal components were visualized using NTsys software (Rohlf 1998), where the least number of principal components that adequately avoided clustering of accessions and explained a high percentage of accession variance (>85 %) were used in visualization of accession relationships (Jolliffe 2005).

DNA extraction and PCR amplification

Leaf samples of each accession were collected, lyophilized, and then ground into fine powder with zinc beads inside extraction tubes by using a Retsch model MM 300 mixer mill (F. Kurt Retsch GmbH and Co., Haan, Germany). Total cellular DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions, and quantified with a Nanodrop Spectrophotometer (ND-1000, NanoDrop Technologies, Inc., Wilmington, DE, USA). The amplified fragment length polymorphism (AFLP) PCR amplification procedure was performed according to Vos et al. (1995) with fluorescently labeled primers, size fractionating, and fragment size analysis according to Jones et al. (2008). The same E.AC/M.CT primers defined by Jones et al. (2008) were used for the pre-amplification, while the primer combinations of E-ACAC/M-CTAC, E-ACAG/M-CTCA, E-ACCA/M-CTAG, E-ACCA/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. About 4 % of the samples were replicated to identify reproducible marker bands and determine their marker error rates. Only the most distinct (i.e., bright) and reproducible (i.e., consistent during repeated PCR amplicon analysis) bands were used for analysis.

AFLP-based cluster analyses

Data matrices [AFLP band present (1) or absent (0)] were constructed and used to create a rooted neighbor-joining dendrogram with PAUP computer software version 4.0b10 (Swofford 2003). The dendrogram was based on band absence and presence frequency by using the Nei and Li distance method (Nei and Li 1979) which estimates average pair-wise genetic distance (GD) values between each pair of accessions. Internal support for cluster groupings was assessed by using 1,000 bootstrap replications (Felsenstein 1985).

Results

For the phenotypic evaluation, significant ($P < 0.001$) main effects of year and accession and the interaction of accession \times year (3 years) were detected for all traits, except for vigor rating (data not shown; Ma 2012). Therefore, results are presented herein by trait for each year.

Total aboveground dry weight

Average dry weight (DW; aboveground) of all accessions was greater in 2011 than in 2009 or 2010 (Table 1). In 2009, DW ranged from 10.13 (W6 30537) to 241.89 ('Coronado') g/plant, with an average of 72.82 g/plant over all accessions. The DW of *F. valesiaca* accessions averaged 49.96 g/plant, and ranged from 10.13 (W6 30537) to 70.67 (W6 30595) g/plant. Commercial cultivars 'Coronado' (tall fescue) and 'Manhattan' (perennial ryegrass) produced significantly ($P < 0.05$) more DW than the fine-leaved commercial control 'Cascade' and *F. valesiaca* accessions, which were similar. In 2010, the DW production of all entries averaged 42.26 g/plant, and ranged from 0.11 (W6 30537) to 117.53 ('Coronado') g/plant. The mean DW production of W6 30588 (73.88 g/plant), PI 659923 (64.57), and W6 30575 (62.65) was higher than 'Cascade' (36.47), but the differences between 'Cascade' and the latter two accessions were not significant. In 2011, the average DW of all entries was 84.60 g/plant, and values ranged from 14.40 (W6 30537) to 154.49 ('Shademaster') g/plant. The *F. valesiaca* accessions produced an average of 85.62 g/plant, with a range from 14.40

(W6 30537) to 126.92 g/plant (PI 659923). Dry weight production in accessions PI 659923 (126.92 g/plant) and W6 30575 (123.11) were higher than ‘Cascade’ (91.23), but not significantly so. The DW mean of the *F. valesiaca* accessions was lower than the overall mean of the accessions in 2009, but this was not the case in 2010 and 2011.

Height

As already noted for aboveground dry weight, plant height was greatest, on average, in 2011 (Table 1). The average plant height among all accessions in 2010 was 48.4 cm, and values ranged from 8.1 (W6 30537) to 75.8 (‘Coronado’) cm. The height of *F. valesiaca* accessions was 49.3 cm on average, and ranged from 8.1 (W6 30537) to 58.0 (W6 30575) cm. Although the mean plant height of *F. valesiaca* accessions W6 30575 (58.0 cm), W6 30506 (56.3 cm), W6 30588 (56.1 cm), and PI 659944 (55.0 cm) were similar to ‘Cascade’ (54.5 cm), they were significantly ($P < 0.05$) shorter than ‘Coronado’ (75.8 cm). In 2011, the mean plant height of all accessions was 60.8 cm, and values ranged from 46.6 (W6 30537) to 78.8 (‘Durar’) cm. The mean height of *F. valesiaca* accessions was 59.9 cm, and ranged from 46.6 (W6 30537) to 66.1 (PI 659944) cm.

Width

In 2010, the mean plant width of all accessions was 32.8 cm, and values ranged from 7.7 (W6 30537) to 47.5 (‘Coronado’) cm (Table 1). The mean width of the *F. valesiaca* accessions was 32.1 cm, and values ranged from 7.7 (W6 30537) to 37.7 (W6 30588) cm. The width of *F. valesiaca* accessions W6 30588 (37.7 cm), W6 30506 (36.1), PI 659944 (36.6), W6 30563 (35.5), W6 30513 (35.4), PI 659923 (34.5), and W6 30575 (35.7 cm) were similar to ‘Cascade’ (36.6). In 2011, the average plant width of all accessions was 46.6 cm, and values ranged from 27.0 (W6 30537) to 61.3 (‘Shademaster’) cm. The width of ‘Coronado’ (56.1 cm) and ‘Shademaster’ (61.3) was significantly ($P < 0.05$) greater than ‘Cascade’ (49.3). The mean width of the *F. valesiaca* accessions was 45.1 cm, ranging from 27.0 (W6 30537) to 52.7 (PI 659944) cm. As a group, the *F. valesiaca* accessions examined were similar in width to ‘Cascade’.

Vigor rating

The average plant vigor rating among accessions in each year, 2010 and 2011, was 2.1, and values ranged from 0.3 (poor vigor; ‘Manhattan’ in 2010) to 3.5 (most vigorous; ‘Shademaster’ in 2011) (Table 1). While the vigor rating of the *F. valesiaca* accessions was near 2.2 in both years, values ranged from 0.4 (W6 30537 in 2011) to 3.1 (W6 30588 in 2010). Although the vigor ratings of all *F. valesiaca* accessions, except PI 659913, W6 30438, W6 30513, and W6 30537, were significantly ($P < 0.05$) higher than ‘Cascade’ in 2010, the vigor of ‘Cascade’ and all *F. valesiaca* accessions was similar in 2011. In contrast, ‘Durar’ and ‘Shademaster’ were more vigorous than ‘Cascade’ in 2011.

Seed number

Differences in average seed number among all entries from 2009 to 2010 (12,780–10,074 seeds/plant) were not significant, while seed number was significantly ($P < 0.001$) greater in 2011 (15,676; Table 1). In 2009, the mean number of seeds per plant of all accessions taken collectively was 12,780, and values ranged from 1,254 (W6 30537) to 24,731 (W6 30588) seeds/plant. For *F. valesiaca* accessions, the average number of seeds per plant was within the same range at 14,800. *Festuca valesiaca* accessions PI 659913 (21,649 seeds/plant), 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) produced significantly ($P < 0.001$) more seeds than did ‘Cascade’ (6,905), but these amounts were similar to those of ‘Coronado’ (18,352). In 2010, the mean seed number of all accessions taken collectively was 10,074 seeds/plant, with a range of 2 (‘Manhattan’) to 33,606 (W6 30588) seeds/plant. The mean seed number of *F. valesiaca* accessions PI 659913 (18,474 seeds/plant), PI 659923 (27,222), PI 659932 (20,204), W6 30563 (16,744), W6 30575 (16,856), W6 30588 (33,606), and W6 30595 (16,445) was significantly ($P < 0.001$) higher than those of ‘Cascade’ (3,212) or ‘Coronado’ (6,031). In 2011, the mean seed number of all accessions was 15,676 seeds/plant, and values ranged from 68 (W6 30537) to 47,963 (W6 30595) seeds/plant. For *F. valesiaca* accessions, the mean seed number was within the same range at 22,208 seeds/plant. The mean seed number of *F. valesiaca*

accessions PI 659913 (28,344 seeds/plant), PI 659932 (32,308), W6 30575 (42,276), W6 30588 (42,539), and W6 30595 (47,963) was significantly ($P < 0.001$) higher than those of ‘Cascade’ (12,875) or ‘Coronado’ (7,209).

Weight per seed

Based on number of seeds (above) and their weight (three replicates of 100 seeds per entry), *F. valesiaca* accessions examined possessed abundant amounts of small seeds. While mean seed weight of all entries was similar in 2009 and 2011, production in these years was significantly higher ($P < 0.001$) than in 2010 (Table 1). For the *F. valesiaca* accessions, the reduction in 2010 was not as large, and seed production was high in 2011, although this trend was not significantly different from the overall means. In 2009, mean seed weight of all accessions was 10.42 g/plant, and values ranged from 0.79 (W6 30537) to 32.43 (‘Coronado’) g/plant. Mean seed weight of the *F. valesiaca* accessions was 8.99 g/plant, and values ranged from 0.79 (W6 30537) to 15.34 (W6 30595) g/plant. The mean seed weight of the *F. valesiaca* accessions PI 659913 (13.49 g/plant), PI 659923 (10.9), PI 659932 (9.84), W6 30563 (8.87), W6 30575 (14.34), W6 30588 (15.10), and W6 30595 (15.34) were significantly greater than ‘Cascade’ (7.87), but significantly lower ($P < 0.001$) than ‘Coronado’ (32.43) and ‘Manhattan’ (27.48). In 2010, the mean seed weight per plant of the all germplasm examined was 6.12 g/plant, and weights ranged from 0.00 (W6 30537) to 18.38 (W6 30588) g/plant. The mean seed weight of *F. valesiaca* accessions was 8.28 g/plant, within the same range. The mean seed weight of PI 659913 (10.90 g/plant), PI 659923 (14.68), PI 659932 (10.52),

W6 30563 (8.72), W6 30575 (13.40), W6 30588 (18.38), and W6 30595 (7.8) was significantly ($P < 0.001$) greater than ‘Cascade’ (2.21). In 2011, the mean seed weight of all accessions was 10.06 g/plant, and values ranged from 0.29 (W6 30537) to 22.42 (W6 30595) g/plant, while the *F. valesiaca* accessions produced an average of 12.22 g of seed per plant within the same range. The mean seed weight per plant of the *F. valesiaca* accessions PI 659913 (15.19 g/plant), PI 659932 (18.33), W6 30575 (22.38), W6 30588 (21.49), and W6 30595 (22.42) were significantly ($P < 0.01$) higher than ‘Cascade’ (13.06).

Trait correlations

The *F. valesiaca* accessions examined possessed abundant amounts of small seed, the weight of which was significantly correlated with the total DW ($r^2 = 0.84$, $P < 0.001$), plant height ($r^2 = 0.58$, $P < 0.05$), and plant vigor ($r^2 = 0.83$, $P < 0.001$) in 2010 (Table 2). Likewise, total DW was significantly correlated with plant height [$r^2 = 0.59$ (2010) and 0.61 (2011), $P < 0.05$], vigor rating [$r^2 = 0.71$ (2010) and 0.88 (2011), $P < 0.01$], seed number [$r^2 = 0.74$ (2010), $P < 0.001$], and width [$r^2 = 0.63$ (2011), $P < 0.05$] depending on year.

Principal component analysis

Morphological relationships among 17 accessions as analyzed by PCA are shown in Fig. 1. Trait values for some of the species examined were disproportionately high [‘Manhattan’ (*L. perenne*), ‘Coronado’ (*F. arundinacea*)] or low [W6 30537 (*F. valesiaca*)] when compared to the rest of the accessions (Table 1) and

Table 2 Pearson correlation coefficients (r^2) between traits of *Festuca* accessions and checks evaluated in 2010 (below diagonal) and 2011 (above diagonal)

	Total dry weight	Height	Vigor rating	Seed number	Seed weight	Width
Total dry weight		0.61**	0.88***	0.36 ^{NS}	0.44 ^{NS}	0.63**
Height	0.59*		0.64**	-0.06 ^{NS}	-0.00 ^{NS}	0.61**
Vigor rating	0.71**	0.75***		0.15 ^{NS}	0.28 ^{NS}	0.76***
Seed number	0.74***	0.54*	0.84***		0.95***	-0.07 ^{NS}
Seed weight	0.84***	0.58*	0.83***	0.98***		0.09 ^{NS}
Width	0.46 ^{NS}	0.78***	0.42 ^{NS}	0.12 ^{NS}	0.20 ^{NS}	

Significant correlations at $P < 0.05$, 0.01, 0.001, and non-significant designated as *, **, ***, and NS, respectively

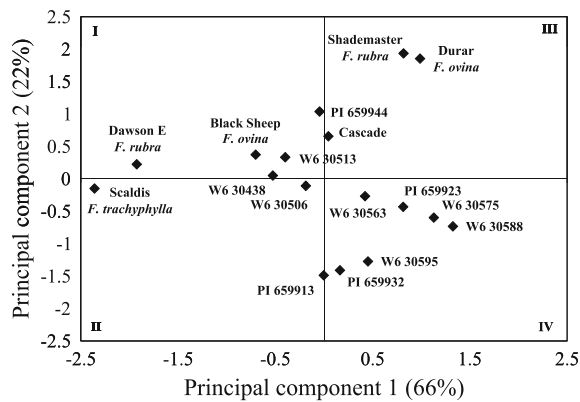


Fig. 1 Plot of the first two principal components from principal component analysis assessing *F. valesiaca* accessions and *Festuca* commercial cultivars (controls) based on three traits in 2009 and six traits in 2010 and 2011 at Blue Creek, UT. Percentage after axis titles indicates the percentage of variation explained for each principal component. The quadrants (I, II, III, IV) illustrate performance over all traits where entries above and to the right of the horizontal and vertical lines, respectively, performed agronomically better than their counterparts

were thus excluded from this analysis. The first two principal components explained large portions of the observed variation (88 %) facilitating a determination of accession relationships (Fig. 1). Commercial cultivars ‘Dawson E’ (*F. rubra*) and ‘Scaldis’ (*F. trachyphylla*) and some accessions of *F. valesiaca* (e.g., PI 659944, W6 30506, W6 30438, and W6 30513) contributed comparatively low values for all traits examined and were positioned in Quadrants I or II. In contrast, commercial cultivars ‘Shademaster’ (*F. rubra*), ‘Durar’ (*F. ovina*) and ‘Cascade’ (*F. rubra*) contributed similar and often comparatively high values for the traits examined and were positioned in Quadrant III. *F. valesiaca* accessions PI 659923, W6 30563, W6 30575, PI 659932, W6 30595, and W6 30588 were similar in trait values and positioned in Quadrant IV, which was largely due to high seed number and weight values.

AFLP-based neighbor-joining tree analysis

Ten AFLP selective amplification primer combinations produced 1,530 consistent, bright bands, 95 % (1,454; Ma 2012) of which were polymorphic among the 20 accessions (Table 1) examined. The average number of polymorphic markers recovered per primer combination was 153. A mid-point rooted neighbor-joining tree constructed from GD estimates resulted in

the partitioning of all but one (W6 30537, 4x) of the *F. valesiaca* accessions, and the *F. ovina* cultivar ‘Durar’, into Cluster 5 and hierarchical Cluster 3 (Fig. 2). Hierarchical Cluster 3, Cluster 4, and Cluster 5 include the taxa *F. ovina*, *F. trachyphylla*, and *F. valesiaca* of the Ovina Complex, and distinguish the fine-leaved fescues from other *Festulolium* turfgrasses. The Ovina Complex is genetically distinct from Cluster 2 (*F. rubra* entries) and the *F. valesiaca* offtype W6 30537. These results indicate that W6 30537 is either misidentified and/or possibly of hybrid origin. As expected, the outgroup taxa, *F. arundinacea* and *L. perenne*, were differentiated from all other taxa (Cluster 1).

Discussion

Festuca valesiaca is a dominant, erect, sod-forming grass on the intensively-grazed, arid rangeland plateaus of Turkmenistan (1,200–1,900 m) (Habibulla et al. 1999) and Central Anatolian Turkey (1,000–2,000 m) (Firincioglu et al. 2007), and on Asian montane grasslands (e.g., the Emin Valley along the China-Kazakhstan border, 2,000 m) (Taft et al. 2011), which resemble some Great Basin ecosystems of North America (Comstock and Ehleringer 1992). This species is an important, abundant, and persistent rangeland forage grass that shapes vegetation patterns because of its heat and drought tolerance (Firincioglu et al. 2009). We provide herein the first report of the agronomic potential of *F. valesiaca* in the arid western US, and preliminary evidence of its relationship to North American *Festuca* species of the Ovina Complex. Substantial genotypic (Fig. 2) and phenotypic (Table 1; Fig. 1) variation exists among the *F. valesiaca* accessions examined. Our AFLP-based, genetic assessment indicates that these *F. valesiaca* accessions share considerable genetic affinities with the *F. ovina* (‘Durar’ and ‘Blacksheep’) and *F. trachyphylla* (‘Scaldis’) accessions examined. However, given the relatively small number of accessions examined, a broader assessment of these and other Ovina Complex *Festuca* taxa (e.g., *F. filiformis*) is needed for a more rigorous determination of such putative relationships.

Plant characteristics associated with plant vigor (e.g., seedling vigor, persistence, sustained green color) and competitiveness (e.g., plant height and width, seed yield) are important prerequisites for turf

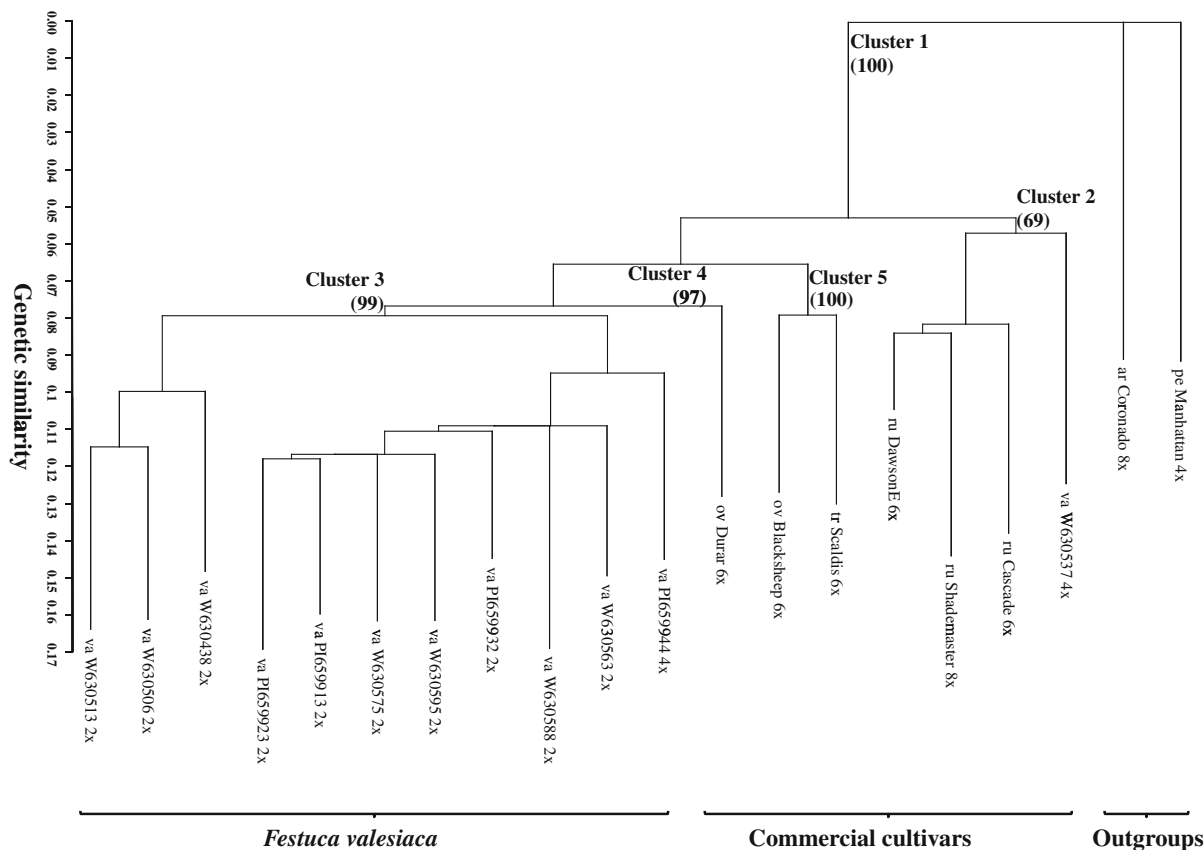


Fig. 2 Rooted neighbor-joining tree illustrating genetic relationships among *F. valesiaca* accessions (va), commercial cultivars, and outgroups based on Nei and Li's (1979) GD among AFLP profiles after 1,000 bootstrap permutations (Swofford 2003). Other two-letter species indicators are

pe = *Lolium perenne*, ar = *F. arundinacea*, ru = *F. rubra*, tr = *F. trachyphylla*, and ov = *F. ovina*. The numbers in parentheses at each cluster indicate bootstrap values after 1,000 permutations

applications (Ruemmele et al. 2003). For urban uses, plant height and width are important associates of mowing frequency (McKernan et al. 2001) and plant competitiveness (Afolayan 1979; Grime 1977). Although plant height was measured at flowering, which is taller than the typical vegetative growth, the height of *F. valesiaca* entries was in the main shorter than those of 'Cascade', 'Durar', and 'Shademaster', but generally resembled 'Black Sheep' and 'Scaldis' in several traits (Table 1). These results suggest that these *F. valesiaca* accessions (i.e., W6 30438, PI 659923, and PI 659932) may have utility as parental stocks in low-maintenance turf improvement programs. Nevertheless, even though these *F. valesiaca* accessions are attractive candidates for use in plant improvement, some accessions (e.g., W6 30537) may require further taxonomic clarification because of their

genetic dissimilarities to a majority of the accessions examined.

Under minimum management, acceptable aesthetic visual attributes, such as greenness and plant uniformity are essential characteristics of low-maintenance turfgrass (Diesburg et al. 1997; Wang et al. 2011). To the same end, substantial plant vigor in the early spring (i.e., transition from winter dormancy) is also desirable (Bertin et al. 2009). The visual rating (color and aboveground biomass) reported herein indicates that *F. valesiaca* accessions PI 659923 (2.65), PI 659932 (2.45), W6 30575 (2.65), and W6 30588 (2.95) were significantly ($P < 0.001$) superior to the commercial control 'Cascade' (2.10) during the 2010 and 2011 growing seasons. Moreover, spring vigor ratings were consistent over years, and mean vigor rating (2010 and 2011) was correlated with plant height ($P < 0.01$;

$r^2 = 0.64\text{--}0.75$) and total dry weight ($P < 0.01$; $r^2 = 0.71\text{--}0.88$) (Table 2).

Traits, such as seed production, biomass production, and plant vigor, in many plants are under quantitative genetic control and can be influenced dramatically by growing environment (Kotecha 1981; van der Heijden and Roulund 2010; Zhang et al. 2006; Brown et al. 2010). Still, such traits can be genetically manipulated during plant improvement (Petr and Frey 1966). In the case of *F. valesiaca*, the level of phenotypic variation (e.g., seed number; Table 1) suggests that potentially useful cross-progeny might be recovered from matings between *F. valesiaca* and cross-compatible elite adapted material. However, even if such hybrids were recovered, they would require extensive field evaluation given the large environmental effects on the traits (e.g., seed weight and number, and DW) evaluated (Table 1; large coefficients of variation). Such testing could be facilitated by employing rigorous experimental designs (e.g., lattice) with extensive replication (more than six) and multi-location testing (more than two).

Vigor-related traits, such as leaf color, can also be dramatically influenced by growing environment (Sărățeanu and Moisuc 2009). The *F. valesiaca* accessions examined herein possess green to bluish-green coloration and could be selected and developed for specific ornamental and golf course applications after rigorous, multi-location testing. Because of the moderate to high correlations between seed weight and total aboveground dry weight, plant height, and plant vigor (Table 2; $r^2 = 0.58\text{--}0.84$), it is likely that recurrent selection for these traits among intraspecific *F. valesiaca* hybrids could result in vigorous, green, tall-statured progeny with abundant seed yield (Fang et al. 2004; Griffiths 1965). This is important given the commercial importance placed on both vegetative (e.g., plant color and size) and seed production (e.g., seed number and weight) characteristics in grasses (Fang et al. 2004).

Under low-precipitation conditions in the Great Basin, the performance of the Kyrgyz *F. valesiaca* accessions PI 659923 (Naryn), PI 659932 (Naryn), W6 30575 (Chu), and W6 30588 (Chu) for plant vigor [i.e., sustained plant growth (biomass) and green color throughout the growing season] and comparatively high seed production was consistent over 2 years of evaluation. These attributes are important for low-maintenance turfgrass applications, including urban

landscapes, golf course fairway fringes/roughs, roadsides, and degraded landscape restoration.

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