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Development of fine-leaved *Festuca* grass populations identifies genetic resources having improved forage production with potential for wildfire control in the western United States

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Abstract Drought and heat tolerant fine-leaved fescue (*Festuca* ssp.) grasses have potential as components in rangeland greenstrips for wildfire control in semi-arid climates, although such grasses have not been evaluated under rangeland conditions. Therefore, 63 geographically diverse *Festuca* accessions of 11 species were evaluated for vigor, color, and biomass in 2009 and 2010 in North Logan, UT to identify grasses for use in U.S. western rangelands. Sixty-two plants representing eight species were selected in 2009 to intermate for further evaluation. Controlled biparental matings among these selections in 2010 produced 18 populations with sufficient seed to be evaluated with three commercial *Festuca* checks in replicated trials between 2012 and 2013 at Malta, ID, Blue Creek, UT, and North Logan, UT, where mean annual precipitation is 265, 362, and 484 mm, respectively. Plants were evaluated for color, relative vigor, biomass, seed yield, persistence, and regrowth over 2 years. Generally, four fine-leaved populations (R4S4, R4S6, R4S22, and R4S32) with parents originating from Turkey (*F. valesiaca* subsp. *valesiaca*), Russia (*F. valesiaca*, *F. valesiaca* subsp. *valesiaca*), Iran (*F. valesiaca*), and the U.S. (*F. ovina*) performed equal to or better than 'Durar' or 'Covar' checks. In Malta (harshest environment), the performance of these four populations compared to 'Durar' was 84–210 % for vigor, 79–90 % for color, 65–562 % for biomass, 64–296 % for seed yield, 92–117 % for persistence, and 164–454 % for regrowth, where R4S22 was superior. AFLP analysis indicated that all four populations were distinct, and that R4S4 and R4S6 grouped near 'Covar', R4S22 clustered near 'Black Sheep' and 'Durar', and R4S32 was genetically unique. These populations exhibit drought tolerance and green leaf color under harsh U.S. western desert conditions that make them amendable for use in greenstrips for wildfire control.

Keywords Abiotic tolerance · AFLP · Breeding · Environmental stress · Germplasm enhancement · Molecular markers

Introduction

Disturbances by wildfire, livestock, wildlife, and humans (including recreational activities) have contributed to degraded conditions on much of America's 800 million acres of rangeland (Yensen 1980; Davison 1996). Increased wildfire frequency, intensity, and unpredictability in the western U.S. is due to the pervasive cyclic accumulation of invasive annual plant species {e.g., cheatgrass (*Bromus tectorum* L.), red brome grass (*Bromus rubens* L.), medusahead [*Taeniatherum caput-medusae* (L.) Nevski], and

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halogeton [*Halogeton glomeratus* (M. Bieb) C.A. Mey.]} that compete with and displace native perennial rangeland species after a fire, leading to reburning (Harris 1967; Harris and Wilson 1970; Pellant 1990). Thus, many plant improvement efforts in semi-arid regions of the Great Basin in the western U.S. focus on developing plant materials that provide novel germplasm to revegetate disturbed areas, increase forage quality and rangeland biodiversity, improve wildlife habitat, and reduce undesirable weed invasion and associated wildfire frequency (Pierson et al. 2011). Such germplasm (i.e., cultivars and pre-variety germplasm) must be increasingly tolerant of abiotic stress, competitive with invasive weeds, easy to establish, and highly persistent under changing climates (Choi et al. 2008).

Wildfire effects and behavior can be modified by plant species planted in strips (typically ~91 m wide, designated as "greenstrips") that control the speed and spread of wildfires (Pellant 1994; Finney et al. 2007). Primary greenstrip component species (shrubs and grasses) must be site adapted, have the ability to establish among annual weeds, produce relatively low amounts of fuel, remain green over the growing season, survive burning, and tolerate grazing by wildlife and livestock (Pellant 1994; Gebhardt et al. 1987; Waldron et al. 2010; Harrison et al. 2002).

Fire-resilient grass vegetation that improves forage production and reduces wildfire spread has been difficult to identify given habitat-specific adaptations, lack of late maturity (i.e., summer greening), and often poor stand establishment and persistence (Monsen 1994; Abella 2010). Although some fine-leaved fescue species possess abiotic stress tolerance and remain green throughout the growing season (Ruemmele et al. 2003; Aronson et al. 1987), many of these have not been rigorously evaluated for their potential use in greenstrips in the western U.S. For instance, although popular 'Covar' (*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*; PI 109497, Konya, Turkey) and 'Durar' [*F. trachyphylla* (Hack.) Krajina; Eurasian origin] possess drought tolerance in some semi-arid rangelands, their adaptation to the harsh climatic conditions of the western desert of the Great Basin is not known. Therefore, a study was designed to evaluate a broad array of fine-leaved fescue species, and then select and hybridize high performance individuals that have potential as greenstrip components. These hybrid populations were subsequently evaluated for their potential as a forage contributor and as greenstrip components under Great Basin desert conditions and the best performing populations were evaluated for their genetic structure and relationships to 'Covar' and 'Durar'. This research allowed for the identification of genetically diverse, high performing germplasm and breeding strategies that will lead to the development and release of elite abiotic stress tolerant fine-leaved fescue grass varieties for use on western U.S. rangelands.

Materials and methods

To identify fine-leaved fescue material with potential for use in greenstrips, a genetically diverse array of 63 accessions representing 11 species was obtained from GRIN and evaluated as an observational trial during 2008–2010 (Table 1). This initial trial screened accessions for their adaptability to semi-arid climates for further characterization. Morphological evaluation of these accessions led to the identification of 62 single-plant selections from 27 different, high-performing accessions (selection intensity = 43 %) that were used in controlled, biparental matings to produce 18 distinct populations. These populations were more rigorously characterized in three ecologically different locations for traits related to rangeland performance (Table 2). The four best performing populations were identified and superior progeny from these selected populations were genotyped with molecular markers to define the genetic similarity among these high performing hybrids and their genetic relatedness to 'Covar' and 'Durar'. This provided a contextual frame for this and future genetic and breeding studies.

Germplasm

We use herein the general taxonomic treatment of Catalán et al. (2004) and that of Barkworth et al. (2007) because of its historical context to North American flora and its recognition of worldwide *Festuca* species that have been introduced to the North American continent. However, Markgraf-Dannenberg's treatment of the Flora of Turkey indicates that *F. ovina* L. does not exist in Turkey (Markgraf-Dannenberg 1980) and perhaps not in the Middle-East (Wilkinson and Stace 1991). Therefore, we classified *F. ovina* accessions the U.S. Department of

Table 1 *Festuca* accessions visually evaluated for relative plant vigor, color, and biomass in 2009 and 2010 at North Logan, UT to identify superior plants for selection

Acccesion	Cultivar	Species	Origin	Ploidy[a]	Plant rating[b]	
					2009	2010
PI 469218	Redondo	*F. arizonica* Vasey	New Mexico	6*x* (42)	0	0
PI 578709	Jemez	*F. arizonica* Vasey	New Mexico	4*x* (28)	0	0
W6 25539		*F. brachyphylla* Schult. & Schult. f.	Greenland	6*x* (42)	6	8
W6 25540		*F. brachyphylla* Schult. & Schult. f.	Greenland	6*x* (42)	3	5
W6 25542		*F. brachyphylla* Schult. & Schult. f.	Greenland	6*x* (42)	4	6
PI 255361		*F. filiformis* Pourr.	Yugoslavia	2*x* (14)	4	4
PI 311045	Magurele	*F. filiformis* Pourr.	Romania	2*x* (14)	3	4
PI 249742		*F. heterophylla* Lam.	Greece	4*x* (28)	0	0
PI 249743		*F. heterophylla* Lam.	Greece	4*x* (28)	0	0
PI 251124		*F. heterophylla* Lam.	Yugoslavia	6*x* (42)	3	3
PI 286209		*F. heterophylla* Lam.	Czech Rep	4*x* (28)	0	0
PI 287552		*F. heterophylla* Lam.	Poland	4*x* (28)	4	4
Fida OR03[c]		*F. idahoensis* Elmer	Oregon	4*x* (28)	4	4
PI 232288		*F. idahoensis* Elmer	Oregon	4*x* (28)	2	2
PI 232293		*F. idahoensis* Elmer	Wyoming	4*x* (28)	3	3
PI 344616		*F. idahoensis* Elmer	Montana	4*x* (28)	0	0
PI 344631		*F. idahoensis* Elmer	Montana	4*x* (28)	2	2
PI 504313		*F. idahoensis* Elmer	Oregon	4*x* (28)	2	2
PI 578731	Nezpurs	*F. idahoensis* Elmer	Idaho	4*x* (28)	2	2
PI 601054	Joseph	*F. idahoensis* Elmer	Idaho	4*x* (28)	4	4
W6 27177		*F. idahoensis* Elmer	Oregon	4*x* (28)	4	4
PI 632485		*F. lenensis* Drobow	Mongolia	2*x* (14)	4	4
PI 636571		*F. lenensis* Drobow	Mongolia	4*x* (28)	3	3
PI 229450		*F. rupicolla* Heuff.	Iran	4*x* (28)	0	0
PI 325329		*F. rupicolla* Heuff.	Russian Fed	2*x* (14)	0	0
PI 440385		*F. rupicolla* Heuff.	Kazakhstan	2*x* (14)	4	4
PI 440387		*F. rupicolla* Heuff.	USSR	2*x* (14)	4	4
PI 440912		*F. rupicolla* Heuff.	Morocco	6*x* (42)	2	2
PI 538964		*F. rupicolla* Heuff.	Russian Fed	2*x* (14)	0	0
PI 251135		*F. trachyphylla* (Hack.) Krajina	Yugoslavia	6*x* (42)	2	2
PI 311064	OH	*F. trachyphylla* (Hack.) Krajina	Sweden	6*x* (42)	3	3
PI 578732	Durar	*F. trachyphylla* (Hack.) Krajina	Washington	6*x* (42)	2	2
PI 229453		*F. valesiaca* Schleich. *ex* Gaudin	Iran	4*x* (28)	0	0
PI 229503		*F. valesiaca* Schleich. *ex* Gaudin	Iran	6*x* (42)	3	3
PI 229533		*F. valesiaca* Schleich. *ex* Gaudin	Iran	6*x* (42)	4	4
PI 237708		*F. valesiaca* Schleich. *ex* Gaudin	Germany	4*x* (28)	4	4
PI 268234		*F. valesiaca* Schleich. *ex* Gaudin	Iran	6*x* (42)	3	3
PI 380846		*F. valesiaca* Schleich. *ex* Gaudin	Iran	6*x* (42)	2	2
PI 502383		*F. valesiaca* Schleich. *ex* Gaudin	Russian Fed	2*x* (14)	2	2
PI 634225		*F. valesiaca* Schleich. *ex* Gaudin	Ukraine	2*x* (14)	2	2
PI 251125		*F. valesiaca* Schleich. *ex* Gaudin subsp. *pseudodalmatica*	Yugoslavia	4*x* (28)	4	4
PI 383654		*F. valesiaca* Schleich. *ex* Gaudin subsp. *pseudodalmatica*	Turkey	4*x* (28)	0	0

Table 1 continued

Acccesion	Cultivar	Species	Origin	Ploidy[a]	Plant rating[b]	
					2009	2010
PI 384861		*F. valesiaca* Schleich. *ex* Gaudin subsp. *pseudodalmatica*	Iran	6*x* (42)	3	3
PI 595160		*F. valesiaca* Schleich. *ex* Gaudin subsp. *pseudodalmatica*	Xinjiang, PRC	5*x* (35)	2	2
PI 632505		*F. valesiaca* Schleich. *ex* Gaudin subsp. *pseudodalmatica*	Turkey	4*x* (28)	4	4
PI 634304		*F. valesiaca* Schleich. *ex* Gaudin subsp. *pseudodalmatica*	Xinjiang, PRC	2*x* (14)	2	2
PI 383652		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Turkey	2*x* (14)	1	2
PI 502380		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Russian Fed	2*x* (14)	3	3
PI 578733	Covar[d]	*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Turkey	2*x* (14)	3	3
PI 595178		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Xinjiang, PRC	2*x* (14)	2	2
PI 618975		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Xinjiang, PRC	2*x* (14)	2	2
PI 659913		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	2	2
PI 659923		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	0	0
PI 659932		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	0	0
PI 659944		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	4*x* (28)	2	2
PI 665579		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	0	0
PI 665580		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	2	2
PI 670359		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	2	2
PI 670362		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	3	3
PI 670364		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	3	3
PI 670366		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	1	1
W6 30438		*F. valesiaca* Schleich. *ex* Gaudin subsp. *valesiaca*	Kyrgyzstan	2*x* (14)	2	2
PI 370273		*Festuca* spp.	USSR	8*x* (56)	3	3

[a] Numbers in parentheses indicate number of chromosomes

[b] The number of plants with a rating (from 0 to 5) of 3 or higher based on relative plant vigor, color, and biomass where 0 = dead; 3 = light green to green with intermediate vigor and biomass; and 5 = dark green with substantial biomass and vigor

[c] *Fida* OR03 is an accession collected near LaGrande cemetery, Union County, Oregon, USA (Jones et al. 2008)

[d] Covar originated from PI 109497, which is from Turkey

Agriculture, Agricultural Research Service, Plant Genetic Resources Unit as identified in its Genetics Resources Information Network (GRIN; http://www.ars-grin.gov) with Middle-Eastern or Asian origins as *F. valesiaca* according to Arndt (2008). The commercial cultivars ‘Durar’ (*F. trachyphylla*; 6*x*), ‘Covar’ (*F. valesiaca* subsp. *valesiaca*; 2*x*), and ‘Black Sheep’ (*F. ovina*; 6*x*) were obtained from Granite Seed Company (Lehi, UT).

Field plot establishment, biparental matings, and experimental design

The 63 accessions in the initial observational trial were chosen as a subset of fine fescue material from GRIN to represent a wide array of species with broad geographic distribution to identify accessions adapted to the western U.S. for further evaluation in multi-location, replicated trials. Seeds of each accession were germinated and seedlings were established and maintained in a greenhouse in Logan, UT under environmental conditions according to Staub et al. (2014). After greenhouse establishment, 14 seedlings of each of the 63 accessions were transplanted in May 2008 to a field nursery in single rows (1.0 m between rows, 0.5 m within rows) without replication in North Logan, Cache County, UT (41°47′9.27″N 111°48′51.76″W; elevation = 1392 m), approximately six km north of Logan, UT. The average annual precipitation during the 2-year period of

Table 2 Biparental *Festuca* cross populations from superior selections evaluated for phenotypic characteristics

Population	Ploidy	Parent 1	Parent 1 species[a]	Parent 2	Parent 2 species[a]
R4S1	2*x*	PI 311045	*F. filiformis*	PI 311045	*F. filiformis*.
R4S3	2*x*	PI 670364	*F. val.* subsp. *val.*	Covar (PI 578733)	*F. val.* subsp. *val.*
R4S4	2*x*	PI 383652	*F. val.* subsp. *val.*	Covar (PI 578733)	*F. val.* subsp. *val.*
R4S5	2*x*	PI 595178	*F. val.* subsp. *val.*	Covar (PI 578733)	*F. val.* subsp. *val.*
R4S6	2*x*	PI 502383	*F. valesiaca*	Covar (PI 578733)	*F. val.* subsp. *val.*
R4S7	4*x*	PI 636571	*F. lenensis*	PI 636571	*F. lenensis*
R4S10	4*x*	PI 287552	*F. heterophylla*	PI 287552	*F. heterophylla*
R4S11	4*x*	PI 237708	*F. valesiaca*	PI 237708	*F. valesiaca*
R4S12	4*x*	PI 251125	*F. val.* subsp. *pseudo.*	PI 251125	*F. val.* subsp. *pseudo.*
R4S14	6*x*	W6 25540	*F. brachyphylla*	W6 25540	*F. brachyphylla*
R4S15	6*x*	W6 25542	*F. brachyphylla*	W6 25542	*F. brachyphylla*
R4S18	6*x*	PI 380846	*F. valesiaca*	Black Sheep	*F. ovina*
R4S19	6*x*	PI 384861	*F. val.* subsp. *pseudo.*	Black Sheep	*F. ovina*
R4S20	6*x*	PI 229503	*F. valesiaca*	Black Sheep	*F. ovina*
R4S21	6*x*	PI 229533	*F. valesiaca*	Black Sheep	*F. ovina*
R4S22	6*x*	PI 268234	*F. valesiaca*	Black Sheep	*F. ovina*
R4S32	2*x*	PI 502380	*F. val.* subsp. *val.*	PI 502380	*F. val.* subsp. *val.*
R4S34	4*x*	PI 632505	*F. val.* subsp. *pseudo.*	PI 632505	*F. val.* subsp. *pseudo.*

[a] Species name abbreviated. For complete name see Table 1

evaluation and selection (2009–2010) of GRIN accessions was 525 mm (PRISM 2015). Each plant from every accession was visually rated in 2009 for relative plant vigor, color, and biomass, with a rating from 0 to 5 where 0 = dead; 3 = light green to green with intermediate vigor and biomass; and 5 = dark green with substantial biomass and vigor. Although plants with a rating of 3 or greater were considered to have rangeland potential (Table 1), only the best performing plants (rating of 4 or 5) were selected for biparental matings. The selections were paired by morphology and species designation, such that similarly performing selections were paired first by accession similarity (same accession; 20 crosses), then by accession diversity (different accessions of the same species; 1 cross), and then with commercial cultivars ‘Covar’ (5 crosses) or ‘Black Sheep’ (5 crosses). Each selection was removed in its entirety in the fall of 2009 and transplanted near the field nursery in close proximity (0.5 m) with its assigned pair for pollination. Both the selections (transplants) and the remnant individuals in the nursery were again visually evaluated in 2010 to verify the performance of characteristics evaluated in 2009.

Biparental matings between phenotypically verified selections were performed in 2010 between a total of 31 crosses representing 62 accessions from eight species (see “Results” section). Controlled biparental pollinations were accomplished using steel cages covered by white pollen exclusion bags erected a few days before anthesis. Although selfing occurs in some fine fescue species as high as 50 %, a rate of 10 % selfing is considered exceptionally high (Auquier 1977; Barker and Stace 1982). Thus, to verify that seed obtained from cage isolation was from cross-pollination, 5–10 inflorescences of each individual plant were placed in pollination bags within the cages to determine the level of self-pollination. Additionally, five spikes of each plant were left outside each cage to verify seed set and estimate optimal harvest date. At harvest, cages were removed, all inflorescences that were within the cage were bulked in paper bags to allow seed to dry, and spikes were subsequently mechanically threshed to separate mature seeds and chaff. Bulking the seeds for each cross (no parental control imposed) allowed for evaluation of the progeny as a single population rather than two separate half-sib families. This reduced the number of entries for evaluation, but did not allow for the elucidation of subtle phenotypic effects that may have been unique to half-sib families (e.g., maternal inheritance and endophyte–plant interactions). Further breeding through recurrent selection will modify such effects, and thus, should be evaluated in more advanced generations. Seeds in pollination bags inside the cage were separately threshed by hand to determine the number of mature seeds per head resulting from self-pollination.

Eighteen biparental crosses (Table 2), representing accessions from *F. valesiaca* Schleich *ex* Gaudin (6), *F. valesiaca* Schleich *ex* Gaudin subsp. *valesiaca* (4), *F. valesiaca* Schleich *ex* Gaudin subsp. *pseudodalmatica* (3), *F. heterophylla* Lam. (1), *F. brachyphylla* Schult. & Schult. f. (2), *F. filiformis* Pourr. (1), and *F. lenensis* Drobow (1), produced sufficient seed for a more rigorous evaluation of performance in the semi-arid western U.S. For this evaluation, seeds from each population and fine-leaved fescue checks, 'Durar', 'Covar', and 'Black Sheep' (Tables 3 and 4), were germinated and individual seedlings were established and maintained in a Logan greenhouse (Staub et al. 2014). Greenhouse established seedlings were transplanted in May 2011 to field nurseries at North Logan, the Utah State University Blue Creek Experimental Farm (41°56′3.14″N 112°26′20.01″W; elevation = 1566 m) in Box Elder County, UT approximately 80 km northwest of Logan, UT, and at the University of Idaho L. A. Sharps Experimental Station (42°18′5.90″N 113°11′49.51″W, elevation = 1476 m) near Malta, ID approximately 176 km northwest of Logan, UT. The soil type at North Logan, Blue Creek, and Malta was a McMurdie silt loam, a Parley's deep silt loam, and a Declo silt loam, respectively (http://websoilsurvey.nrcs.usda.gov/). The average annual precipitation during the experiment (2011–2013) at North Logan, Blue Creek, and Malta was 440, 362, and 265 mm, respectively, while the 30-year averages at these locations were 484, 407, and 291 mm, respectively (PRISM 2015).

Plants were arranged in a randomized complete block design (RCBD) with ten (North Logan and Malta) or five (Blue Creek) plants per plot in six replications spaced at 0.5 m within the rows and 1 m between rows (~20,000 plants/ha) with plants of PI 659984 (*F. rubra* L.; 8*x*; Qinghai, China) used as end- and side-borders. No water or fertilizer was applied to plants at planting, during establishment, or at any point during the experiment and plots were hand-weeded from May to August. Broadleaf weeds were also controlled with herbicide [mixture of 2,4-D (30.56 %), mecoprop-p (8.17 %), and dicamba (2.77 %); MEC Amine-D; Loveland Products, Greeley CO; U.S. Environmental Protection Agency (EPA) registration no. 34704-239] application once in April or May of each year at a rate of 3.0 (2,4-D), 0.8 (mecoprop-p), and 0.3 (dicamba) g/ha a.i.

Phenotypic trait evaluation

On March 30, 2012, May 1, 2012, and May 23, 2013, the relative vigor of each plant for all accessions at each location was assessed using an 11-point visual rating scale (from 0 to 5 with 0.5 as units), where plant spring green-up (size and transition from winter to spring growth) was defined as 0 = plant dead, 3 = plants possessing comparatively moderate biomass or leaf blade length with tussock evident, and 5 = plants with the greatest above ground biomass and/or long leaf blade length. Relative plant color was assessed for each plant on May 1, 2012 using a 1–5 scale based on the Royal Horticulture Society (RHS) color chart designations (edition V; rhscf.orgfree.com), where 1 = silver grey (RHS 188A), 2 = grey (RHS N155A), 3 = blue/green grey (RHS 97B), 4 = light to medium green (RHS 140B), and 5 = dark green (RHS 140A). Because of variation in leaf color among entries, light and medium green were given equal value, and color hue (intensity) was considered in the determination of relative plant color. On May 11, 2012 and May 23, 2013, leaves and seed stalks of the first half of each plot (the first 5 plants of the plot in North Logan and Malta and the first 3 plants in Blue Creek) were harvested just before anthesis and oven dried at 60 °C to estimate dry weight (g) biomass. The second half of the plot was similarly harvested when seeds were mature (June 26, 2012 to June 30, 2012 and June 27, 2013 to July 1, 2013) as a second, later measure of biomass and to estimate seed yield. Samples were dried, weighed, and mechanically threshed to separate mature seeds and chaff including poorly developed or aborted seeds, and seeds were weighed. Seed number per plant was estimated from seed weight using 0.0574 g per 100 seeds [average of *F. valesiaca* entries determined by Ma et al. (2014)], which is representative of the parental species utilized in the biparental crosses. Persistence was calculated as

Table 3 Mean values of phenotypic traits of selected biparental *Festuca* cross populations and checks evaluated in 2012 at three locations (Malta, ID, Blue Creek, UT, and North Logan, UT)

Entry	Vigor (rate 0–5)	Early biomass (g/plant)	Late biomass (g/plant)	Number seeds per plant	Persistence	Color (rate 1–5)	Early regrowth (g/plant)	Late regrowth (g/plant)
R4S1	1.5	11.22	6.38	1039.80	0.87	3.0	4.72	0.62
R4S3	2.4	16.08	21.53	7720.39	0.96	2.8	6.43	0.70
R4S4	2.4	18.13	34.68	2318.11	0.93	2.6	6.91	0.51
R4S5	3.0	25.80	32.29	16,093.00	0.95	2.7	7.26	0.58
R4S6	2.8	20.46	28.29	7572.36	0.93	2.9	6.69	0.81
R4S7	2.3	16.50	14.57	1868.46	0.89	3.4	10.18	1.32
R4S10	3.6	22.92	41.24	14,727.00	0.95	3.7	14.84	2.92
R4S11	2.4	13.56	19.42	5030.01	0.97	3.2	6.93	1.38
R4S12	2.1	15.01	20.62	4155.07	0.97	3.4	10.25	1.81
R4S14	2.6	16.91	25.34	3482.76	0.94	4.1	16.12	2.86
R4S15	3.1	19.27	30.45	4144.19	0.92	4.4	18.29	5.00
R4S18	2.3	19.34	24.14	7424.27	0.96	2.7	6.25	1.08
R4S19	2.6	16.48	20.42	3064.40	0.94	3.1	5.90	1.05
R4S20	2.7	19.92	21.43	3864.64	0.93	3.0	8.49	2.65
R4S21	2.5	19.53	23.52	5997.09	0.99	2.9	6.93	1.06
R4S22	3.3	32.19	36.99	10,792.00	0.92	2.9	16.96	4.28
R4S32	2.8	21.49	32.63	8642.39	0.93	3.1	11.68	1.77
R4S34	1.6	15.79	21.21	6591.13	0.88	2.5	5.69	0.12
Black Sheep	2.3	18.80	23.50	10,057.00	0.97	3.1	7.75	2.01
Covar	2.2	18.95	30.04	8365.97	0.90	2.9	9.80	2.68
Durar	2.4	20.85	28.49	8131.40	0.93	3.3	11.52	3.13
Grand mean	2.6	18.11	25.60	7027.63	0.95	3.1	9.34	1.45
LSD (α = 0.05)	0.7	7.32	10.31	3859.31	0.11	0.4	4.08	0.57
CV (%)	26.5	32.35	43.03	56.77	10.26	13.3	46.81	35.09

the percentage of plants alive in each plot at seed harvest. On October 10, 2012 and October 10, 2013, regrowth was harvested separately on plants from the early biomass and later biomass/seed harvests in each plot at the same height as the original harvest. Thus, data for vigor and color were recorded on a per-plant basis, while early and late biomass, seed yield, persistence, and early and late regrowth data were recorded by plot. Data for late biomass, seed yield, and regrowth were not available for the Malta location in 2013 due to significant rabbit (*Lepus californicus deserticola* Means) predation throughout the trial after the first biomass harvest.

Phenotypic trait analysis

Morphological trait data (over 2 years) were analyzed on per-plot means using a linear mixed models analysis under which residuals for all traits were tested for normality using PROC UNIVARIATE in SAS software (Version 9.3 for Windows, SAS Institute, Cary, NC). Homogeneity of variance was evaluated by plotting the residuals against the predicted values. Due to a significant deviation from normality in regrowth data at the late harvest, a natural log transformation was used in the analysis (Draper and Cox 1969). Although this transformation improved

Table 4 Mean values of phenotypic traits of selected biparental *Festuca* cross populations and checks evaluated in 2013 at three locations (Malta, ID, Blue Creek, UT, and North Logan, UT)

Entry	Vigor (rate 0–5)	Early biomass (g/plant)	Late biomass (g/plant)[a]	Number seeds per plant[a]	Persistence	Early regrowth (g/plant)[a]	Late regrowth (g/plant)[a]
R4S1	0.6	10.21	18.17	5725.07	0.54	6.92	0.68
R4S3	2.6	24.83	59.61	9022.80	0.97	11.50	4.00
R4S4	3.2	33.60	73.19	4317.32	0.92	10.53	1.78
R4S5	3.2	42.47	73.17	22,473.00	0.93	13.04	2.33
R4S6	2.7	28.71	63.67	4525.07	0.95	13.90	3.87
R4S7	1.8	15.40	41.99	6347.99	0.81	25.20	2.09
R4S10	3.5	20.32	67.95	9378.38	0.92	24.80	8.91
R4S11	1.4	7.45	27.39	4191.71	0.88	9.37	3.60
R4S12	1.9	11.32	29.79	1320.42	0.97	14.61	4.70
R4S14	2.1	13.78	39.26	2584.82	0.98	24.88	9.47
R4S15	1.6	12.25	37.03	879.14	0.94	26.88	15.69
R4S18	3.2	35.91	81.68	8740.68	0.94	13.52	5.63
R4S19	3.0	28.31	38.39	4117.75	0.94	12.98	4.84
R4S20	3.0	31.49	58.99	6200.97	0.90	16.04	6.61
R4S21	3.1	32.92	61.37	6278.22	0.98	15.71	4.51
R4S22	3.9	53.97	105.07	18,656.00	0.92	31.85	10.53
R4S32	3.3	30.21	67.90	8363.40	0.93	14.56	4.88
R4S34	2.2	27.93	60.87	9987.16	0.80	12.11	1.36
Black Sheep	2.1	12.05	42.13	9208.21	0.93	14.12	4.06
Covar	2.4	25.11	59.21	10,287.00	0.81	18.51	7.94
Durar	3.0	31.92	71.15	10,830.00	0.87	22.66	9.60
Grand mean	2.6	21.23	52.39	7523.64	0.89	16.26	5.48
LSD ($\alpha = 0.05$)	0.7	7.32	19.89	6563.85	0.13	6.07	3.82
CV (%)	26.8	27.60	25.26	68.42	10.90	32.53	60.53

[a] Data for late biomass, number seeds per plant, early regrowth, and late regrowth was not available in Malta in 2013 because of rabbit predation

normality (data not presented), the results are reported herein in their original scale for biological relevance and interpretation. Data distributions for all other traits approached normality and could be used directly for ANOVA.

The main effects of year, location, ploidy, and entry within ploidy were tested using a repeated measures model with compound symmetry covariance structure using year as the repeated factor and each plot as the subject. Year was assumed to be a fixed effect because inferences for years were limited to the 2 years (2012 and 2013) under evaluation (Smith and Casler 2004) and these 2 years may not be representative of multiple growing seasons of perennial species. Location, ploidy, and entry were also considered fixed effects because inferences were made for the specific levels evaluated. Replicates were considered random effects. Due to missing data from rabbit predation, data for late biomass, seed yield, and regrowth were analyzed separately by year as a RCB design using PROC MIXED in SAS with data only from North Logan and Blue Creek in 2013. Entry means were separated using Fisher's protected least-significant difference (LSD) test by applying the lsmeans statement in SAS. Multivariate principal component analysis (PCA) was performed on lsmean values of all traits using PROC FACTOR in SAS to define relationships among entries and to identify those traits that led most to entry discrimination (Kutner et al. 2004). Pearson product-moment correlation coefficients were produced using PROC CORR in SAS to assess the strength of associations among the traits examined (Székely et al. 2007).

Ploidy determination

The determination of ploidy level of parental stocks is critical to strategic selection of parents for hybrid production in grasses (Ruemmele et al. 2003). Thus, the ploidy level of each accession was determined by cytological examination of mitotic chromosomes at metaphase following the methodology of Jensen et al. (2006). At least three samples from each accession were treated and chromosomes were visually counted (Table 1).

Genetic similarity and structure analysis

Analyses of within-population mean genetic similarity and model-based Bayesian clustering were used to assess genetic relatedness of the best performing biparental cross populations employing amplified fragment length polymorphism (AFLP) markers. To extract genomic DNA for the AFLP procedure, leaf samples of multiple (>10) plants within each accession/population were collected, lyophilized, and then ground into fine powder with zinc beads inside extraction tubes using a mixer mill (Retsch model MM 300, F. Kurt Retsch GmbH and Co., Haan, Germany). Total cellular DNA was extracted using a DNA extraction kit (DNeasy Plant Mini Kit, QIAGEN, Venlo, Netherlands) according to the manufacturer's instructions, and quantified with a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, Waltham, MA). The AFLP polymerase chain reaction (PCR) amplification procedure was performed following 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 pre-amplification, while the primer combinations of E-ACAC/M-CTAC, E-ACAG/M-CTCA, E-ACCA/M-CTAG, E-ACCA/M-CTTC, and E-ACCT/M-CTCT were used for selective amplification. At least 10 % of the samples were replicated to identify reproducible marker bands and determine marker error rates (data not presented). AFLP data were visualized and scored according to Staub et al. (2014).

The similarity index (Dice 1945; Leonard et al. 1999) was used to estimate the within-population mean genetic similarity. The similarity index is defined as: $S_{xy} = 2N_m/N_x + N_y$, where N_m is the number of marker bands that are shared between individuals x and y, and N_x and N_y are the total number of bands present in each respective individual. Standard errors of the mean similarity values were computed according to Leonard et al. (1999). The genetic diversity (pairwise Φ_{PT} distances) among cultivars was computed using an analysis of molecular variance (AMOVA) implemented in GenAlEx v6.4 (Peakall and Smouse 2012). Principal coordinate analysis (PCoA) was conducted using the standardized covariance matrix of the Φ_{PT} distances, also in GenAlEx.

Population structure was assessed by employing STRUCTURE v2.2 (Falush et al. 2007) without advanced assignment of plants into groups. The data were analyzed with the "Recessive Alleles" option and the "Admixture Model" with correlated marker frequencies. Probabilities of the dataset for $K = 1$ through $K = 12$ groups were tested with three replications for each level of K. The Markov chain Monte Carlo (MCMC) procedure was used with 10,000 burn-in and 100,000 MCMC steps after burn-into determine the probability of each structure model. The average estimated log probability of the data was plotted against the K values to observe structural fit of the data, and the second order rate of change of log probability (ΔK) between two successively tested models (Evanno et al. 2005) was used to determine the optimal K value.

Results

Phenotypic trait evaluations of accessions and biparental matings

Observations were made on the general performance of 63 fine-leaved fescue accessions representing 11 species in North Logan in 2009 (Table 1). Fourteen plants of each accession were rated for relative plant vigor, color, and biomass, and the number of plants with rangeland potential (rating of 3 or greater) ranged from 0 (all plants from several accessions died) to 8. Only those plants with a rating of 4 or 5 were selected as parents for biparental populations. A total of 62 plants from 27 accessions representing eight species (no selections from *F. arizonica*, *F. idahoensis*, and *F. rupicolla*) were selected as parents and these were transplanted as pairs in 31 crosses. Most crosses (20) were parents of the same accession, but five selections were paired with 'Covar' as intraspecific crosses, five selections were paired with 'Black Sheep' as interspecific crosses (*F. valesiaca* × *F. ovina*), and PI 659923 was paired with PI 670362 (intraspecific cross). Crosses of PI 634225 × PI 634225, PI 659944 × PI 659944, PI 659923 × PI 670362, and PI 634304 × 'Covar' could not be made as one or both of the parents did not produce inflorescences. Intra-accession crosses using PI 311045, PI 370273, W6 25539, W6 25542, and W6 25540 did not produce any viable seed; the PI 255361 intra-accession cross did not produce adequate seed quantities for multi-location evaluation; and seed germination of crosses with PI 670364 and W6 25539 was poor. Thus, 18 crosses (Table 2) produced seed in sufficient quantity and quality for evaluation in replicated trials. Of the seed heads covered to measure self-pollination, 6.1 seed heads yielded only 7.2 seeds on average, indicating the seed collected (>1000 seeds/cross) was largely cross-pollinated.

For the field evaluation of the biparental cross populations, the main effects of year and year by location interaction were significant ($P < 0.05$) for all traits, except vigor ($P = 0.83$ and 0.11, respectively). Overall mean trait values were greater in 2013 than 2012 for early biomass, late biomass, early regrowth, and late regrowth, whereas the mean values for persistence and seed number decreased. The main effect of location was significant ($P < 0.05$) for all traits except vigor ($P = 0.17$) and color ($P = 0.79$), with mean trait values at each location as North Logan > Blue Creek > Malta for early biomass, late biomass, and seed number, and North Logan = Blue Creek > Malta for persistence, early regrowth, and late regrowth. The main effect of ploidy was significant ($P < 0.05$) for vigor, early biomass, color, early regrowth, and late regrowth, but the highest performing ploidy levels were not generally consistent over traits, indicating further evaluations are required to characterize the effect of ploidy on these traits. The main effect of entry was highly significant ($P < 0.006$) for all traits examined, as was entry by year interaction effects, except for early and late regrowth across years at North Logan and Blue Creek (no data for Malta over both years). Entry by location interaction effects were also significant ($P < 0.05$) for all traits.

To provide a visual representation of the performance of all entries for all traits over locations, the least squares means in each year were used in a PCA analysis and the values for each entry for the first two principal components were plotted (Fig. 1). The variance explained by the first two components was 74 % for 2012 and 78 % for 2013. Based on the absolute value of the eigenvectors, the traits that had the greatest influence on the first component over all locations in 2012 were vigor, early regrowth, late biomass, early biomass, and late regrowth, in that order. In 2013, the traits with the greatest influence were late biomass, vigor, early biomass, and seed number, in that order. The PCA plots (Fig. 1) indicate that, although most populations perform similarly, several populations (R4S1, R4S14, R4S15, R4S5, and R4S22) did not group with the other populations examined. Population R4S1 consistently performed comparatively low, while R4S22 was a high performer across locations and years. Population R4S5 performed well for most traits, except for color, early regrowth and late regrowth, while R4S14 and R4S15 were average performers for most traits, low for seed production, and high for color and early and late regrowth (Tables 3 and 4). Other than R4S22, there were not any entries that were clearly superior in all locations over all years. Based on this variation, the best performing populations at the harshest environment (lowest precipitation, Malta), which best represents arid rangelands in the Great Basin, were chosen for morphological and molecular analyses.

During the first year of biparental cross-progeny evaluation, four of the 18 populations examined (22 %) were identified that out-performed the cultivar check 'Durar' at Malta. Over the two evaluation years, the average performance of these four populations (R4S4, R4S6, R4S22, and R4S32) at Malta was, when compared to 'Durar', 84-210 % for vigor, 79-90 % for color, 65-562 % for biomass, 64-296 % for seed yield, 92-117 % for persistence, and 164-454 % for regrowth, with R4S22 demonstrating the best performance overall (mean values Tables 3 and 4 and Fig. 1; % data not shown). We present the data by year over locations (Tables 3 and 4) since the

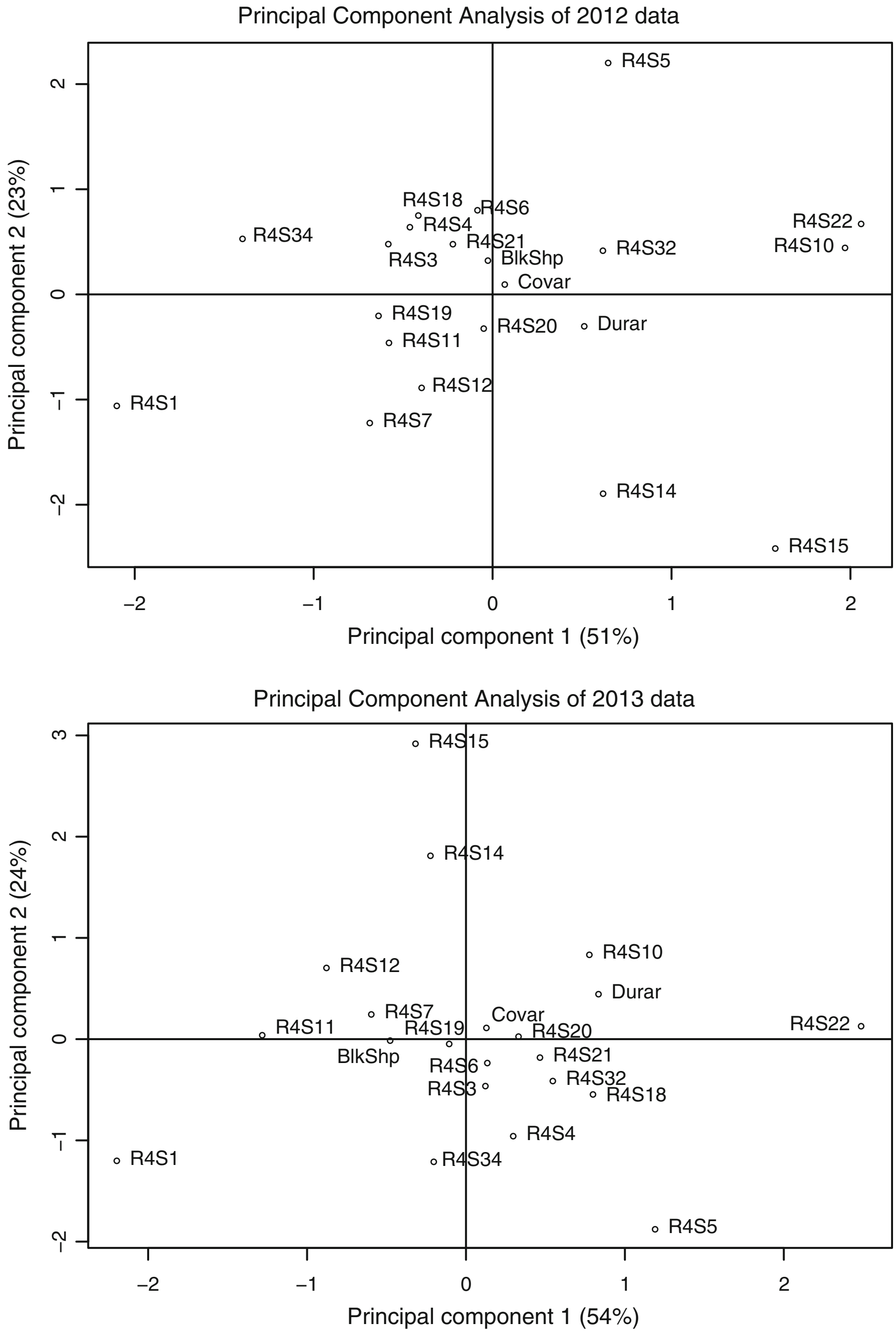

Fig. 1 The first two components of principal component analysis of biparental *Festuca* cross populations and checks evaluated over three locations (Malta, ID, Blue Creek, UT, and North Logan, UT) in each of 2 years. *Parenthetical numbers* on each axis indicate the percent variation explained by each component. *BlkShp* indicates the cultivar 'Black Sheep'

differences in location were mainly of magnitude rather than rank. Of the check cultivars that were evaluated, both 'Durar' (greatest values for vigor, early biomass, color, early regrowth, and late regrowth) and 'Black Sheep' (greatest values for seed number, and persistance) performed the best in 2012 (Table 3), whereas, 'Durar' (greatest values for vigor, early biomass, late biomass, seed number, early regrowth, and late regrowth) was the best performer in 2013 (Table 4). In 2012, the four selected biparental cross populations (R4S4, R4S6, R4S22, and R4S32) did not differ from the highest check for most traits. Population R4S22, however, performed significantly better than 'Durar' for vigor rating, early biomass, early regrowth, and late regrowth. In 2013, R4S22 performed better than 'Durar' for all traits, except for persistence and late regrowth, which were not significantly different. In fact, R4S22 mean trait values for vigor rating, early biomass, late biomass, and early regrowth were the highest among all entries, though not always significantly different than other entries. The other biparental cross populations (R4S4, R4S6, and R4S32) were not significantly different from 'Durar', except for exhibiting lower mean values for early and late regrowth.

When considered across locations for each year, several trait values were consistently and significantly correlated. Vigor rating, early biomass, and late biomass were highly positively correlated over both years as were early and late regrowth (Table 5). Early and late regrowth were positively correlated with vigor rating, early biomass, and late biomass in 2012, but only late biomass and early regrowth were positively correlated in 2013. The number of seeds per plant was moderately positively correlated with vigor, early biomass, and late biomass over years, but more so in 2013 than in 2012. However, seed number was not correlated with early or late regrowth. Persistence was highly correlated with vigor rating and moderately correlated with late biomass in 2013, but not correlated with any trait in 2012. Plant color, which was only evaluated in 2012, was not correlated with any trait, except a moderate correlation with vigor rating.

Molecular marker assessment

Because our objective was to indentify and genetically characterize the best performing hybrid populations, we present the molecular characterization (Table 6; Figs. 2 and 3) for these 4 populations only. To determine the genetic similarity of plants within a population, and genetic relatedness between the four selected biparental populations, AFLP data from five primer combinations were analyzed using a similarity index and AMOVA, respectively. Similarity indexes (with standard errors) were 0.543 (0.019) for 'Covar', 0.567 (0.018) for 'Black Sheep', 0.588 (0.012) for R4S6, 0.643 (0.020) for R4S4, 0.646 (0.010) for R4S22, 0.662 (0.015) for 'Durar', and 0.755 (0.006) for R4S32 indicating that 'Covar' possessed the most within-cultivar diversity while R4S32 was the least diverse. The Φ_{PT} genetic distances indicated that each population and accession were significantly distinct (Table 6). These genetic distances were utilized in a PCoA analysis (Fig. 2), where 52 % of the variation was explained by principal coordinates one and two. The four populations and cultivars ('Durar', 'Covar',

Table 5 Pearson correlation coefficients of 8 traits of *Festuca* populations evaluated at three locations (Malta, ID, Blue Creek, UT, and North Logan, UT) in 2012 (top diagonal), and 2013 (bottom diagonal)

	Vigor	Early biomass	Late biomass[a]	Number seeds per plant[a]	Persistance	Color[b]	Early regrowth[a]	Late regrowth[a]
Vigor		0.723***	0.769***	0.512*	0.374	0.425*	0.664***	0.550*
E. bio.	0.825***		0.754***	0.598*	0.175	−0.061	0.511*	0.499*
L. bio.	0.872***	0.907***		0.641*	0.204	0.116	0.543*	0.424*
No. seeds	0.526**	0.700***	0.679***		0.192	−0.113	0.134	0.048
Persist.	0.611**	0.332	0.456*	0.057		0.188	0.124	0.058
Color							0.757***	0.631**
E. reg.	0.319	0.265	0.418*	0.208	0.353			0.876***
L. reg.	0.222	0.090	0.234	−0.029	0.390		0.772***	

[a] Data for late biomass, number seeds per plant, early regrowth, and late regrowth was not available in Malta, ID in 2013 because of rabbit predation

[b] Color was not evaluated in 2013

The symbols *, **, and *** denote significance at $P < 0.05$, 0.001, and 0.0001, respectively

Table 6 Pairwise Φ_{PT} genetic distances between biparental *Festuca* cross populations and check cultivars determined by AFLP data

	R4S22	R4S6	R4S32	R4S4	Covar	Durar
R4S6	0.358					
R4S32	0.438	0.389				
R4S4	0.401	0.295	0.493			
Covar	0.307	0.196	0.462	0.227		
Durar	0.302	0.334	0.438	0.435	0.328	
Black Sheep	0.277	0.354	0.433	0.410	0.313	0.278

All differences were significant at $P < 0.01$

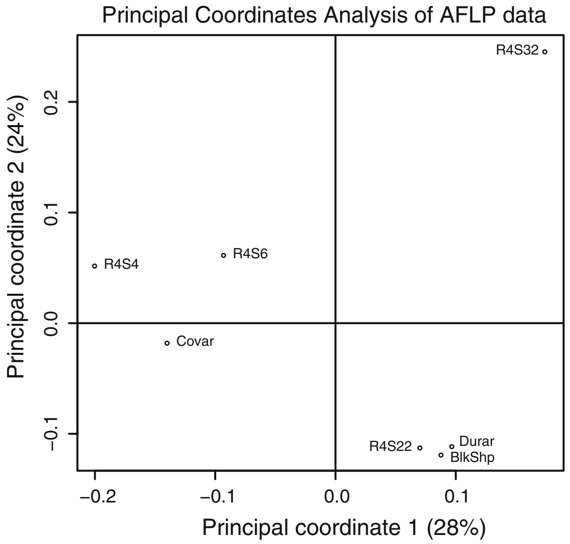

Fig. 2 The first two coordinates of principal coordinates analysis of AFLP data of biparental *Festuca* cross populations and commercial checks. *Parenthetical numbers* on each axis indicate the percent variation explained by each coordinate. *BlkShp* indicates the cultivar 'Black Sheep'

and 'Black Sheep') clustered into three distinct groups. The R4S4 and R4S6 populations grouped near the check cultivar 'Covar', the R4S22 population clustered near 'Black Sheep' and 'Durar', and the R4S32 population did not group near any check cultivar (Fig. 2).

To further test the accessions and populations for genetic relationships, a Bayesian structure analysis was conducted without a priori assigning plants into populations/accessions. The $K = 3$ model showed a distinct spike in the second order differences, and the average log likelihood value improved only marginally thereafter, indicating that $K = 3$ is the most optimal K value. For this model, the relationships of populations to check cultivars (Fig. 3) mirrored that of the PCoA clustering (Fig. 2). Additionally, the parents of R4S22, R4S32, and R4S4 were included (R4S6 parents died before tissue was collected). All the parental plants included co-ancestry of the variety 'Covar', with R4S4 mainly derived from 'Covar', which was one of its parents (Table 2). Population

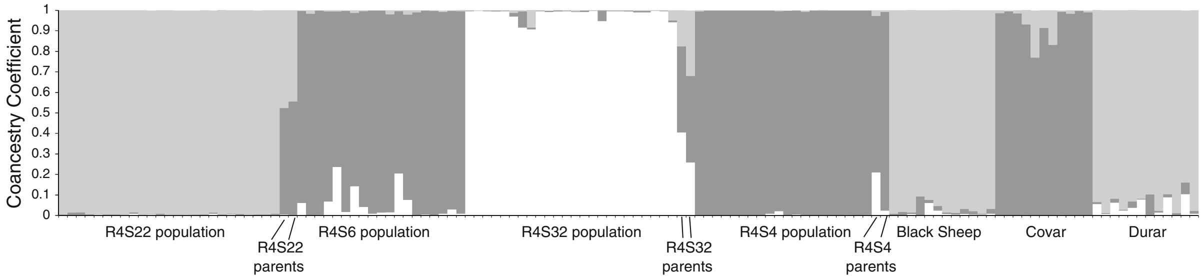

Fig. 3 Coancestry coefficients from Bayesian cluster analysis of AFLP genotypes of biparental *Festuca* cross populations, their parents, and commercial checks with the optimal number of clusters ($K = 3$)

R4S22 included ancestry of ‘Black Sheep’ (or ‘Durar’), which was also consistent with its pedigree, and R4S32 (*F. valesiaca* × *F. valesiaca*) included significant percentages of co-ancestry from ‘Covar’, ‘Black Sheep’/’Durar’, and another unknown source.

Discussion

The semi-arid and arid rangelands and irrigated pastures of the western U.S. provide a broad array of ecosystem services, including livestock forage, pollinators, animals, and recreational activities (Wisdom and Chambers 2009; Booth et al. 2003). However, extensive and repeated wildfires in the western U.S. have resulted in the invasion and expansion of undesirable annual weeds into many ecosystems (Brooks and Pyke 2001; Abella 2010). Thus, the identification and development of high performance drought and heat tolerant narrow-leaved fescue germplasm possessing fire resilience for use in greenstrips and rehabilitation of degraded landscapes could increase rangeland sustainability. This study evaluated narrow-leaved North American and Old World *Festuca* species that form an *F. ovina* aggregate called the “Ovina Complex” (Arndt 2008; Ruemmele et al. 2003; Table 1). The comparatively shallow root system, shorter plant canopy, slower growth, and lower transpiration rates of some narrow-leaved *Festuca* species of the Ovina Complex (e.g., *F. ovina* and *F. valesiaca*; Ma et al. 2014) make them candidates for drought tolerant plant material with potential use in arid environments where low productivity and species diversity create susceptibility to wildfires. Although some of the parental materials selected from the observational trial reported herein and used for hybrid production might have been predicted based on their origins and previous performance in arid Great Basin environments (Ma et al. 2014), the initial choice of some species for parental stocks from arctic and alpine environments [e.g., *F. lenensis*, *F. trachyphylla* (synonym *F. lemanii*), and *F. brachyphylla*] was not expected.

Based on predicted climate change models for semi-arid regions, growing environments in the Great Basin will become hotter and drier, increasing the already high rate of rangeland and pasture degradation and reductions in forage productivity (Karl et al. 2009; Brooks and Minnich 2006). Some of the hybrid populations compared favorably with the performance of ‘Covar’ and ‘Durar’ (Tables 3 and 4), where R4S22 (*F. valesiaca* × *F. ovina*; Table 2) was vegetatively more vigorous and persistent than either check in the harshest environment, Malta, ID. ‘Durar’ (*F. trachyphylla*, synonyms *F. brevipila* Tracey, *F. lemanii*, and *F. ovina*; Barkworth et al. 2007) is derived from a 1934 collection made in a grass planting in Union, eastern WA, and was released in 1949 after several generations of mass selection for phenotypic uniformity (Experimental P-251) by Washington State University (WSU) and the U.S. Department of Agriculture Natural Resources Conservation Service Plant Materials Center (USDA-NRCS) at Pullman, WA (USDA 1994). In contrast, ‘Covar’ (*F. valesiaca* subsp. *valesiaca*) was derived from PI 109497 (Turkey; collected 1934), where it was selected from spaced plants and released jointly in 1977 by WSU and the USDA-NRCS. Populations R4S4, R4S6, R4S22, and R4S32 are derived from *F. valesiaca* subsp. *valesiaca*; *F. valesiaca* × *F. valesiaca* subsp. *valesiaca*; *F. valesiaca* × *F. ovina*; and *F. valesiaca* subsp. *valesiaca* matings, respectively (Table 2). Although R4S4, R4S6, and R4S22 were derived from biparental matings using either commercial *F. valesiaca* subsp. *valesiaca* (‘Covar’) or *F. ovina* (‘Black Sheep’) cultivars as a parent, all hybrid populations have genetic contributions from Eurasian PI’s of diverse origin [i.e., PI 268234 (Iran), PIs 502383 and 502380 (Russia), and PI 383652 (Turkey)]. These PIs were collected mainly from high steppe grasslands in Russia (PI 502383; Stavropol Province, elevation = 196 m; annual precipitation = 562 mm) on calcareous soils and in Turkey (PI 383652; Erzurum Province, elevation = 2000 m; annual precipitation = 400 mm) on gravelly silt loam soils. Such collection environments, however, are not as harsh (i.e., annual precipitation and temperature) as growing environments at Blue Creek or Malta, and, thus, it appears that these parental materials may possess genes for abiotic stress tolerance (e.g., heat, drought) not predicted given their origins.

Some fine-leaved *Festuca* species [e.g., *F. rubra* var. *commutata* Gaud.; *F. ovina* var. *duriuscala* (L.) Koch] remain relatively green under high temperatures (e.g., >30 °C) and drought conditions (Ruemmele et al. 2003; Aronson et al. 1987). Although largely untested in rangeland settings, these and other characteristics make fine-leaved *Festuca* phenotypes attractive for use in rangeland greenstrips for wildfire control from June to August in the western U.S. when wildfires are most prevalent (Dibble et al. 2007; Littell et al. 2009). Green color in leaves is indicative of chlorophyll content and active photosynthetic capacity (Gates et al. 1965). “Glaucous” leaf coloration (bluish-grey/green to silvery white) typically present in “blue fescue” (e.g., *F. glauca* auct. non Vill.; Auquier and Kerguélen 1977; Wilkinson and Stace 1991) accessions is due to a waxy leaf coating, which reduces transpiration under abiotic stress conditions (e.g., water and salt) (Li et al. 2010). Blue fescue types are physiologically complex, where their grey to blue green grey leaf color (intensity and hue) can increase under abiotic stress due to cuticular changes. Even though parental selections used in hybrid development were chosen, in part, for their green color (each parent had a combined vigor and color rating of 4 or 5) in North Logan (data not shown), some hybrid progeny demonstrated grey to blue/green grey color (color rating = 1–3) at Blue Creek and Malta. While the color of most plants (>95 %, data not presented) in populations R4S22, R4S32, and R4S6 was blue/green grey or similar to ‘Covar’ (light green), plant color in R4S4 individuals grown at Malta varied between silver grey to grey (data not shown). Thus, it appears that cuticlar wax can develop in some plants of these populations in response to harsh abiotic conditions, which may be an adaptive mechanism (Li et al. 2010). In contrast to population R4S4 (mean rating = 2.6), plants of R4S22, R4S32, and R4S6 (mean ratings = 2.9, 3.1, and 2.9, respectively) were blue/green grey to dark green and vigorous at Malta, and, thus, likely possess leaf responses (e.g., stomatal adjustments and leaf rolling) that provide for lower transpiration rates under abiotic stress (O’Toole and Cruz 1980; Bonos and Murphy 1999). Such leaf characteristics along with their continued green coloration (e.g., relatively high water content) from June to August are characteristics that make these populations amendable for further evaluation for use as greenstrip components.

Some of the most effective greenstrip plant species grow in bunches with relatively large spaces between plants to disrupt fuel continuity (Pellant 1994; Finney et al. 2007). The bunch type habit of fine-leaved fescue species and their ability to remain green through early summer periods (July–August) when western U.S. rangeland fires typically occur makes them particularly amenable for use as a greenstrip component. Both the phenotypic and genotypic evaluations of R4S4, R4S6, indicate that the progeny from these biparental populations are similar to their parental cultivar, ‘Covar’. Although the R4S32 population does not contain ‘Covar’ as a parent and the AFLP analysis indicates that this population does not group with ‘Covar’, its phenotypic performance is not significantly different from ‘Covar’ for most traits examined. Thus, R4S4, R4S6, and R4S32 would likely be useful in areas where ‘Covar’ is utilized, but they do not represent a significant improvement in currently available fine-leaved plant material. Population R4S22 is genetically similar to ‘Durar’ and its parent, ‘Black Sheep’, and might be expected to perform similar to these cultivars. The performance of R4S22, however, was superior to both cultivars (Tables 3 and 4), indicating that this population should be further selected for persistence, drought tolerance, biomass production, regrowth, and leaf color uniformity. Given the trait correlations presented herein, emphasis during continued selection in R4S22 should be placed on plant vigor, which is moderately to highly positively correlated with early- and late-season growth characteristics (Table 5). Uniform selections from such evaluations should be intermated with the resulting progeny (e.g., half-sib families) being evaluated for their specific greenstrip attributes including resiliency to fire, interaction with commonly used subshrub components of greenstrips such as forage kochia [*Bassi prostrata* (L.) A.J. Scott., synom. *Kochia prostrata* (L.) Schard], and their competitiveness with annual invasive weeds. Such genetically diverse refined populations will likely perform better than current narrow-leaved *Festuca* cultivars (e.g., ‘Durar’) in greenstrips, and, thus, their use will improve rangeland productivity and sustainability, and assist in reclaiming degraded landscapes.

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