

# PLANT GENETIC RESOURCES

## Identification of Western Wheatgrass Cultivars and Accessions by DNA Fingerprinting and Geographic Provenance

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### ABSTRACT

Western wheatgrass [*Pascopyrum smithii* (Rydb.) Löve (= *Agropyron smithii* Rydb)] is an allogamous North American range grass cultivated for seed production, hay, low-maintenance turf, and soil stabilization. The USDA maintains western wheatgrass cultivars, synthetic multiple-origin germplasms, and source-identified single-origin accessions in the National Plant Germplasm System. The objective of this study was to test similarity of amplified fragment length polymorphism (AFLP) genotypes among individual plants classified by accession and geographic provenance, and compare rates of DNA variation between single-origin and multiple-origin germplasm. Individual plants from 36 of 39 source-identified single-origin germplasm sources group strictly by accession, on the basis of the average proportion of shared DNA fragments between individual genotypes. Conversely, individual plant genotypes from three of the four synthetic multiple-origin accessions did not cluster by accession and displayed higher rates of DNA variation than did single-origin accessions. Although different accessions of the same cultivar generally group together, off-types were detected in six of the nine cultivar accessions. Pairwise comparisons of interpopulation genetic distances ( $\phi_{st}$ ) among 39 single-origin accessions were correlated with geographical distances among the original collecting sites ( $r = 0.66$ ). Genotypes representing these accessions form three natural groups on the basis of  $\phi_{st}$  that correspond to three geographic regions of the USA: northern Great Plains, northern Rocky Mountains, and southern Rocky Mountains. Therefore, geographically significant sources of DNA variation were detected and maintained within and among these *ex situ* germplasm sources. These results document genetic identity and diversity in the USDA western wheatgrass germplasm collection and support the premise that geographic provenance contributes to germplasm integrity.

WESTERN WHEATGRASS is an ecologically dominant, native perennial range grass in the northern Great Plains (Hart et al., 1996). Cultivars of western wheatgrass are also planted for pasture, hay, and soil stabilization in this region (Asay and Jensen, 1996). However, the distribution of western wheatgrass further extends throughout much of temperate North America (Hitchcock, 1951; Cronquist et al., 1977), including dry sagebrush (*Artemisia* spp.) deserts and foothills of western mountain regions, where it also displays considerable

ecotypic variation (Asay and Jensen, 1996). Although forage quality rapidly declines as plants mature, it is highly palatable to all classes of livestock during the early growing season and naturally cured leaves provide good winter forage. Western wheatgrass rapidly regenerates from rhizomes following wildfire and other disturbances (USDA Forest Service, 2001), and forms a durable sod that is frequently used to stabilize disturbed soils. This species is naturally abundant on heavy alkaline soils characteristic of intermittent swale bottoms, shallow lake beds, or areas subjected to periodic flooding (Rogler, 1973). It is also well suited to reclamation of saline sites (Beetle, 1955; Halvorson and Lang, 1989; Scheetz et al., 1981).

Western wheatgrass is an allooctoploid ( $2n = 8x = 56$ ) species comprised of the SSHHNNXX genome combination (Dewey, 1975). On the basis of cytological evidence as well as ecological, morphological, and reproductive characteristics in related allotetraploid ( $2n = 4x = 28$ ) species and their hybrids, this species arose through hybridization between NXXX *Leymus triticoides* (Buckl.) Pilger and SSHH *Elymus lanceolatus* (Scribn. J. G. Sm.) Gould, or closely related perennial Triticeae species with similar genome combinations (Dewey, 1975). Although most western wheatgrass plants are highly self incompatible, some individual clones may be self fertile (Jensen et al., 1990). Therefore, genetic heterogeneity should be maintained within and among individual plants. Like its putative ancestral species, western wheatgrass is strongly rhizomatous and individual plants may form large recognizable clones in nature.

The USDA has made substantial efforts and commitments to acquire, preserve, evaluate, document, and distribute plant germplasm, primarily through the National Plant Germplasm System (NPGS). Although much of this effort has been devoted to cultivated field crops, a substantial number of nondomesticated species including natural and cultivated forages are also maintained. Currently, NPGS preserves 48 western wheatgrass accessions including cultivars and other potentially useful germplasm sources. Most of these NPGS accessions are described as single-origin accessions originally derived from well-defined localities. However, multiple-origin cultivars and germplasm sources (Alderson and Sharp, 1994; Barker et al., 1995; Smoliak and Johnston, 1984) have been derived from several broad-based west-

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**Abbreviations:** AFLP, amplified fragment length polymorphism; AMOVA, analysis of molecular variance; FRRL, Forage and Range Research Laboratory; GRIN, Germplasm Resources Information Network; NPGS, National Plant Germplasm System; PMC, Plant Material Center; WRPIS, Western Region Plant Introduction Station.

ern wheatgrass collections (Barker et al., 1983; Johnston et al., 1975). Most of these cultivars and germplasm sources originate from the Great Plains region of Alberta, Kansas, North Dakota, Nebraska, South Dakota, and Saskatchewan, where this grazing-tolerant species is naturally abundant. However, the NPGS and ARS Forage and Range Research Laboratory (FRRL) also maintain accessions for several other cultivars and natural germplasm sources originally obtained from the Rocky Mountain region of Montana, Wyoming, Utah, Colorado, Arizona, and New Mexico. Even though all of these cultivars and accessions have relatively recent and well-defined natural origin, the genetic identity and diversity of many germplasm sources of western wheatgrass may have been intentionally or inadvertently affected by breeding and/or regeneration. In any case, DNA variation within and among these germplasm sources has not been evaluated or documented.

Attention to plant genotype and population genetics is needed to improve the role of seed banks in germplasm conservation (Brown et al., 1997; Schoen and Brown, 2001; Ford-Lloyd, 2001). The AFLP method of DNA fingerprinting (Vos et al., 1995) has been used to characterize genetic identity and diversity for heterogeneous cultivars and natural populations of cross-pollinating crested wheatgrass (*Agropyron* spp.) (Hu et al., 2001), bluebunch wheatgrass (*Pseudoroegneria spicata*) (Larson et al., 2000), and ryegrasses (*Lolium* spp.) (Rolán-Ruiz et al., 2000). The primary objective of this study was to test the genotypic identification of plants classified by accession and geographic provenance, including duplicated sources of several cultivars, by the AFLP technique. Another related objective was to compare rates of DNA variation among single-origin and multiple-origin western wheatgrass accessions.

## METHODS

### Plant Materials

Seed from 39 NPGS accessions (Table 1) obtained from the Western Regional Plant Introduction Station (WRPIS) plus 13 additional FRRL accessions (Table 1) were germinated on moist blotter paper and grown in single plant containers. Duplicated accessions for P727 and cultivars Arriba, Barton, and Rodan (Alderson and Sharp, 1994) were obtained from FRRL and WRPIS (Table 1). The P727.2 accession was donated to NPGS by the Pullman NRCS Plant Material Center (PMC) in 1977, whereas P727.1 was regenerated in 1982 (2 g) and 1993 (15 g) by FRRL. The P727 selection was originally collected by NRCS workers from a naturalized railway sward near George, WA, and regenerated by bulk seed increase after comparisons with other accessions (Alderson and Sharp, 1994; Mark Stander, personal communication, 2000). However, the source population of P727 may not be native to Washington. The accession cv. Arriba.1 (PI 578777) was donated to NPGS from the Los Lunas PMC in 1977, whereas cv. Arriba.2 was increased by FRRL in 1985. The accession cv. Barton.1 (PI 421274) was submitted to NPGS by the Manhattan PMC in 1977, whereas cv. Barton.2 (PI 578778) was submitted to NPGS by the Manhattan PMC in 1974. The single-origin cultivars Arriba and Barton were essentially developed by bulk seed increases of seed collected from well-defined populations (Alderson and Sharp, 1994). The acces-

sion cv. Rodan.1 (PI 477993) was submitted to NPGS by the Bismark PMC in 1983, whereas cv. Rodan.2 (PI 578780) was submitted to NPGS by the ARS Northern Great Plains Research Laboratory (Mandan, ND) in 1984. Although the pedigree of Rodan is well-defined (Barker et al., 1984), the exact natural origin its base population is uncertain.

Nine single-origin seed accessions from the southern Rocky Mountain region of Arizona, Colorado, New Mexico, Utah, and Wyoming (Table 1) were collected from relatively small natural swards (less than 1 ha) and regenerated via bulk seed increases by FRRL. Likewise, three single-origin NPGS accessions from the northern Rocky Mountain region (MT-Broadwater, MT-Deerlodge, and MT-Teton), collected by USDA workers F.J. Herman and B.M. Leese in 1955, were presumably donated as seed collected directly from relatively small natural swards. The single-origin MT-Yellowstone accession (Table 1), submitted by the NRCS in 1975, was presumably regenerated by bulk seed increase of a superior natural germplasm collection from Yellowstone Co., Montana. Similarly, the cultivar Rosana was regenerated by bulk increase of seed harvested along a tributary of the Yellowstone River in Rosebud Co., Montana (Alderson and Sharp, 1994).

Twenty-six single-origin NPGS accessions from North and South Dakota (Table 1) were originally submitted to NPGS as half-sib progenies from 30 open-pollinated clones. These source-identified clones were pollinated by 2000 other clones selected from a base population of 5150 plants from 1040 sites in western North and South Dakota (Barker et al., 1983; J.D. Berdahl, personal communication, 2002). The multiple-origin WWG931 population (Table 1) was selected from the same broad-based collection of 5150 genotypes (Barker et al., 1995) and displayed heritable variation for agronomic and morphological traits (Ray and Harms, 1994). Similarly, the 20-clone synthetic cultivar Walsh (Smoliak and Johnston, 1984) and synthetic germplasm WWG932 (Barker et al., 1995) accessions (Table 1) were both selected from a multiple-origin collection of 468 ecotypes from western Canada (Johnston et al., 1975). The multiple-origin base population of Walsh and WWG932 also displayed significant agronomic variability (Johnston et al., 1975). Another multiple-origin cultivar, Flintlock (Table 1), was derived from 100 clones selected from a base population of 30 accessions from western Nebraska and Kansas synthetic (Alderson and Sharp, 1994). Seed from multiple-origin cultivars Walsh and Flintlock was available at FRRL (Table 1), but not NPGS.

### DNA Analysis

Total leaf DNA was extracted from seven individual seedlings per accession. Approximately 100 mg of leaf tissue was collected in 2-mL microcentrifuge tubes containing two 5-mm steel bearings. Sample tubes were frozen under liquid nitrogen and vortexed to pulverize tissues into a fine powder. One milliliter of prewarmed (65°C) extraction buffer [2% (w/v) hexadecyltrimethylammonium bromide, 1.4 M sodium chloride, 20 mM ethylenediaminetetraacetic acid, 100 mM Tris-HCl pH 8.0, 0.2% (v/v)  $\beta$ -mercaptoethanol, 0.1 mg/mL RNase] was added to the frozen leaf powder and incubated in 65°C water bath for at least 1 h. A 24:1 (v/v) solution of chloroform-isoamyl alcohol was added and mixed to remove organic solubles prior to phase separation by centrifugation (14 000  $\times$  g for 5 min). The upper aqueous phase containing DNA was transferred to a 1.5-mL microcentrifuge tube and mixed with 0.7 mL cold isopropanol. The DNA was removed via glass pipette and washed in 70% (v/v) ethanol/10 mM ammonium acetate, air dried, and dissolved in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0).

Table 1. Description 52 USDA-ARS western wheatgrass accessions analyzed by the AFLP DNA fingerprinting technique.

ID†	Accession	Seed Source‡	Origin	Type	Plants analyzed	DNA fragments per plant avg. (min.-max.)	DNA Polymorphisms avg. (SE)
cv. Arriba.1	PI 578777	WRPIS-95o	Kit Carson Co., CO	cultivar, single-origin	7	417.3 (397-428)	240.6 (17.5)
cv. Arriba.2	Arriba	FRRL-85i	Kit Carson Co., CO	cultivar, single-origin	7	422.1 (413-435)	217.6 (26.0)
AZ-Navajo	D-3289	FRRL-93i	Navajo Co., AZ	single-origin	4	441.0 (422-457)	206 (35.0)
cv. Barton.1	PI 421274	WRPIS-81i	Barton Co., KS	cultivar, single-origin	7	418.3 (404-445)	221.6 (24.0)
cv. Barton.2	PI 578778	WRPIS-95o	Barton Co., KS	cultivar, single-origin	7	414.7 (404-428)	238.1 (11.1)
CO-Huerrfano	D-3293	FRRL-86i	Huerrfano Co, CO	single-origin	6	445.3 (428-461)	247.7 (39.9)
CO-Larimar	D-3295	FRRL-86i	Larimar Co., CO	single-origin	6	412.8 (392-434)	226.6 (57.2)
CO-RioBlanco	KJ-48	FRRL-93i	Rio Blanco Co., CO	single-origin	7	396.0 (380-414)	143.8 (20.2)
cv. Flintlock	Flintlock	FRRL-90i	Nebraska and Kansas	cultivar, multiple-origin	6	438.5 (419-449)	221.1 (16.8)
MT-Broadwater	PI 232120	WRPIS-86i	Broadwater Co., MT	single-origin	7	426.4 (414-439)	194.1 (20.2)
MT-DeerLodge	PI 232122	WRPIS-81i	Deer Lodge Co., MT	single-origin	7	404.6 (375-422)	186.7 (22.9)
MT-Teton	PI 232121	WRPIS-83i	Teton Co., MT	single-origin	6	405.8 (384-418)	192.2 (40.0)
MT-Yellowstone	PI 432401	WRPIS-81i	Yellowstone Co., MT	single-origin	5	420.8 (405-430)	203.4 (12.5)
ND-Bowman	PI 598377	WRPIS-95i	Bowman Co., ND	half-sib, single-origin	6	415.5 (396-441)	205.8 (12.4)
ND-Burleigh	PI 598380	WRPIS-95i	Burleigh Co., ND	half-sib, single-origin	7	422.1 (406-434)	210.4 (19.9)
ND-DivideA	PI 577674	WRPIS-95i	Divide Co., ND	half-sib, single-origin	5	413.8 (402-421)	236.1 (26.9)
ND-DivideB	PI 598375	WRPIS-95i	Divide Co., ND	half-sib, single-origin	6	415.8 (381-433)	230.1 (14.1)
ND-GoldenValley	PI 610967	WRPIS-97i	Golden Co., ND	half-sib, single-origin	7	424.3 (414-441)	216.9 (16.3)
ND-Grant	PI 598376	WRPIS-95i	Grant Co., ND	half-sib, single-origin	7	423.6 (385-444)	207.4 (12.5)
ND-McIntosh	PI 577673	WRPIS-95i	McIntosh Co., ND	half-sib, single-origin	7	418.4 (405-437)	212.6 (19.6)
ND-McKenzie	PI 598373	WRPIS-95i	McKenzie Co., ND	half-sib, single-origin	7	420.3 (399-445)	220.6 (20.5)
ND-McLeanA	W6 3282	WRPIS-95i	McLean Co., ND	half-sib, single-origin	6	409.3 (389-428)	212.5 (13.9)
ND-McLeanB	W6 3283	WRPIS-95i	McLean Co., ND	half-sib, single-origin	6	423.3 (413-435)	212.7 (17.2)
ND-Morton	PI 598374	WRPIS-95i	Morton Co., ND	half-sib, single-origin	7	416.0 (398-427)	213.4 (16.7)
ND-Oliver	PI 598378	WRPIS-95i	Oliver Co., ND	half-sib, single-origin	7	421.0 (397-438)	212.2 (18.5)
ND-Slope	PI 598379	WRPIS-95i	Slope Co., ND	half-sib, single-origin	6	418.8 (412-428)	236.3 (24.2)
ND-Ward	PI 610971	WRPIS-97i	Ward Co., ND	half-sib, single-origin	6	431.0 (424-444)	204.4 (12.5)
WWG931	PI 576178	WRPIS-93o	North and South Dakota	multiple-origin	7	415.5 (405-439)	253.7 (10.8)
WWG932	PI 576179	WRPIS-93o	AB, SK	multiple-origin	6	414.6 (379-436)	231.0 (18.2)
NM-RioArriba	EPC-360	FRRL-87i	Rio Arriba Co., NM	single-origin	7	411.9 (387-439)	245.9 (43.0)
NM-Taos	EPC-181	FRRL-87i	Taos Co., NM	single-origin	3	391.3 (388-394)	230.0 (24.3)
cv. Rodan.1	PI 477993	WRPIS-85i	seed field (Mandan, ND)	cultivar, single-origin	7	429.1 (415-445)	229.4 (23.4)
cv. Rodan.2	PI 578780	WRPIS-95o	seed field (Mandan, ND)	cultivar, single-origin	6	413.0 (386-431)	196.3 (13.0)
cv. Rosana	PI 469236	WRPIS-85i	Rosebud Co., MT	cultivar, single-origin	5	425.6 (415-438)	236.4 (21.3)
SD-CorsonA	PI 598385	WRPIS-95i	Corson Co., SD	half-sib, single-origin	7	416.0(407-426)	215.3 (19.0)
SD-CorsonB	PI 598386	WRPIS-95i	Corson Co., SD	half-sib, single-origin	7	408.6 (388-420)	226.2 (23.2)
SD-CorsonC	PI 610970	WRPIS-97i	Corson Co., SD	half-sib, single-origin	7	396.6 (380-417)	210.1 (19.8)
SD-Dewey	PI 598384	WRPIS-95i	Dewey Co., SD	half-sib, single-origin	6	414.2 (397-431)	217.5 (15.8)
SD-Haakon	PI 598387	WRPIS-95i	Haakon Co., SD	half-sib, single-origin	6	425.2 (418-434)	217.8 (15.9)
SD-Meade	PI 598383	WRPIS-95i	Meade Co., SD	half-sib, single-origin	7	435.1 (424-443)	223.5 (9.6)
SD-PerkinsA	PI 598372	WRPIS-95i	Perkins Co., SD	half-sib, single-origin	5	431.4 (419-441)	230.2 (21.5)
SD-PerkinsB	PI 598381	WRPIS-95i	Perkins Co., SD	half-sib, single-origin	6	420.2 (416-430)	210.2 (18.7)
SD-PerkinsC	PI 598382	WRPIS-95i	Perkins Co., SD	half-sib, single-origin	4	421.8 (409-439)	235.5 (13.4)
SD-Potter	PI 610969	WRPIS-97i	Potter Co., SD	half-sib, single-origin	5	421.6 (397-447)	216.0 (16.9)
SD-Stanley	PI 610968	WRPIS-97i	Stanley Co., SD	half-sib, single-origin	5	431.6 (419-450)	221.4 (15.9)
SD-Ziebach	PI 577675	WRPIS-95i	Ziebach Co., SD	half-sib, single-origin	3	414.3 (410-420)	204.0 (25.5)
UT-UintahA	EPC-8	FRRL-87i	Uintah Co., UT	single-origin	6	434.8 (400-455)	185.9 (21.5)
UT-UintahB	KJ-47	FRRL-90i	Uintah Co., UT	single-origin	7	412.0 (389-432)	231.3 (30.9)
P727.1	P-727	FRRL-93i	Grant Co., WA	single-origin	7	417.4 (389-442)	96.6 (21.9)
P727.2	PI 578779	WRPIS-95o	Grant Co., WA	single-origin	7	425.7 (396-458)	223.3 (13.3)
cv. Walsh	Walsh	FRRL-84i	AB, SK	cultivar, multiple-origin	7	413.6 (398-431)	235.7 (12.9)
WY-Sweetwater	D-3300	FRRL-86i	Rio Arriba Co., NM	single-origin	5	420.4 (409-431)	233.8 (46.1)

LSD = 17.3

† Decimal numbers used to distinguish different accessions of the same germplasm source, otherwise accessions considered distinct.

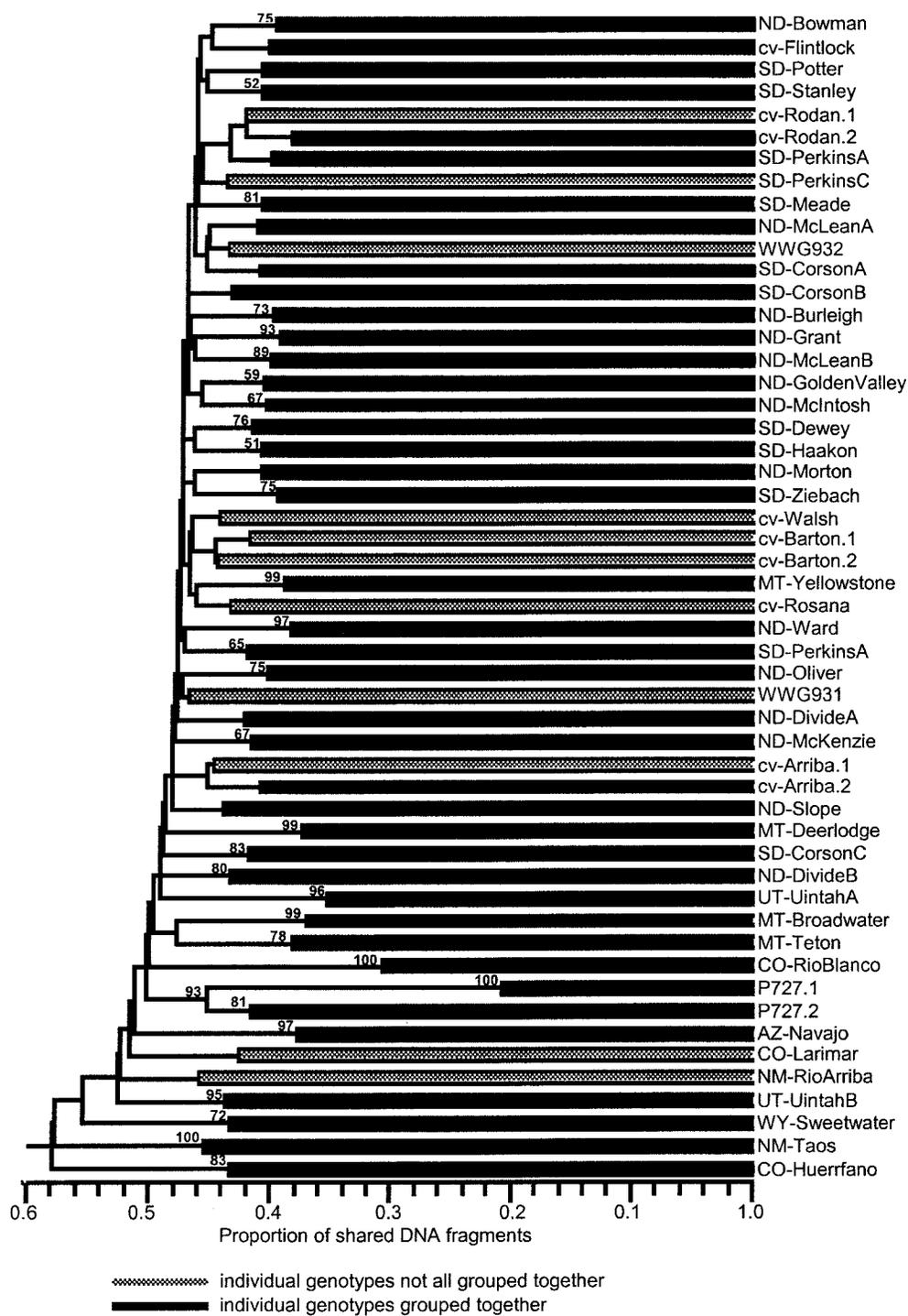
‡ WRPIS = (National Plant Germplasm System) Western Region Plant Introduction Station, Pullman, WA; FRRL = Forage and Range Research Laboratory, Logan, UT; numbers indicate year of original (o) seed acquisition or year of last seed increase (i).

DNA fingerprinting was conducted by the AFLP technique according to the methods of Vos et al. (1995), except that *EcoRI* selective amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein) label on the 5' nucleotide. Selective amplifications were performed with five primer pairs (e.g., E.ACC//M.CAG, E.ACC//M.CTA, E.ACC//M.CTG, E.ACG//M.CTG, and E.AGG//M.CTG), where E and M designate the *EcoRI* and *MseI* adapters with three selective nucleotides as described by Vos et al. (1995). Selective amplifications for three primer combinations were fractionated and detected with an ABI373XL instrument (PE Applied Biosystems, Foster City, CA) using 34-cm well-to-read polyacrylamide gels formulated with 5.75% (w/v) Long Ranger Syngel packs (FMC, Rockland, ME), 7 M urea, and 1× TBE running buffer. Each sample lane included the GS500-ROX (PE Applied Biosystems) internal lane size standards (labeled with

rhodamine X). DNA fragments between 50 and 500 base pairs (bp) were identified by means of GeneScan 3.1 software (PE Applied Biosystems). Selective amplifications for two other primer combinations were fractionated and detected with an ABI3100 instrument using 50-cm capillaries, POP6 polymer, and GS400-ROX size standards (PE Applied Biosystems). DNA fragments between 50 and 400 bp were identified by means of GeneScan software. All GeneScan sample files were subsequently analyzed for the presence and absence of DNA fragments by means of Genographer version 1.5 (Benham et al., 1999).

### Data Analysis

Pairwise comparisons of the proportion of shared DNA fragments between individual genotypes (plants) were deter-



**Fig. 1.** UPGMA analysis based on pairwise comparisons of genetic similarities among 320 individual plants (AFLP genotypes) classified and summarized by accession. The length of shaded bars reflects the average proportion of shared DNA fragments per accession. Bootstrap confidence levels are shown for groups in the 50% consensus tree.

mined by the Jaccard (1908) coefficient, where  $J = \text{matching fragments} / (\text{matching} + \text{polymorphic fragments})$ . The resulting genetic similarity coefficients were subsequently averaged within and among accessions and analyzed by the UPGMA and Tree plot procedures of NTSYS-pc, version 2.02 (Exeter Software, Setauket, NY). A dendrogram (Fig. 1) was modified to include bootstrap confidence levels recovered from the 50% majority-rule consensus of 1000 UPGMA searches for the 320 individ-

ual genotypes (Table 1) by PAUP\* version 4.0b8 (Sinauer Associates, Inc. Publishers, Sunderland, MA).

The apportionment of genotypic variance among accessions was examined by analysis of molecular variance (AMOVA) (Excoffier et al., 1992). As described by Huff (1997), a Euclidean distance matrix (total number of polymorphic bands for pairwise comparisons of individual plants) was used as data input for AMOVA. The AMOVA procedure was used to

calculate values of interpopulation distance ( $\Phi_{st}$ ), which is equivalent to the proportion of total genotypic variance partitioned between populations (Excoffier et al., 1992). A neighbor-joining tree, based on pairwise comparisons of  $\Phi_{st}$ , was developed by means of PAUP\* and TREEVIEW (Page, 1996). The AMOVA procedure was also used to examine the partitioning of genotypic variance among geographic groups of populations (Excoffier et al., 1992).

Matrix correlation between  $\Phi_{st}$  and geographical distances among source locations of single-origin accessions was evaluated by the Mantel (1967) test statistic (Z), by mean of the MXCOMP procedure of NTSYS-pc (Rohlf, 1998). Significance levels for correlations between genetic and geographical data were determined by comparing observed values to values obtained by 1000 random permutations (Smouse et al., 1986). Therefore, the upper tail probability ( $p$ ) that 1000 random Mantel test statistic (Z) values are (by chance) less than observed values of Z equals 0.002. Geographical distance (km) matrices were computed from geographical coordinates, by the formula described in Math Forum (1997): distance =  $\text{Arccos}[\cos(\text{LAT1})\cos(\text{LONG1})\cos(\text{LAT2})\cos(\text{LONG2}) + \cos(\text{LAT1})\sin(\text{LONG1})\cos(\text{LAT2})\sin(\text{LONG2}) + \sin(\text{LAT1})\sin(\text{LAT2})] * r$ , where LAT1, LONG1 and LAT2, LONG2 are the latitude, longitude (expressed in radians) for the two accessions (1 and 2) being compared and  $r$  is 6378 (km), the radius of Earth. A spatial map of collecting sites for the 39 single-origin accessions was developed by principle coordinates analysis by transforming the symmetric geographic distance matrix to a scalar product (Gower, 1966) by means of the DCENTER procedure and computing eigenvectors using the EIGEN procedure (Rohlf, 1998). The goodness of fit for the resulting spatial map was evaluated by constructing a distance matrix from the eigenvector matrix, by means of the SIMINT procedure, and comparing this distance matrix with the original geographic distance matrix by the MXCOMP procedure (Rohlf 1972; Rohlf 1998).

## RESULTS AND DISCUSSION

Complete and relatively similar AFLP profiles were obtained, for all five primer pairs, from 320 of the 364 samples tested. The number of DNA fragments per plant was reasonably consistent among these 320 genotypes, ranging from 372 to 461 fragments per plant with an overall average of approximately 419 fragments per plant (Table 1). Selective amplifications that occasionally displayed weak or incomplete AFLP profiles were repeated. Occasional PCR failures during selective amplification can be attributed to problems such as evaporation from damaged PCR tubes or pipetting. However, 44 samples reproduced faint and/or incomplete profiles for all five primer combinations. Deficiencies of these 44 samples may be attributed to one or more procedures of the AFLP method including DNA extraction, restrictions, ligations, and preamplifications. These suspect samples were excluded from any further analyses.

Individual genotypes (plants) of most single-origin accessions clustered together relative to other heterogeneous accessions (Fig. 1). Conversely, individual plants of multiple-origin accessions do not group together and tend to display less genotypic similarity, averaged within accessions (Fig. 1). Therefore, individual plants of genetically narrow or geographically well-defined source populations generally can be classified by accession, whereas

individual plants of relatively geographically broad-based source populations do not classify by accession. The multiple-origin accession WWG931 displayed the highest rate of DNA polymorphism observed in this study (Table 1). The average number of DNA polymorphisms (SE) within and among the 26 half-sib single-origin accessions from North and South Dakota was 217.1 (19.5) and 261.9 (14.9), respectively. Although DNA polymorphism among these 26 single-origin accessions, selected from the base population of WWG931, was greater than DNA polymorphism within WWG931 (Table 1), this difference may be balanced by increased heterozygosity within individual plants of accession WWG931. The average number of DNA polymorphisms (SE) within other single origin accessions (Table 1) was 206.8 (45.4). Therefore, rates of DNA polymorphism in the multiple-origin accessions WWG931, WWG932, cv. Walsh, and cv. Flintlock (Table 1) tended to be greater than single-origin accessions.

Duplicated accessions for cultivars Arriba, Barton, and Rodan generally group together, by cultivar, relative to many other heterogeneous populations examined in this study (Fig. 1). However, individual genotypes of four duplicated accessions (cv. Rodan.1, cv. Barton.1, cv. Barton.2, and cv. Arriba.1) do not classify precisely as expected (Fig. 1), and display relatively high rates of DNA polymorphism (Table 1). Nevertheless, AMOVA detected only 3.6, 5.5, and 6.7% difference between these duplicated accessions of Barton, Rodan, and Arriba, respectively. The average difference among 39 single-origin accessions was 21.0% (Table 2). Therefore, differences between duplicated sources of cultivars Arriba, Barton, and Rodan were considerably less than the average difference among single-origin accessions. Although AMOVA detected 35.1% difference between duplicated accessions of the NRCS selection P727, individual genotypes of P727.1 and P727.2 cluster together relative to other accessions examined in this study (Fig. 1). Moreover, DNA polymorphism in P727.1 was considerably less than all other accessions, including half-sib families (Table 1; Fig. 1). The accession P727.2 is original seed from the NRCS (Table 1), thus we speculate that accession P727.1 incurred a genetic bottleneck

**Table 2. Analysis of Molecular Variance (AMOVA) for 39 natural single-origin USDA western wheatgrass accessions, classified by geographic region.**

Source of Variation	df	Sums of squares	Variance components	% Total	P-value
<b>Northern Great Plains†</b>					
Among accessions	25	6 130	22.5	17.2	<0.001
Within accessions	132	14 309	108.4	82.8	<0.001
<b>Northern Rocky Mountains</b>					
Among accessions	3	979	37.0	27.7	<0.001
Within accessions	21	2 030	96.6	72.3	<0.001
<b>Central Rocky Mountains</b>					
Among accessions	8	3 202	52.0	32.6	<0.001
Within accessions	42	4 520	107.6	67.4	<0.001
<b>Overall</b>					
Among regions	2	1 240	5.8	4.0	<0.001
Among accessions (regions)	36	10 310	30.0	21.0	<0.001
Within accessions	211	20 859	107.0	75.0	<0.001

†Half-sib families from open-pollinated source-identified clones.

that reduced DNA variation within this accession and increased genetic divergence between P727.1 and P727.2. The amount of seed regenerated from P727.1 in 1982, by FRRL, was unusually low and may have been produced by selfpollination of one plant. Self fertile plants are occasionally observed in western wheatgrass (Jensen et al., 1990).

Excluding multiple-origin populations, cultivars, and P727, the 39 remaining source-identified single-origin accessions form three natural groups on the basis of  $\phi_{st}$  (Fig. 2), which correspond to three geographic regions (Fig. 3). For reference, a geographic map of these source locations was reconstructed from the geographic distance matrix by principle coordinates analysis, with the approximate state boundaries superimposed (Fig. 3). As expected, a very good fit between the resulting spatial map and the original geographic distance matrix was confirmed by the cophenetic correlation test ( $r = 1.00000$ ). Interestingly, interpopulation genetic distances ( $\phi_{st}$ ) among 39 single-origin accessions (Fig. 2) showed a significant ( $P \leq 0.001$ ) positive correlation ( $r = 0.66$ ) with geographical distances among corre-

sponding source populations (Fig. 3). However, this correlation may be affected by the different methods of regeneration for these source-identified single-origin accessions. Compared with variation among Rocky Mountain accessions, the apportionment of DNA variation was noticeably less among the 26 half-sib northern Great Plains accessions (Table 2). These half-sib families were derived from 26 source-identified clones open-pollinated by 2000 diverse genotypes from North and South Dakota, whereas the Rocky Mountain accessions were presumably regenerated via isolated bulk seed increases of natural seed collections. Therefore, correlation between  $\phi_{st}$  and geographic provenance, within this relatively large group of northern Great Plains accessions, was probably obscured by out-crossing of source clones of the 26 accessions from North and South Dakota. Conversely, genetic identities among these 26 northern Great Plains accessions were probably enhanced by open-pollination of the source clones, which may inflate the apparent correlation between  $\phi_{st}$  and geographic provenance. However, only 4% difference was detected among natural groups of single-origin western wheat-

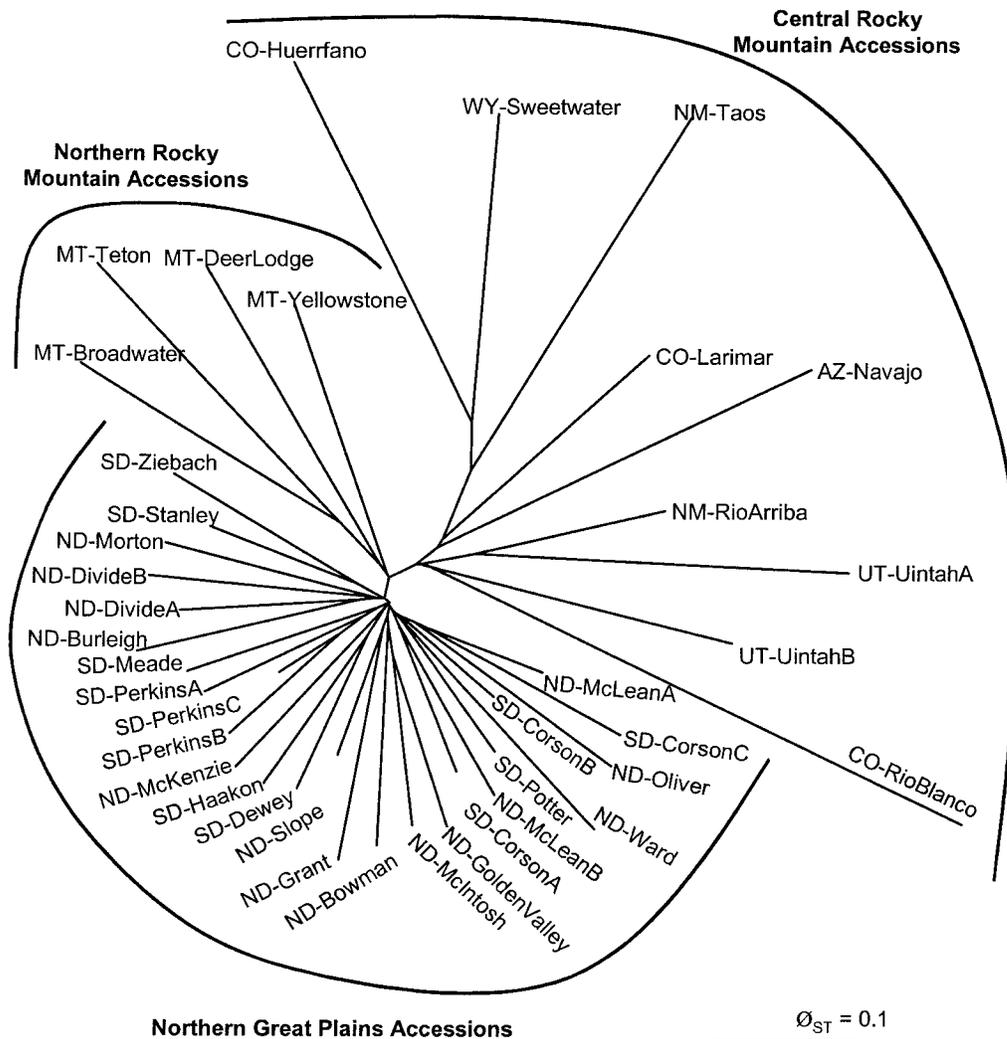


Fig. 2. Unrooted neighbor-joining tree based on interpopulation genetic distances ( $\Phi_{ST}$ ) among 39 single-origin western wheatgrass accessions, identified by geographic provenance.

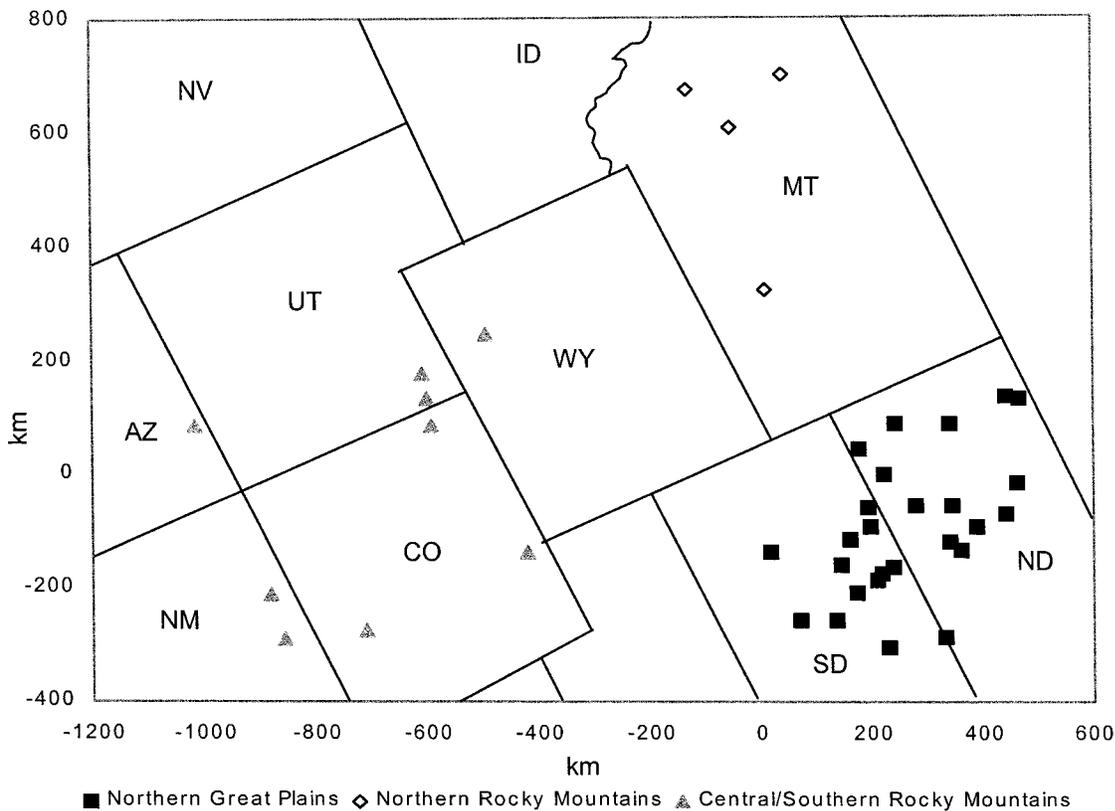


Fig. 3. Spatial map of collecting sites for 39 single-origin western wheatgrass accessions, classified by geographic regions of the USA.

grass accessions from the northern Great Plains, northern Rocky Mountain, and central Rocky Mountain regions (Table 2).

## CONCLUSIONS

The USDA has made substantial efforts and commitments to acquire, preserve, evaluate, document, and distribute plant germplasm, including out-crossing native perennial grass species such as western wheatgrass. The clustering of individual plant genotypes by accession and relatively low divergence among duplicated accessions provide evidence that genetic identity has been effectively maintained in the western wheatgrass collection. The correlation of  $\phi_{st}$  and geographical provenance provides additional evidence that genetic integrity of the original germplasm sources has been maintained *ex situ* and supports the premise that geographic origin contributes to germplasm identity. Many of the western wheatgrass accessions originate from the northern Great Plains region, where this species is naturally abundant. As might be expected, the apportionment of DNA variation among single-origin accessions regenerated by isolated bulk seed increases from well-defined localities was greater than the apportionment of DNA variation among half-sib families that once shared a common pollen source. Nevertheless, significant DNA variation was detected among these selected half-sib germplasm sources from North and South Dakota. As expected, higher levels of DNA polymorphism were detected in the multiple-origin germplasm sources than

detected in single-origin accessions. These observations also support the premise that geographic origin may contribute to germplasm identity and diversity. All of these single-origin and multiple-origin germplasm sources were intended to provide heritable sources of agronomic and morphological variation (Barker et al., 1983, 1995; Johnston et al., 1975; Ray and Harms, 1994; Smoliak and Johnston, 1984). Combined with other cited passport data, results of this study provide evidence that genetically unique, diverse, and potentially useful western wheatgrass accessions have been procured and maintained by the USDA.

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