



**Improved establishment characteristics of 'Vavilov II' Siberian
whetgrass**

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Complete List of Authors:	JENSEN, KEVIN; USDA-ARS-FRRL Palazzo, Antonio Waldron, Blair; USDA-ARS, Forage and Range Res. Lab. Robins, Joseph; USDA-ARS Forage and Range Bushman, Bradley; USDA-ARS, Forage and Range Research Lab Johnson, Douglas; USDA-ARS, Forage and Range Res. Lab. Ogle, Dan
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Improved Establishment Characteristics of ‘Vavilov II’ Siberian Wheatgrass

Kevin B. Jensen^{a*}, Anthony J. Palazzo^b, Blair L. Waldron^a, Joseph G. Robins^a,
B.Shaun Bushman^a, Douglas A. Johnson^a, and Dan G. Ogle^c.

^aUSDA-ARS, Forage and Range Research Laboratory, 695 North 1100 East., Logan, UT 84322-6300; ^bUS Army Corps of Engineers, Engineering Research and Development Center, Hanover, NH 03755; ^c USDA-Natural Resources Conservation Service, 9173 W. Barnes Dr., Suite C, Boise, ID 83709. Utah Agric. Exp. Stn. Journal Paper No. _____. Received _____.

*Corresponding author kevin.jensen@ars.usda.gov.

ABSTRACT

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‘Vavilov II’ Siberian wheatgrass (*Agropyron fragile* (Roth) Candargy) (Reg. No. CV-___, PI_____). Vavilov II was developed for reseeding disturbed rangelands dominated by annual weeds as a result of severe disturbance, frequent fires, and soil erosion. Selection emphasis in Vavilov II was on seedling establishment and plant persistence. During the establishment year, Vavilov II had significantly ($P<0.05$) higher numbers of seedlings per unit area (m^2) using a frequency grid when planted at a rate of one pure live seed (PLS) cm^{-1} than Vavilov at Yakima, WA (est. fall 2002; 52 vs 23%); Fillmore, UT (est. fall 2004; 79 vs 54%); Dugway, UT (est. fall 2005; 79 vs 52%); and Curlew Valley, ID (est. fall 2002; 70 vs 40%). In persistence after establishment, Vavilov II was significantly more persistent than Vavilov at Yakima, WA (68 vs 44%); Fillmore, UT (84 vs 62%); Curlew Valley, ID (69 vs 55%), and Malta, ID (97 vs 91%). Seed of Vavilov II is available through the Utah Crop Improvement Association and the University of Idaho Foundation Seed Program.

Abbreviations: DMY, dry matter yield. AFLP, amplified fragment length polymorphisms. UPGMA, unweighted pair group method with arithmetic mean. USDA-ARS, U.S. Department of Agriculture-Agricultural Research Service. USDA-NRCS, U.S. Department of Agriculture – Natural Resource Conservation service. NPGS, National Plant Germplasm System. PAUP, phylogenetic analysis using parsimony.

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3 Vast areas of semiarid rangeland in the western U.S. are severely disturbed, frequently burned,
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5 increasingly eroded, and subsequently infested with troublesome weeds such as cheatgrass
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7 (*Bromus tectorum* L.), medusahead (*Taeniatherum caput-medusae* (L.) Nevski) and others.
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9 Reseeding disturbed rangelands with genetically improved plant materials that are competitive
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11 enough (seedling establishment and persistence) to replace existing undesirable vegetation is
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13 often the most plausible and economically feasible way to reclaim such sites (Asay et al., 2003).
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15 A failure to develop improved plant materials that can restore these degraded rangelands to
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17 perennial vegetation will result in increased fire frequency, loss of soil structure (Norton et al.,
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19 2004), increased soil erosion, and economically unproductive rangelands. One species frequently
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21 used in rangeland revegetation is Siberian wheatgrass (*Agropyron fragile* (Roth) Candargy).
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27 In its native habitat, Siberian wheatgrass is more drought-resistant and better adapted to
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29 medium to coarse textured soils than either Standard (*A. desertorum* (Fisch. ex Link) Schultes) or
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31 Fairway (*A. cristatum* L.) type crested wheatgrass. Siberian wheatgrass is recommended for
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33 semiarid ecological sites receiving between 150 to 300 mm of annual precipitation at elevations
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35 up to 2,150 m. When drilled under dryland range conditions, a seeding rate of 8 kg h⁻¹ is
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37 recommended (Jensen et al., 2001). In recent studies on six ecological sites, Siberian wheatgrass
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39 was one of the easiest to establish, more productive, more persistent, and more defoliation-
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41 tolerant under severe water stress than other rangeland grasses (Asay et al., 2001).
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46 The first released Siberian wheatgrass cultivar 'P-27' was released in 1953 by the NRCS
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48 and was selected from a large evaluation nursery for persistence (Alderson and Sharp, 1994). At
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50 present, the most widely used Siberian wheatgrass cultivar is Vavilov, which was released in
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52 1994 and exhibits increased retention of plant color, vegetative vigor under extreme drought,
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54 seedling establishment, and seed yield compared to P-27 (Asay et al., 1995). To combat the
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3 increasing spread of invasive annual weeds on western rangelands, it is critical to develop
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5 improved plant materials with increased seedling establishment and persistence (Asay et al.,
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8 2003). The principle objective of this research was to develop a Siberian wheatgrass cultivar
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10 with increased establishment and persistence characteristics under harsh, dry environments of the
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12 western U.S.A.
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MATERIALS AND METHODS

Breeding History

The parent material for Vavilov II Siberian wheatgrass was selected from evaluation trials at three locations: 1) Yakima, WA, 2) Curlew Valley, ID, and 3) Lakeside, UT. At Yakima, WA, an 832-plant nursery of the cultivar Vavilov was established in 1998. Based on visual plant vigor, total seed yield, and seedling establishment (ability to emerge from a 7.6-cm planting depth in a greenhouse) in 1999, 15 genotypes were selected. At Curlew Valley, ID (USDA-FS-Curlew National Grasslands), an evaluation nursery was established in 1998 that included Vavilov and collections made from the steppes of Kazakhstan in 1988 by Drs. Kay Asay and Douglas Johnson. Based on visual plant vigor, five genotypes of Vavilov and one from the steppes of Kazakhstan (PI 598668) were selected. At Lakeside, UT, the cultivar Vavilov and two bulk populations (low and medium annual precipitation) from Kazakhstan were evaluated. The low annual precipitation population originated by equal bulking by weight of original seed from PIs 598682, 598683, 598684, 598685, 598686, 598687, 632475, and collection JA 68 that originated from sites that receive annual precipitation ranging between 100 to 130 mm (Jensen et al., 2008). The medium annual precipitation population originated by equal bulking by weight of original seed from PIs 598664, 598665, 598666, 598667, 598689, 598690, 598691, 598692, 598693, 598694, 598696, 598697, 598698, 598714, 598715, and collection JA 74 that originated from sites that receive annual precipitation of 150 mm (Jensen et al., 2008). Twenty-one genotypes of Vavilov and eight genotypes from the Kazakhstan collection (four each from the different annual precipitation populations) were selected from the Lakeside site.

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3 Clones (20 replications) from the 50 selected genotypes from Yakima, WA; Curlew
4 Valley, ID; and Lakeside, UT were established in 2000 at the Blue Creek, UT research station
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6 and designated as the Vavilov II breeder seed.
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9 10 **Morphological Characterization**

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12 Twelve morphological characters were evaluated (Table 1) from at least 40 different
13 plants each of Vavilov II, Vavilov, and P-27 at Nephi (39°38'43" N, 111°52'11" W; elevation
14 1,600 m) and Blue Creek (41°56'02" N, 112°26'20" W; elevation 1,563 m), UT. All data were
15 subjected to analysis of variance using GLM procedures as a fixed model. Mean separations
16 were made on the basis of least significant differences (LSD) at the 0.05 probability level (SAS
17 Institute Inc., 1999).
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26 27 **Molecular Characterization**

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29 Twelve plants from each of four Siberian wheatgrass cultivars/breeding populations
30 (Vavilov II, Vavilov, P-27, and a population originating from the JA collections called Kazak)
31 were screened for DNA polymorphisms with six AFLP primer pairs: E.AGC_M.CAG,
32 E.AGC_M.CAT, E.AGC_M.CTG, E.AGG_M.CAA, E.AGG_M.CAC, and E.AGG_M.CAG.
33 AFLP reactions were conducted according to Vos et al. (1995), except that fluorescently labeled
34 primers were used and detected on an ABI3730 (Applied Biosystems, Foster City, CA). Size
35 standards were spiked into each reaction to assure band-length validity. Estimates of similarity
36 were obtained using the method described by Leonard et al. (1999), and analysis of molecular
37 variance and diversity were estimated as *per* Excoffier et al. (1992). Dendograms were
38 constructed using UPGMA cluster methods in PAUP (Swofford, 2001).
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Seeded Trials

Between 2002 and 2005, fall-seeded evaluation trials were established to compare seedling establishment, persistence, and forage yield of Vavilov II and Vavilov at Yakima, WA (46°50'47" N, 120°22'18" W; elevation 704 m); Guernsey, WY (42°15'46" N, 104°48'01" W; elevation 1,429 m); Fillmore, UT (39°10'50" N, 112°14'27" W; elevation 1,769 m); Stone, ID (42°01'15" N, 112°39'09" W; elevation 1,432 m); and Malta, ID (42°14'21" N, 113°08'09" W; elevation 1,650 m). Plots were arranged in a randomized complete block with four replications. Entries were seeded at a rate of one pure live seed cm⁻¹ at a seeding depth of 0.63 cm in five rows. Plot size was 1.5 m wide by 8 m long.

Seedling establishment and persistence were measured as plant density frequency using the grid system described by Vogel and Masters (2001). Frequency was determined by laying a grid of 5.1 x 5.1-cm quadrats over the drilled rows and determining the percentage of quadrats containing at least one seedling (100% stand if a seedling/plant occurred in every quadrant [48]). This was repeated three times along the 8-m row for a total of 144 quadrats.

Seedling Establishment

Selection for improved seedling establishment followed the methods reviewed by Johnson and Asay (1993), where they showed a strong correlation between a plant's ability to emerge from a deep planting depth in a greenhouse and its ability to germinate and establish rapidly when planted at 0.63 cm deep under dryland conditions. One hundred seeds (PLS) of each entry were placed at the bottom of 7.6-cm trenches and then covered with soil. Benches were watered daily, and rate of emergence was determined according to Maguire (1962).

RESULTS and DISCUSSION

Vavilov II Siberian wheatgrass (*Agropyron fragile* (Roth) Candargy) Reg. No. CV-___, PI_____) was developed for use on arid and semiarid rangelands as a rapid establishing revegetation grass in the Intermountain West, Great Basin, and Northern Great Plains Regions of western U.S.A. (Ogle et al., 2007; St. John, 2008). Vavilov II was evaluated in field trials as Vavilov-Select, SERDP Siberina wheatgrass, and 9076515 (NRCS). Vavilov II is a broad-based 50-clone synthetic that was developed and tested as part of the Strategic Environmental Research and Development Program (SERDP) project CS-1103 to identify resilient plant characteristics and develop wear-resistant plant cultivar(s) for use on military training lands. Vavilov II was evaluated in field trials as Vavilov-Select, SERDP Siberian wheatgrass, and 9076515 (NRCS). Vavilov II was selected for persistence and overall plant and seedling establishment in response to drought. Vavilov II expands the genetic base of the cultivar Vavilov, has been evaluated extensively on ecological sites in the western U.S.A., and has superior seedling establishment compared to the commercially available cultivar Vavilov.

Morphological Characterization

When combined across locations (Nephi and Blue Creek, UT) there are significant differences for flag leaf length and spike length between Vavilov II and Vavilov (Table 1). Vavilov II has significantly shorter leaves (10.2 cm) than Vavilov (11.7 cm), but significantly longer leaves than P-27 (8.5 cm). Spikes of Vavilov II were significantly ($P<0.05$) shorter (8.2 cm) than Vavilov (9.1 cm), but similar to P-27 (7.8). Vavilov II is significantly ($P<0.05$) taller (64.2 cm) than P-27 (58.2 cm), but similar in plant height to Vavilov (63.4 cm). Flag leaves in Vavilov II were oriented significantly ($P<0.05$) higher on the culm (40.3 cm) and were wider (3.1 mm) than P-27. Vavilov II had significantly ($P<0.05$) longer lemmas and glumes than P-

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3 27, but was similar to Vavilov (Table 1). Lemma and glume awn lengths in Vavilov II were
4 significantly ($P<0.05$) longer than P-27. The ratio of spike length/width was not highly
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6 diagnostic in separating Vavilov II, Vavilov, and P-27 (Table 1). Non-diagnostic morphological
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8 traits included spike width, lemma width, and glume width. Heading and flowering dates at Blue
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10 Creek and Nephi, UT were the third week in May and first week in June, respectively. Vavilov
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12 II is an autotetraploid ($2n=4x=28$; PPPP) and has the same ploidy level and genomic composition
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14 as P-27 and Vavilov.
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20 **Molecular Characterization**

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22 Amplified fragment length polymorphisms (AFLP) were used to compare Vavilov II to
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24 Vavilov, P-27, and a breeding population Kazak Siberian wheatgrass. The six AFLP primer pairs
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26 amplified 728 bands that were present in more than 5% and less than 95% of the individuals. The
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28 average number of AFLP bands ranged from 239 for P27 to 255 for Kazak Siberian wheatgrass
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30 and Vavilov II. The average number of pairwise differences within the different
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32 cultivars/populations ranged from 203 to 218 bands, while the average number of pairwise
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34 differences between cultivars/populations was 226 to 247 bands. Across all four
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36 cultivars/populations, 88% of variation was within, while 12% was between. The most similar
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38 cultivars were Vavilov and Vavilov II. The results are consistent with the pedigree of the four
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40 cultivars/populations. A dendrogram was generated using cluster analysis (UPGMA) based on
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42 the average pairwise differences between the cultivars/populations (Fig. 1). The significant
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44 variation among cultivars/populations allows for molecular markers that distinguish the varieties
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Seedling Establishment

Rapid seedling establishment is one of the primary keys to successful revegetation in the western U.S.A. Selection emphasis in Vavilov II was on seedling establishment and plant persistence. During the establishment year, Vavilov II had significantly ($P<0.05$) higher numbers of seedlings per unit area (m^2) using a frequency grid when planted at a rate of one pure live seed (PLS) cm^{-1} at Yakima, WA (est. fall 2002; 52 vs 23%); Fillmore, UT (est. fall 2004; 79 vs 54%); Dugway, UT (est. fall 2005; 79 vs 52%); and Curlew Valley, ID (est. fall 2002; 70 vs 40%) (Fig. 2). Vavilov II was more persistence after establishment (as measured by percent stand) Vavilov at Yakima, WA (68 vs 44%); Fillmore, UT (84 vs 62%); Curlew Valley, ID (69 vs 55%); and Malta, ID (97 vs 91%) (Fig. 3). Dry matter yields (64-cm x 38-cm plot) combined across Yakima, WA and Guernsey, WY, were significantly ($P<0.05$) greater in Vavilov II (53 g $plot^{-1}$) than Vavilov (39 g $plot^{-1}$). Vavilov II germinated in seven days compared to 10 days on sandy loam, loam, and sandy soil types.

NRCS planted Vavilov II along with 58 accessions of grasses, forbs, and shrubs at the Coffee Point test site about 40 km northwest of Aberdeen, ID (13 cm precipitation) in November 2006. Plant density data were collected using a frequency grid on 1 May 2007. Vavilov II had the highest plant density of all accessions evaluated at 15.9 plants m^{-2} compared to 8.0 plants m^{-2} for Vavilov. On 7 September 2007 the second evaluation was completed, and Vavilov II had 15.7 plants m^{-2} and Vavilov had 7.4 plants m^{-2} . The development of Vavilov II gives land managers new plant materials with enhanced seedling establishment and persistence on dry harsh rangelands.

Seed Availability

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A Foundation Seed production field was established at the Aberdeen Plant Materials Center in August 2005. In 2006, seed was harvested from the field and yielded 748 kg (740 kg ha⁻¹). Breeder, Foundation, Registered, and Certified seed classes will be recognized. Breeder and Foundation seed will be maintained by the USDA-ARS Forage and Range Research Laboratory at Logan, UT and the USDA-NRCS Plant Materials Center at Aberdeen, ID. Protection under the U.S. Plant Variety Protection Act of 1994 will be applied for, with the requirement that seed of Vavilov II can be marketed only as a class of certified seed. No seed will be distributed without written permission for 20 years from the date of release 11 March 2008 by the USDA-Agricultural Research Service, at which time seed will also be available from the National Plant Germplasm (NPGS). Foundation seed is available through the following contacts: 1) Utah Crop Improvement Association (435-797-2082; sayoung@mendel.usu.edu) and 2) University of Idaho Foundation Seed Program (208-423-6655; Williams@kimberly.uidaho.edu).

Footnotes: Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA or Utah State University.

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Figure Headings

Figure 1. UPGMA cluster analysis of the average pairwise differences among four Siberian wheatgrass accessions. Twelve plants per accession were used in the analysis.

Figure 2. Seedling establishment (% stand) measured during the establishment year at five western range sites comparing Vavilov II with Vavilov. Mean separations were made on the basis of least significant differences (LSD) at the 0.05 probability level.

Figure 3. Persistence (% stand) measured after the establishment year at five western range sites comparing Vavilov II with Vavilov. Mean separations were made on the basis of least significant differences (LSD) at the 0.05 probability level.

Table 1. Morphological summary of morphological traits at Nephi and Blue Creek, UT (planted April 2004). Analysis included 10 reproductive culms (different plants) per replication (four-replications).

Morphological traits		Vavilov II	Vavilov	P-27	LSD (P<0.05)
Plant height (cm)	Mean	64.2	63.4	58.2	3.7
	Range	38.5-130.2	26.0-100.2	35.0-87.3	
Flag leaf height (cm)	Mean	40.3	39.3	34.0	3.3
	Range	15.1-68.2	7.0-63.5	11.4-55.8	
Flag leaf length (cm)	Mean	10.2	11.7	8.5	0.8
	Range	3.2-21.0	4.0-24.7	2.5-22.0	
Flag leaf width (mm)	Mean	3.1	2.8	2.6	0.4
	Range	1.5-6.0	2.0-5.0	1.0-6.0	
Spike length (cm)	Mean	8.2	9.1	7.8	0.7
	Range	5.0-12.5	4.8-22.4	4.0-18.1	
Spike width (mm)	Mean	1.1	1.0	0.9	NS
	Range	0.5-2.2	0.6-1.6	0.5-2.7	
First lemma length (mm)	Mean	7.4	7.2	6.4	0.4
	Range	5.0-12.0	4.5-10.5	5.0-12.0	
First lemma width (mm)	Mean	1.1	1.2	1.1	NS
	Range	1.0-2.0	0.9-2.5	0.9-2.0	
First lemma awn length (mm)	Mean	0.9	0.8	0.4	0.3
	Range	0.0-4.0	0.0-3.5	0.0-4.5	
First glume length (mm)	Mean	6.1	5.6	5.4	0.5
	Range	3.0-10.0	3.5-9.0	2.2-9.5	
First glume width (mm)	Mean	1.0	1.1	1.1	NS
	Range	0.6-2.0	0.5-2.0	0.6-2.0	
First glume awn length (mm)	Mean	1.9	1.7	1.2	0.4
	Range	0.0-6.0	0.0-4.5	0.0-4.5	
Spike length/width	Mean	8.2	9.5	9.5	NS
	Range	3.7-15.8	4.3-22.4	2.4-30.2	

Table 2. Gene frequencies of four AFLP markers for four Siberian wheatgrass accessions.

	Gene frequencies			
	Kazak	P27	Vavilov	Vavilov II
E.AGC_M.CAT_171.4	0.08	0.58	0.67	1.00
E.AGC_M.CAT_111.1	0.17	0.08	0.33	0.83
E.AGG_M.CAG_364.7	0.25	0.00	0.00	0.58
E.AGG_M.CAG_190.3	0.00	0.50	0.50	0.83

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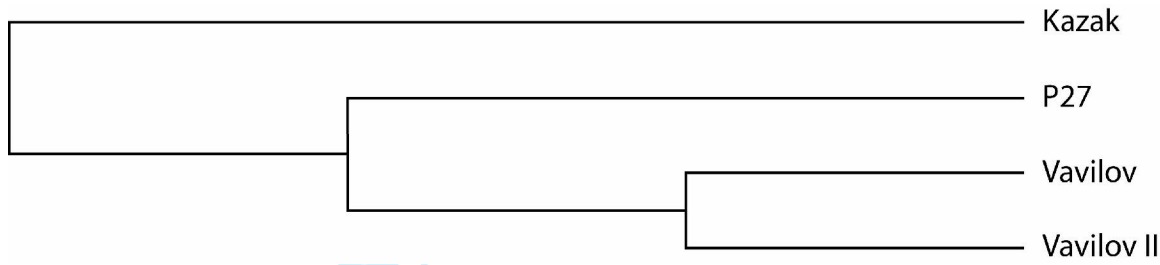


Figure 1

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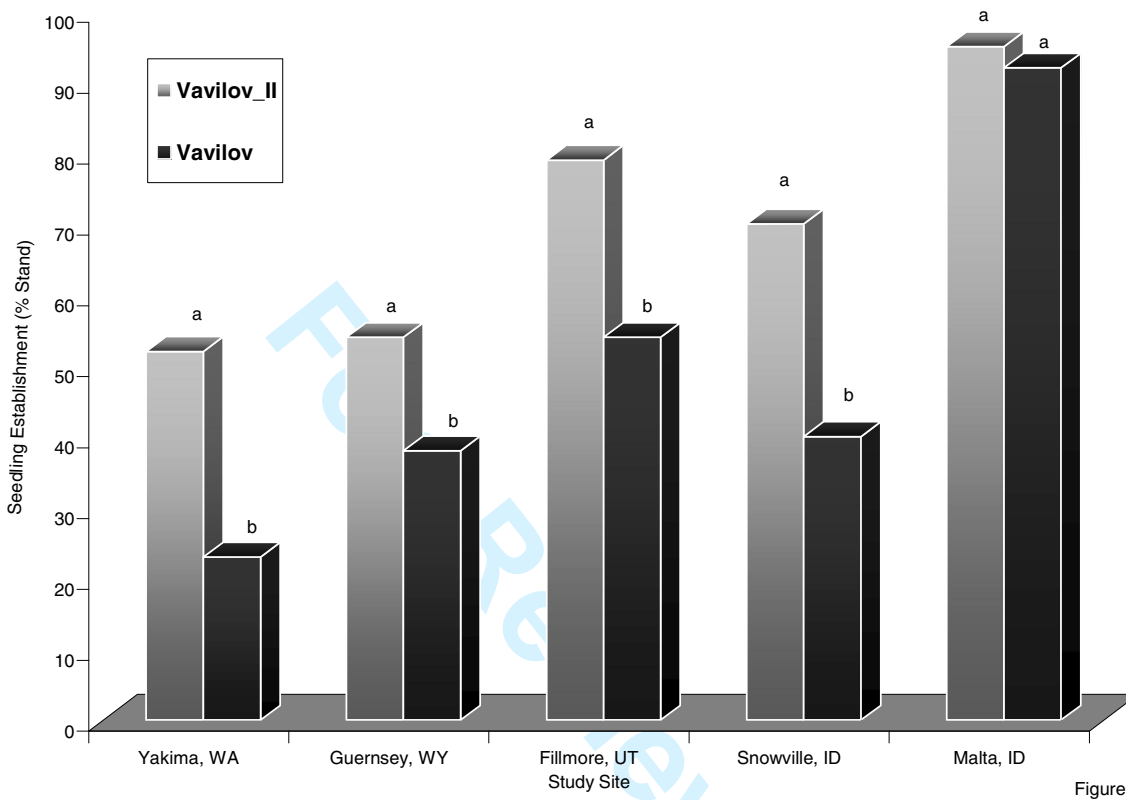


Figure 2.

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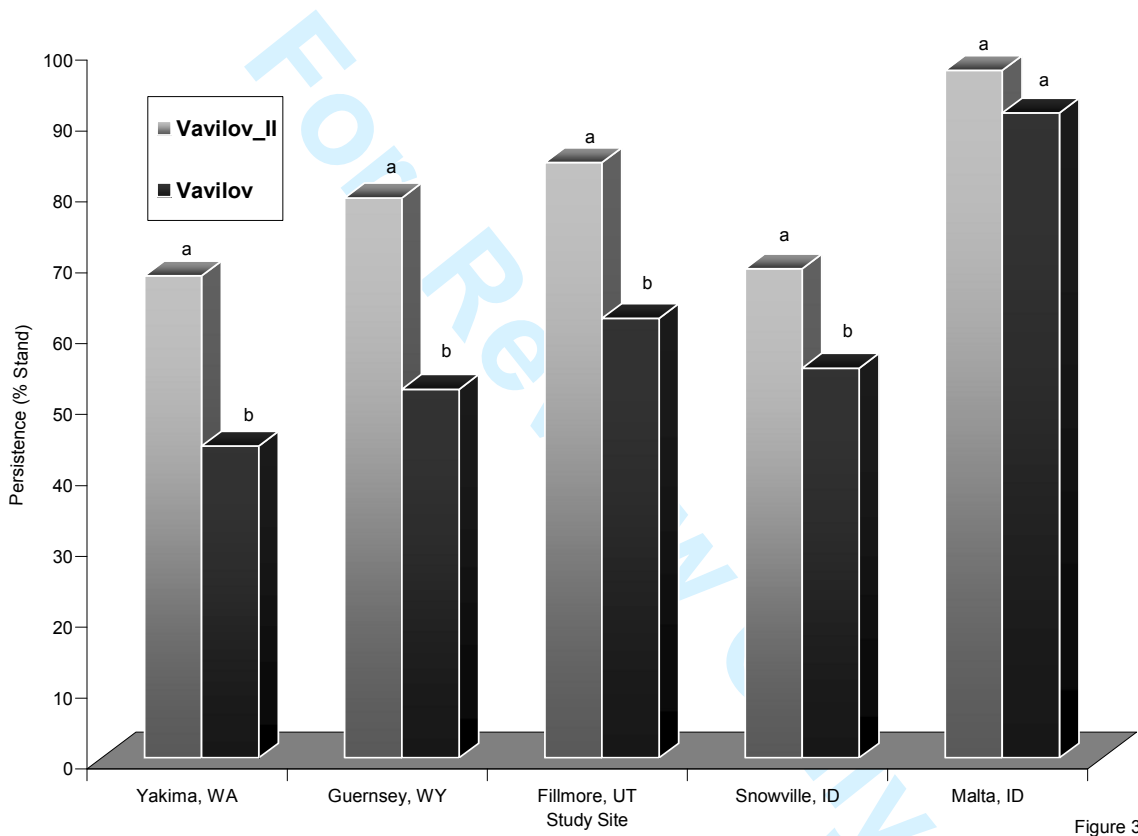


Figure 3.