10-2020

Breeding Alfalfa for Semiarid Regions in the Northern Great Plains: History and Additional Genetic Evaluations of Novel Germplasm

Arvid A. Boe

Kevin D. Kephart

John D. Berdahl

Mike D. Peel

E. Charles Brummer

See next page for additional authors

Authors
Arvid A. Boe, Kevin D. Kephart, John D. Berdahl, Mike D. Peel, E. Charles Brummer, Lan Xu, and Yajun Wu

agronomy

MDPI

Review

Breeding Alfalfa for Semiarid Regions in the Northern Great Plains: History and Additional Genetic Evaluations of Novel Germplasm

Arvid Boe [1,*], Kevin D. Kephart [1], John D. Berdahl [2], Michael D. Peel [3], E. Charles Brummer [4], Lan Xu [5] and Yajun Wu [6]

1 Department of Agronomy, Horticulture, and Plant Science, South Dakota State University, Brookings, SD 57007, USA; kevin.kephart@sdstate.edu
2 USDA-ARS Northern Great Plains Research Laboratory, Mandan, ND 58554, USA; jolob1@bis.midco.net
3 USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, UT 84322, USA; mike.peel@usda.gov
4 Department of Plant Sciences, University of California, Davis, CA 95616, USA; ecbrummer@ucdavis.edu
5 Department of Natural Resource Management, South Dakota State University, Brookings, SD 57007, USA; lan.xu@sdstate.edu
6 Department of Biology & Microbiology, South Dakota State University, Brookings, SD 57007, USA; yajun.wu@sdstate.edu
* Correspondence: arvid.boe@sdstate.edu; Tel.: +1-6-(05)-688-4759

Received: 2 September 2020; Accepted: 26 October 2020; Published: 30 October 2020

check for updates

Abstract: Yellow-flowered alfalfa (*Medicago sativa* subsp. *falcata*) (also known as sickle medic) has been the cornerstone for breeding alfalfa for dual grazing and hay production in the semiarid regions of the northern Great Plains in the US and Canada. Most, if not all, of the cultivars developed for the northern Great Plains during the 20th century, had parentage tracing back to introductions by Niels Ebbesen Hansen that were obtained from expeditions to Russia, primarily the province of Siberia, on behalf of the United States Department of Agriculture during the early 1900s. The *M. falcata* genome contains alleles for high levels of drought-tolerance, winter hardiness, and tolerance to grazing, but is generally deficient for commercial seed production traits, such as non-shatter, compared with common alfalfa (*M. sativa*). A naturalized population, tracing to USDA plant introductions to Perkins County South Dakota by N.E. Hansen in early 1900, and subsequently, facilitated by the determined seed increase and interseeding of a population by a local rancher, Norman 'Bud' Smith, has shown highly desirable in situ characteristics for improving rangelands in the northern Great Plains. This includes adequate seed production to build a seed bank in the soil for natural seedling recruitment and population maintenance/expansion and support the production of a commercial seed source. This review documents the seminal events in the development of cultivars to date and describes novel germplasm with potential for new cultivars in the future.

Keywords: *Medicago sativa* subsp. *falcata*; pasture legumes; grazing alfalfa; stress tolerance; forage breeding and genetics; grassland improvement; creeping rooted

1. History

Breeding alfalfa (*Medicago sativa* L.) for semiarid regions of the northern Great Plains, predominantly North Dakota (ND), South Dakota (SD), southern Saskatchewan and southern Alberta west of the 100th meridian and the eastern half of Montana, arguably began with the first intentional introduction of yellow-flowered alfalfa (*Medicago sativa* subsp. *falcata* (L.) Arcang.) (hereafter *M. falcata*) for forage purposes in cold and dry environments by Niels Ebbesen Hansen (hereafter N.E Hansen) to SD in

1906 [1,2]. N.E. Hansen, from his travels throughout northern Europe and Asia, recognized *M. falcata* had the traits (i.e., tolerance of growing-season drought and cold and dry winters, palatability to livestock, and endurance to grazing) that he was convinced were useful for "solving the alfalfa problem in the Prairie Northwest". The earliest breeding work for alfalfa, in general, was from 1903 to 1915 [3]. During that time, 'Grimm', 'Baltic', 'Cossack', and 'Ladak' were developed; all, except for Grimm, selected/developed in SD. The parental material of Baltic, developed by W.A. Wheeler, South Dakota State University (SDSU), was likely Grimm [3]. Grimm, Baltic, and Cossack were hybrids (*M. sativa* nothosubsp. varia (Martyn) Arcang. (syn. *M. media* Pers.) (hereafter *M media*) between *M. falcata* and *M. sativa*.

The first variety trials of alfalfa in the semiarid northern Great Plains, conducted during the first three decades of the 20th century, were necessarily composed of those few that were commercially available [4]. A trial that ran from 1917 through 1930 compared Cossack, Grimm, 'SD Common', 'Semipalatinsk', and 'Turkestan' on the SDSU Cottonwood Experiment Farm near Wall, SD (43°57′59″ N, 101°54′20″ W) (average annual precipitation = 419 mm). Results of that long-term trial indicated Turkestan (*M. sativa*), introduced by N.E. Hansen, from a single plant in Russia in 1912, to be substantially superior for forage production (2105 kg ha^{-1}). Cossack, a hybrid (*M. media*) developed from a "spoonful" of seeds that Hansen brought back from southeastern Russia, in 1906, was increased to thousands of bushels in western SD by 1916 [5] and was thought by Hansen to be superior to Turkestan. Grimm (*M. sativa*), of course, was famously introduced to western Minnesota near Chaska by Wendelin Grimm, a dairy farmer from Baden, Germany, in 1857. Its introduction, followed by natural selection, provided a source of winter hardiness, previously not available, for the northern Midwest. With the involvement of experiment stations, Grimm was widely available, desired, and distributed by 1900 [3]. However, bacterial wilt (*Clavibacter michiganense* ssp *insidious*) ended its dominance.

Semipalatinsk (*M. falcata*) was selected from the most vigorous form of this widespread species N.E. Hansen observed during his travels in western Siberia [6]. 'Teton', developed by SDSU, traces back to a cross made by N.E. Hansen in 1914 between Turkestan and Semipalatinsk (PI 24455) from Siberia [7].

Ladak, one of the earliest developed cultivars in the northern Great Plains, was selected at Redfield, SD in 1914 by H.L. Westover and Samuel Garver of the USDA from an introduction from Ladakh Province in northern India [8]. The seeds were labeled *M. falcata*, and plants were predominantly yellow-flowered; however, some variegation occurred and increased in successive generations [9]. This cultivar increased forage production in monoculture and in mixtures with perennial wheatgrasses (Triticeae) in long-term studies near Swift Current, Saskatchewan, where the average annual precipitation is 350 mm [10]. Ladak was also the most productive, vigorous, and persistent of 10 cultivars evaluated in hay and grazing systems at three semiarid locations in ND during the early 1950s. It was superior to Grimm, 'Nomad' (rhizomatous cultivar developed in Oregon in 1941), Semipalatinsk, 'Rhizoma' (developed by the University of British Columbia in 1950), and 'Ranger' (developed by USDA and Nebraska Agricultural Experiment Station in 1942), for those traits. However, Semipalatinsk was superior for the crown spread and filling in of rows [11].

Ladak was also rated higher than Semipalatinsk, Nomad, Ranger, Grimm, and Cossack for stand, crown health, and vigor in grazing trials on the SDSU Cottonwood Range Station near Wall, SD during the early 1950s [12]. It was chosen as a parent in crosses with *M. falcata* in the development of creeping-rooted grazing cultivars by the Canada Department of Agriculture [13–15]. That same parentage of Ladak × *M. falcata*, with the addition of remnants of N.E. Hansen's introductions to western SD, was used to develop the 10-clone synthetic cultivar 'Travois' [16].

Breeding of hybrid synthetic cultivars with various concentrations of yellow-flowered parentage began, for the most part, during the late 1940s and continued through the early 1970s in both the USA (e.g., Teton) and Canada (e.g., 'Rambler'). Selection criteria generally included drought-tolerance, winterhardiness, creeping rootedness, and in some cases, bacterial wilt resistance. The resulting grazing-type cultivars were vastly superior to standard hay-type cultivars for forage production and persistence in semiarid haylands and grazing lands [17], due to the incorporation of the creeping rooted trait [13,18,19] from *M. falcata*, introduced by N.E. Hansen from Semipalatinsk, Siberia [5]. Rambler was an early synthetic cultivar composed of clones derived from crosses between Ladak and Hansen's Siberian from a 1934 planting that had survived the "Great Drought" of the thirties [13]. These new cultivars were, for the most part, adapted to areas where precipitation limited forage production to a single harvest taken during mid-to full bloom [15,20].

Long-term comparative evaluations of alfalfa germplasms in the semiarid northern Great Plains demonstrated cultivars and experimental strains with a high percentage of *M. falcata* parentage were better adapted, and therefore, superior to the conventional hay-types (*M.sativa*) for inter-seeding rangelands [17,21]. Misar et al. [22] reported the commercial first-generation seed increase (produced by Wind River Seed Co., Manderson, Wyoming) of Norman Smith's '*falcata*' [23] and selections from progenies from seeds collected from the Grand River National Grassland (GRNG) [24] had higher survival and productivity than conventional hay-and grazing-type alfalfas under mob grazing on the mixed-grass prairie, within 50 km of the Norman Smith Ranch in northwestern SD.

In addition, particularly novel germplasms developed during the 1970s were (1) C-6 derived in Colorado in the 1970s from drought-resistant selections by A.C. Dillman between 1910 and 1914 at Belle Fourche, SD from N.E. Hansen's original introductions [25], and (2) 'Wisfal' developed in Wisconsin in the 1970s by backcrossing tetraploidy into diploids using 2n eggs [26]. The C-6 Alfalfa Germplasm was composited from seeds of drought-resistant USDA National Plant Germplasm System plant introduction accessions (PIs) produced in SD that had survived for 55 years in an unheated, uninsulated building at Mandan, ND [27]. The composition of the germplasm included 24 selections from eight PIs from Russia and Siberia, Cossack, 'Canadian Variegated', 'Cherno', and 'Sibturk', and seven additional selections made at Mandan, ND from N.E. Hansen's Semipalatinsk introductions. Two cycles of selection were made for vigor at Fort Collins, Colorado [25]. This germplasm showed promise for forage yield in a delayed single-harvest system at Highmore, SD [20]. Wisfal was also productive in that management system in South Dakota [20].

Smith [23] described highly successful interseedings, and subsequent, natural recruitment from the seeds of yellow-flowered alfalfa descended from introductions made by N.E. Hansen to the mixed-grass prairie in northwestern South Dakota during the early 20th century. Smith harvested seeds from small patches of exclusively yellow-flowered plants and found those lots to be superior to cultivars, such as Teton and Travois, for establishment, persistence, and productivity. Smith sold the seeds of this population, which he named '*falcata*' to interested ranchers. A seed increase of this population produced in isolation is currently marketed by Wind River Seed Company. He also observed widespread natural recruitment on his ranch and on federally owned land (i.e., GRNG) adjacent to his ranch, and for which he had long-term grazing permits. This natural spread presumably resulted from seed rain, due to livestock and wildlife grazing and other natural agents, such as wind and surface water movement. This reseeding characteristic was not evident in several interseedings of grazing-type cultivars that Smith had conducted. Xu et al. [24] recently described the distribution pattern and forage production of that naturalized population related to landscape position on the GRNG. The GRNG population has particular value for breeding purposes because it represents selection pressure under long-term livestock stocking systems prescribed by the USDA Forest Service. As pointed out by Ries [28], for alfalfa to become part of a rangeland and maintain itself, it must have the capacity to reseed because periodical reseeding is impractical.

In addition to Travois and Teton, Smith [23] also interseeded another local population, in addition to his selected 'falcata' population, of predominantly yellow-flowered alfalfa, introduced by N.E. Hansen from Siberia. The seeds of this population was produced for sale (SDSU Seed Testing Laboratory Records) during the 1940s and 1950s by Claude Foster near Coal Springs (a ghost town since 1954), SD, about 80 km southeast of the Norman Smith Ranch in Perkins County. Heinrichs [29] noted that this population of *M. falcata* was one of several in the US and Canada that survived the drought of the 1930s with little loss of stand and that it had a high expression of creeping-rootedness. The seeds of 'Foster's Yellow Blossom' (also called 'Foster's Siberian', 'Foster's Semipalatinsk', or '*M. falcata* from Coal

Springs, SD' [30]) was quite widely distributed in the western US in the mid-20th century by Claude Foster. It was compared with other creeping-rooted or otherwise prominent pasture and dryland alfalfa cultivars (e.g., Grimm, Ladak, Nomad, Ranger, Rhizoma) in dryland pasture in northern Utah (UT) (average annual precipitation 200 mm) from 1954 to 1979 [31,32]. Although this population had the poorest initial stand rating, it was second only to Nomad for plants ha^{-1} in 1977. Given Foster's Yellow Blossom was interseeded on the Norman Smith Ranch during the 1960s, it feasibly may have contributed to the genetic composition of the present-day naturalized population on the GRNG.

Since about 1970, the emphasis has been on developing new yellow-flowered alfalfa (*M. falcata*) cultivars in the USA and Canada. This trend began with 'Anik', released in Canada in 1971 [33] and resulted in three more cultivars (Yellowhead [34], Don [35], and Sholty (tested as SDSU Experimental Population SD201)); [20] and two improved germplasms (IAMF101 and IAMF102 [36]) to date.

Anik was selected for winterhardiness north of 57° N in northwestern Canada [33] and is recommended for pasture, hay, and ecological plantings in Northwestern Canada [20]. Yellowhead was developed from recurrent selection for bacterial wilt resistance within wild populations near Swift Current, Saskatchewan [34]. Yellowhead had the highest survival, out of 16 alfalfa populations composed of hay- and grazing-type cultivars, after five years of mob grazing in Mandan, ND [37], and was recommended for incorporating into rangelands in the northern Great Plains [38].

Don was selected from a strain collected by N.E. Hansen in the Don Province of southeastern Russia in 1906 [39,40]. The strain persisted well at Brookings, SD, and seeds collected from surviving plants by M.D. Rumbaugh composed the original population from which 96 plants were selected for upright stature, fine-leaf type, and yellow flower color in 1999 at Logan, UT. Don is recommended for mixtures with grasses on Intermountain West rangelands [35].

Sholty is a 41-clone putative diploid synthetic cultivar composed of 29 genotypes from seven PIs, six genotypes from experimental populations from remnant rangeland collections that trace back to introductions by N.E. Hansen in SD, five genotypes from 'Kuban', and one genotype from Anik. The selection was for vigor, tolerance to potato leafhopper (*Empoasca fabae*) yellowing, leaf retention/indeterminate flowering, and semi-erect erect growth habits [20,41]. It has produced more forage than the other *M. falcata* cultivars in long-term yield trials at Brookings, SD, and has reseeded from transplants in grass swards (A. Boe, personal observations). Sholty was persistent and productive (9.7 Mg ha^{-1}) annually for six years in monoculture and binary mixtures with pubescent wheatgrass (*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey) and Fairway crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) at Bison, SD [42].

IAMF101 is 36-genotype tetraploid germplasm developed from two cycles of recurrent phenotypic selection for persistence, autumn regrowth, and vigor from about 20 PIs and experimental populations. IAMF102 is 32-genotype tetraploid germplasm developed by test crossing 104 plants from about 15 PIs to four elite *M. sativa* populations and evaluated in field trials at Ames, Iowa (IA) [36].

The 'Spredor' series of proprietary cultivars began with the release of 'Spredor' in 1977 by Northrup King and is currently in the fifth generation, 'Spredor 5', marketed by NEXGROW™ alfalfa developed by Forage Genetics International. These cultivars have a high frequency of creeping rooted plants, characterized by stems produced from laterally spreading roots, from a Vernal, Rambler, and Travois parentage [43]. Spredor 5 has high resistance to crown, root, and leaf diseases and is salt tolerant and winterhardy. Spredor 3 was comparable to Rangelander and Yellowhead for persistence in continuous stocking trials in Manitoba [44].

Boe et al. [20] conducted evaluations of: (1) Most of the creeping-rooted grazing-type cultivars, (2) several *M. falcata* PI accessions from the National Plant Germplasm System, USDA Plant Introduction Station, Pullman, WA, (3) aforementioned experimental populations of *M. falcata* from University of Wisconsin, Madison (E.T. Bingham) and USDA-ARS/Colorado State University, Fort Collins, Colorado (C.E. Townsend), and (4) several conventional hay- and pasture-type cultivars in spaced-plant, single-row transplant, and seeded plots in eastern and central SD. Data collected were forage yield, crude protein, in vitro dry matter digestibility, height, growth habits [41], and potato leafhopper yellowing. Results revealed extensive among and within cultivar/population/strain/PI variation for morphological, physiological, and forage quality traits useful in breeding programs for grassland improvement.

2. Additional Genetic Evaluations

Variation for desirable traits for breeding new cultivars of alfalfa for hay production and grazing systems in the semiarid northern Great Plains [45] were evident in situ in the GRNG population [24] (Figure 1). In addition, this population demonstrated a capacity for recruitment from reseeding under grazing, putatively facilitated by sporadic production of large crops of hard seed, which composed nearly 100% of the alfalfa soil seed bank [24]. To our knowledge, these critically important traits have not been reported for any other alfalfa germplasms [23]. However, Rumbaugh [32] observed reseeding enough to maintain plant density in pure stands of alfalfa over a 25-year period in UT, where plants were grazed early spring and seeds were produced from regrowth. However, seed production by the GRNG population in northwestern SD is more episodic, depending on when the annual grazing period occurs and the intensity of insect feeding that destroys floral meristems (alfalfa weevil (*Hypera postica*)) or developing seeds (alfalfa seed chalcid (*Bruchophagus roddi* Guss.)).

Figure 1. Yellow-flowered alfalfa in full bloom on the Grand River National Grassland near Lodgepole, South Dakota (45°45′42″ N, 102°40′25″ W).

Another important attribute of the GRNG population is the capacity to establish naturally within stands of crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.). This introduced cool-season grass was widely planted in the semiarid northern Great Plains to revegetate and permanently stabilize abandoned cropland as directed by federal programs, such as the Bankhead-Jones Farm Tenet Act of 1937, prior to the formation of the National Grassland System [24]. Both species are highly competitive and relatively impervious to the introduction and long-term establishment of other plant species in semiarid areas [45,46]. About eight million hectares of crested wheatgrass, much in monoculture, occur in the

US. The benefits of incorporating alfalfa include economic and environmental parameters [46] such as increased forage production, improved forage quality, and enhanced wildlife habitat.

Mortenson et al. [47] reported relatively uniform alfalfa plant densities of 5.5 plants m^{-2} and 4.4 plants m^{-2} in interspaces between interseeded rows (152-cm row spacing) in 2004 from interseedings in mixed-grass prairie made by Norman Smith [23] in 1965 and 1987, respectively. This suggested the alfalfa populations had reached equilibrium, through natural reseeding, with the native grass-dominated plant community under the grazing management system used on the Norman Smith Ranch for several decades. Forage production in interspace areas containing alfalfa from natural reseeding was 67% greater in the 1965 interseeding and 240% greater in the 1987 interseeding than in the control areas. In the 1998 interseeding, forage production within 1 m^2 spanning the interseeded row was 42% greater than the adjacent area, where alfalfa had not yet reseeded. The alfalfa plant population density in planted rows in the 1998 interseeding was 2.2 plants m^{-2} in 2004. They also reported increases in soil nitrogen and soil organic carbon, and suggested climate change mitigation aspects, on the Norman Smith Ranch in association with inter-seeding adapted yellow-flowered alfalfa [48].

Therefore, to establish a base population for a breeding program, the seeds were collected and kept separate from about 230 individual plants across about 5 km^2 on the GRNG in 2000. The amounts of seeds collected ranged from <0.25 g to >3.5 g $plant^{-1}$. From that seed collection, half-sib family nurseries were established in 2001 at Highmore, SD (SDSU), Mandan, ND (USDA-ARS, Northern Plains Research Center), and Ames, IA (Iowa State University) and at Millville, UT (USDA-ARS, Forage and Range Research Center) in 2003. Data collected in subsequent years included forage production, growth habit, flower color, pod shape, and potato leafhopper yellowing. This was a collaborative USDA NIFA Multi-State Research Objective (NE 1010 Breeding and Genetics of Forage Crops to Improve Productivity, Quality, and Industrial Uses).

Presently, the GRNG population is also proving useful for finding genetic sources of drought-resistance [49] and freezing tolerance [50] in alfalfa. Plants grown from seeds collected on the GRNG maintained root growth, relative water content, and chlorophyll content, with increasing water use efficiency, as deficit became severe, better than 10 other alfalfa accessions.

Oakley and Garver [1] determined that forage production of *M. falcata* was greater in hills and space-planted stands compared with broadcast or seeded stands, presumably due to plasticity in crown diameter and shoot density traits. They pointed out in its natural habitats in Europe and Asia, plants of *M. falcata* were generally widely spaced, relative to populations of *M. sativa*. Research is needed to elucidate the relationship between plant population (i.e., inter-plant spacing) and forage production. A recent study of interseeding alfalfa in semiarid native grassland in Texas revealed an advantage of wide row (71 cm) spacing compared with narrow row (36 cm) spacing for water use and forage production and quality [51].

Our objectives here are to report results for: (1) The Multistate Research half-sib family evaluation trials for adaptation, forage production, pest resistance, and morphological traits at multiple locations (Highmore, SD, Mandan, ND, Millville, UT, and Ames, IA), and three ancillary experiments at Brookings, SD. The three ancillary experiments: (1) Compared morphological characteristics of plants derived from five bulk-seed samples collected from different topographical subdivisions of the GRNG, (2) compared eight cultivars/strains/experimental populations of predominantly *M. falcata*, including populations from divergent phenotypic selection for pod shape, and four standard hay-type and pasture-type cultivars for three years in a delayed single-harvest forage management system, and (3) evaluated forage production of 'Sholty' *M. falcata* with an *M. media* collection from rangeland in western SD, at low plant population densities. All three experiments were conducted in transplant nurseries at Brookings, SD.

2.1. Materials and Methods

2.1.1. GRNG Half-Sib Families

Genetic evaluation nurseries of the GRNG population were transplanted at Highmore, SD (44°31′7 N, 99°26′28″ W), Mandan, ND (46°49′44 N, 100°53′28″ W), and Ames, IA (42°02′05″ N, 93°37′12″ W) during 2001 and Millville, UT (41°41′36″ N, 111°49′55″ W, elevation 1381 m) during 2003. Each investigator/location used standard forage breeding spaced-plant nursery designs appropriate for their specific environments. For example, at Highmore, SD, the alfalfa nursery was broadcast-seeded with crested wheatgrass after transplanting in 2001 and again in April 2002 to provide strong interspecific competition typical of what would occur in inter-seeded situations in rangelands in the northern Great Plains (Figure 2). At the other locations, transplanting was into tilled soil, with weed control by tillage, hand, or herbicides.

Figure 2. Half-sib family rows of the Grand River National Grassland population growing with crested wheatgrass at Highmore, South Dakota (44°31′7″ N, 99°26′28″ W).

At Highmore, the experimental design was two replications of 224 half-sib families in a randomized complete block design. Smith's falcata, '5454', and 'Vernal' were included as checks. Each family row was composed of seven plants with 0.3-m interplant spacing. Alley and inter-row spacings were 0.9 m. Soil type was a Glenham (fine-loamy, mixed, superactive, mesic Typic Argiustolls)-Java (fine-loamy, mixed, superactive, mesic Entic Haplustolls)-Prosper (fine-loamy, mixed, superactive, mesic Pachic Argiustolls) loam. Plots were harvested at 10-cm stubble height for dry matter forage production at late bloom to well-developed pod stage during late July for each of 2002 and 2005. During late July in each of 2003 and 2004, the standing crop was swathed and removed from the experimental area. This system provided estimates of forage production at early-and fully-established (alfalfa and over-sown desert wheatgrass) stages. Data for growth habits (scale was 1 = prostrate, 2 = semisprawling, 3 = bowl-shaped, 4 = fully erect) was devised to coincide with Sinskaya's [41] ecoelement designations based on heritable morphological variation for growth habits in *M. falcata* in Russia. Variation for flower color was described as the frequency of families that occurred for each of 11 color class combinations (i.e., monochrome purple, variegated, monochrome cream, and monochrome yellow and their two-way and three-way combinations). Data for growth habits and flower color were collected at full to late bloom on 25 July 2005.

At Mandan, ND, two replications of single-row plots of 128 families were transplanted in a randomized complete block design on 12 August 2002. Each family row had eight plants with 0.6-m interplant spacing. Inter-row spacing was 1.8 m. Soil type was a Parshall (coarse-loamy, mixed, superlative, frigid, Pachic Haplustolls)-Manning (coarse-loamy, mixed, superactive, frigid Typic Haplustolls) sandy loam. Plots were harvested for dry matter forage production on 12 August 2003 and 2 August 2004, when plants were in late bloom to well-developed pod stages. Growth habits were determined on an individual plant basis using the same scale as for Highmore. Flower color was defined using a 1 = purple, 2 = variegated, 3 = cream, 4 = yellow, 5 = white scale developed by Barnes [52]. Data for growth habits and flower color were collected on 20 June 2003. Mean pod shape, determined using a graduated scale from 1 = sickle to 3 = two complete coils based on Sinskaya [41], was estimated for a sample of 3 random plants within each family row on 2 August 2004. The number of plants in row^{-1} was also determined at that time. Individual plant data for flower color and pod shape were subjected to analyses of variance to partition the total variance into within and among family components and generate intraclass correlation coefficients.

At Millville, UT, two replications of 84 half-sib families were transplanted in 10-plant single-row plots with 0.5-m intra-row and 1.0-m inter-row spacing on 13 May 2003. The soil at the Evans Farm is a Nibley silty clay loam (fine, mixed, mesic, Aquic Argiustolls). Area annual precipitation averages 472 mm (1980–2011), with July and August being the driest and hottest months (http://www.usclimatedata.com, 8 June 2020). Plots were harvested for dry matter forage production on 17 June and 5 August 2004 and 1 July and 19 September 2005. Data for growth habits were as described for Highmore [41]. Flower color was determined using the same scale used at Mandan, ND, but was based on the most frequently occurring color in a 10-plant family plot.

At Ames, IA, two replications in a randomized complete block design of seedlings of 212 half-sib families, Smith's falcata, 'Sholty', Vernal, and 5454 were transplanted during mid-May 2001 in single-row plots with 1 to 8 plants plot^{-1} (mean was 6.5 plants plot^{-1}) with 0.3-m intra-and 0.9-m inter-plot spacing. The soil type was a Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). Data were collected for growth habits and potato leafhopper yellowing (PLY) tolerance on 14 July 2003 and for regrowth (1 = low to 5 = high) on 4 August 2004 for forage accumulated since harvest at mid-bloom during June. The primary trait of interest was tolerance to potato leafhopper yellowing. The expectation was that stockpiling forage until mid-July in south-central Iowa would provide an ideal situation for the development of heavy leafhopper infestation.

Analyses of variance were used to partition phenotypic variation into sources associated with fixed (year, entry, and year × entry) effects for Highmore and Mandan for dry matter forage production, growth habit, and pod shape. For Millville, the same analyses were conducted for dry matter forage production and growth habit.

The number of families and plants within families evaluated at each location varied by amounts of seeds available and local limitations for field plot area and support staff. Traits measured at each location were based on the preferences of individual investigators and time limitations. Ultimately, the dataset was intended to provide the value of diverse environments for measuring the plasticity of phenotypic expression and appropriateness to target environments in the semiarid northern Great Plains and perhaps beyond.

2.1.2. Standard Cultivars and Selected *M. falcata* Populations

Transplant nurseries of the same populations evaluated by Misar et al. [22] in northwestern SD were established in Kentucky bluegrass (*Poa pratensis* L.) sod at Brookings, SD in June 2006. Soil type was a Vienna (fine-loamy, mixed, superactive, frigid, Calcic Hapludolls)-Brookings (fine-silty, mixed, superactive, frigid, Pachic Hapludolls) complex. Annual precipitation at Brookings (615 mm) exceeds the upper threshold of semiarid, but the nurseries were on soils that had provided mid-summer drought stress in alfalfa in previous studies (A. Boe, personal observations). The experimental design was three replications of 5-plant plots of eight cultivars and three experimental populations transplanted in single rows with 0.3-m intra-plot and 0.9-m inter-plot spacing. Plots were harvested for dry matter forage yield on 24 July 2007 and 2008 and on 16 June and 17 August 2009.

2.1.3. Phenotypic Characteristics of Progeny Related to Landscape Position on the GRNG

The majority of seeds were collected from about 25 random plants within each of five subdivisions of the GRNG population, based on proximity to the Norman Smith Ranch and topographical heterogeneity. From those five bulks, seedlings were transplanted to a Kentucky bluegrass sod at Brookings, SD, in a completely randomized design on 22 June 2004. Soil type was a Vienna (fine-loamy, mixed, superactive, frigid, Calcic Hapludolls)-Brookings (fine-silty, mixed, superactive, frigid, Pachic Hapludolls) complex. The number of plants bulk^{-1} transplanted ranged from 40 to 81, with 324 plants in the nursery. Plants were spaced on 0.9-m centers. Data collected were g dry matter plant^{-1}, flower color, and growth habits in mid-July during each of 2005 and 2006.

2.1.4. Effect of Population Density on Forage Yield of *M. falcata*

Seedlings from 'Sholty' and from bulk seeds collected in 2009 from 10 plants, hereafter referred to as the Thunder Butte Creek population (TBC), were transplanted in tilled soil at two sites, Brookings and Aurora, in eastern SD during June 2010. The seeds of the TBC population was collected in 2009 in a pasture near the site of commercial seed production for Foster's Yellow Blossom alfalfa during the mid-20th century. At Brookings, for each alfalfa population, two population densities (3.0 plants m^{-2} and 1.8 plants m^{-2}) were evaluated in 1.8-m long rows with 0.9-m inter-row spacing; whereas, at Aurora, a single population density, 2.4 plants m^{-2}, was evaluated for each of the two alfalfa populations. The experimental design at each site was a randomized complete block with 10 replications. Plots were harvested for dry matter yield on 9 August 2011 and 16 July 2013 and 14 August 2011 and 20 July 2013 at Brookings and Aurora, respectively.

2.2. Results and Discussion

2.2.1. Forage Production

Factorial analyses of variance showed highly significant differences ($p < 0.001$) between years and among families at Highmore, SD, and Mandan, ND in the northern Great Plains. At Highmore, mean dry matter yield in late July 2002 was 15% greater than in late July 2005. March through September precipitation was below the 20-year average (420 mm) for both 2002 (383 mm) and 2005 (343 mm). The distribution varied widely between the two years. In 2002, 55% fell during July and August, whereas, in 2005, 73% fell during May and June. However, the desert wheatgrass was well established and likely competed very strongly for the early growing season precipitation in 2005. At Mandan, mean dry matter yield in August 2004 was 2.7 times the yield in August 2003. Total precipitation during June and July for 2004 was 130 mm (99% of average), compared with 41 mm (31% of average) for 2003 (Table 1). The cessation of top growth during severe drought by *M. falcata* is an adaptation whereby plants devote energy to the production of rhizomes for resumption of growth when conditions become favorable [1].

Check cultivars at each location were 'Vernal', '5454', and 'Smith's *falcata*'. At Highmore, Smith's falcata ranked 52, whereas Vernal and 5454 ranked higher than 150 out of 224 entries. At Mandan, Smith's falcata ranked 33, Vernal ranked 75, and 5454 ranked 112 out of 128 entries.

Table 1. Dry matter forage production (Mg ha^{-1}) for half-sib families of the Grand River National Grassland population of yellow-flowered alfalfa in North Dakota, South Dakota, and Utah.

Location	30-yr Average Precipitation (mm)	Harvest Date	No. Families	Family Means	
				Grand	Range
Highmore, SD	539	25 July 2002	224	2.5	0.7–4.9
		29 July 2005	224	2.1	0.9–4.1
Mandan, ND	456	12 August 2003	128	1.1	0.2–2.2
		2 August 2004	128	2.8	1.1–6.0
Millville, UT	472	17 June 2004	84	5.6	3.7–8.2
		5 August 2004 Regrowth	84	1.9	0.7–3.1
		1 August 2005	84	6.9	5.2–8.9
		19 September 2005 Regrowth	84	1.4	0.4–3.4

At Millville, UT in the Intermountain West, dry matter forage production from a two-harvest system was about four times that of a delayed single harvest in the northern Great Plains. The first harvest was taken at early bloom near peak standing crop. The checks at Millville were hay-type (*M. media*) cultivars, 'Ladak 65', 'Saranac', 'Vernal', and '5454', and 'Smith's *falcata*'. Ranking of all 84 entries for forage production from initial growth placed only Ladak 65 in the top 10 for each of 2004 (rank = 4) and 2005 (rank = 1). On the other hand, a ranking of all 84 entries for forage production from regrowth placed all four checks in the top 10 for each of 2004 and 2005. For example, 5454 ranked 6 in 2004 and 1 in 2005, and Saranac ranked 2 in 2004 and 4 in 2005. Smith's *falcata* ranked 50 for both initial growth and regrowth in 2004 and 31 and 22 for the same in 2005. These data pointed out the superiority of numerous GRNG families, compared with conventional hay-type cultivars, for forage production from initial spring growth. However, the data also showed that several families exhibited regrowth potential comparable to the hay-types.

Since only two replicates of single rows of spaced plants were established at each location, caution should certainly be taken when declaring differences among families as a basis for selecting for forage production, in particular. However, at this initial stage of germplasm evaluation, our goal was to estimate the breadth of variation in the GRNG population for morphological and forage production traits. By collecting seeds from individual plants on the GRNG, rather than bulking, we had an identifiable source (i.e., family) useful in partitioning the variation and providing valuable insights for future more detailed genetic studies.

2.2.2. Growth Habit

Mean growth habits for 1875 individual plants, from 128 families, at Mandan was 2.9 ± 0.01. In comparison, when scored on a family-row basis, mean growth habits were 3.1 ± 0.06 for 84 families at Millville and 2.5 ± 0.02 for 224 families at Highmore. At Ames, mean growth habits score was 3.0 ± 0.03 for 215 families. These results quantified the visual descriptive assessment that the preponderance of plants in this population had a distinctive bowl-shaped (i.e., ascending) morphology [1,41] that was consistent across a wide geographical area. Sholty was selected for this morphotype [20] for stockpiling until mid-July in the northern Great Plains for combined forage and wildlife habitat purposes.

2.2.3. Flower Color

The occurrence of flower color types data at Highmore, Mandan, and Millville are presented in Table 2. These data were generated from different approaches at each location; however, the interpretations are similar. At Mandan, the intraclass correlation, $\rho = \sigma^2_F/(\sigma^2_F + \sigma^2_E)$, where

$σ^2_F$ is the variance component associated with among family variance and $σ^2_E$ is the variance component associated with within-family variance, was ρ = 0.3733/(0.3733 + 0.6807) = 0.35. This is the correlation between two randomly chosen individuals from the same family. This indicated a significant amount of variation among and within families.

Table 2. Occurrence (%) of flower color types in the Grand River National Grassland population, based on individual plant (Mandan) or family row (Highmore and Millville) basis.

	Location		
Color Type [1]	**Highmore (224)** [2]	**Mandan (1875)** [3]	**Millville (84)** [2]
Purple	0.04 [1]	12.5 [1]	9.6 [1]
Variegated	20.5	44.7	31.1
Cream	0.0	0.1	3.0
Yellow	12.1	42.3	56.3
Mixtures			
Purple/Variegated	9.3		
Yellow/Variegated	57.6		

[1] Purple flowered plants were from check cultivars (i.e., 5454, Vernal, Ladak 65, and Saranac). [2] Number of families scored; [3] Number of individual plants scored.

Although >90% of the plants on the GRNG are yellow-flowered (Figure 1), variegated-flowered plants occur most noticeably with desert wheatgrass in swales [24]. The Norman Smith Ranch population contains a low frequency of plants with variegated flowers [46], presumably from intercrossing with past inter-seedings of Teton and Travois on the ranch, and seedings of hay-type cultivars in the immediate region [23].

2.2.4. Pod Shape

Significant ($p < 0.001$) differences were found among families for pod shape, estimated on a graduated scale (0.1 units) from 1.0 to 3.0, at Mandan. Ninety-five percent of the plants had pods that were in the range of 1.0–1.7 (i.e., <1 complete coil). Only five percent had pods with from one to two complete coils. These results provided strong morphological evidence of a major contribution of *M. falcata* to the parentage of the population. The intraclass correlation was 0.51, which is the correlation between two individuals within the same family. As expected from the magnitude of the within-family variation for flower color, a significant amount of variation for pod shape occurred among and within families.

2.2.5. Potato Leafhopper Yellowing (PLY) and Regrowth

These two traits were measured only at Ames, IA, PLY in 2003, and regrowth in 2004. The grand mean for the half-sib families was 2.7 for PLY, indicating general moderate resistance/tolerance. Twelve out of 215 families had a PLY < 2.0. On the other hand, the check standard hay-type cultivars, Vernal and 5454, were highly susceptible, with ratings of 4. Garver [8] observed in early 20th century cultivar trials at Redfield, SD that Ladak was more resistant to PLY than cultivars, such as Grimm and Ranger, which had smaller contributions of *M. falcata*, relative to Ladak, in their parentage. Of course, consideration needs to be given to differences in regrowth between the families and the checks contributing to attractiveness for leafhoppers, as the simple linear correlation between PLY and regrowth was highly significant ($r = 0.52$, $p < 0.01$).

2.2.6. Standard Cultivars and Selected *M. falcata* Populations

Results in the 2006 transplant nursery at Brookings mirrored, in some respects, what Misar et al. [22] reported for the same germplasms in northwestern South Dakota. That is, Smith's *falcata*, a selection for sickle-shaped pods from the GRNG population, and Sholty produced more forage than conventional hay- and pasture-type cultivars (i.e., Alfagraze, 5454, and 6200 HT) in a delayed 1-harvest system in eastern South Dakota during each of 2007 and 2008 when early summer precipitation was severely lacking (Table 3). However, forage production of Vernal was similar to Smith's *falcata*, Sickle pod, and Sholty in both years (Table 3). Morphologically, the yield advantage of the *M. falcata* populations could be attributed to wider crowns, compared to conventional types, at a uniform population density. During May through July of the transplant year (2006), total precipitation was 40% of the 30-yr average, with <6% of the normal amount for July. Similarly, total precipitation during May through July 2007 was only 47% of normal, with <3% of the normal amount for July. In addition, during May through July 2008, precipitation was 95% of normal, but was still <50% for July (Table 3).

Table 3. Dry matter forage production (Mg ha^{-1}) of eight cultivars and three experimental populations of alfalfa harvested on 24 July in each of 2007 and 2008 at Brookings, South Dakota.

	Harvest Date	
Population [1]	**2007**	**2008**
Smith's falcata (WR)	2.49	3.23
Don (C)	1.52	1.46
Sholty (C)	2.40	2.87
Alfagraze (C)	0.85	1.56
Coiled pod (SD)	1.69	2.35
Mandan 1991 (ARS)	1.99	2.51
6200 HT (C)	0.91	1.98
Sickle pod (SD)	2.19	3.04
5454 (C)	0.79	1.89
Vernal (C)	1.98	2.85
Travois (C)	1.35	2.40
Least significant difference (0.05)	1.10	1.04

[1] WR, commercial increase of seed produced by Norman Smith, Lodgepole, SD; C, Cultivar; SD, South Dakota State University selection for pod shape from GRNG population; ARS, selection for yield and persistence by USDA-ARS, Mandan, ND.

However, May through July precipitation was 88% of normal in 2009, with 100% of normal in July. When a 2-harvest system was applied in 2009, Smith's *falcata* and selections for pod shape from it produced just as much forage as the highest yielding hay types. In addition, these three germplasms exhibited regrowth similar to the top-yielding hay-and pasture-types. As expected, the two diploid *M. falcata* cultivars, Don and Sholty, produced less forage from regrowth than standard hay- and pasture types under favorable early summer precipitation totals (Table 4).

Table 4. Dry matter forage production (Mg ha^{-1}) of eight cultivars and three experimental populations of alfalfa harvested on 18 June and 17 August 2009 at Brookings, South Dakota.

	Harvest Date	
Population [1]	**18 June**	**17 August**
Smith's falcata (WR)	8.44	3.19
Don (C)	6.10	0.95
Sholty (C)	6.77	1.72
Alfagraze (C)	6.82	2.81
Coiled pod (SD)	7.05	2.19
Mandan 1991 (ARS)	7.67	3.34
6200 HT (C)	6.43	2.95
Sickle pod (SD)	8.92	2.76
5454 (C)	6.91	3.10
Vernal (C)	8.44	3.10
Travois (C)	8.06	3.58
Least significant difference (0.05)	1.76	0.95

[1] WR, commercial increase of seed produced by Norman Smith, Lodgepole, SD; C, Cultivar; SD, South Dakota State University selection for pod shape from GRNG population; ARS, selection for yield and persistence by USDA-ARS, Mandan, ND.

2.2.7. Phenotypic Characteristics of Progeny Related to Landscape Position on the GRNG

Significant differences were found among the five bulks for flower color and growth habit, but not for g dry matter plant^{-1}. Plants derived from seeds collected adjacent to the Smith Ranch and plants high on the landscape (i.e., shoulder position) produced more yellow-flowered plants than bulks collected across swales that had Kentucky bluegrass or desert wheatgrass as co-dominant species (Figure 2). This indicated genetic variation for morphological traits of interest and perhaps for physiological traits associated with spatial environmental variation in soil and vegetation (native vs. predominantly introduced plant communities) related to landscape position on the GRNG [24].

2.2.8. Effect of Population Density on Forage Yield of *M. falcata*

Significant ($p < 0.01$) differences occurred between populations and between plant population densities for forage dry matter yield at Brookings in 2011. Sholty produced 25% more forage (5.6 Mg ha^{-1}) than the TBC population. The 3.0 plants m^{-2} population density produced 30% more forage (5.7 Mg ha^{-1}) than the 1.8 plants m^{-2} population density. Similarly, in 2013, mean dry matter yields were significantly ($p < 0.01$) greater for Sholty (7.6 Mg ha^{-1}) than TBC (5.6 Mg ha^{-1}); however, no difference ($p > 0.05$) was found between the two population densities (6.5 Mg ha^{-1}). The population × plant density was non-significant in both years.

Significant ($p < 0.01$) differences occurred between Sholty and TBC at a plant population density of 2.4 plants m^{-2} in both 2011 and 2013 at Aurora. Mean dry matter yield averaged across the two years was 6.3 Mg ha^{-1} for Sholty and 3.4 Mg ha^{-1} for TBC.

The TBC population had variegated flowers and narrower crowns than Sholty; however, both populations showed no effect of differences in population density on forage yield during the fourth growing season. The yield advantage of Sholty over TBC increased at both locations between the second (1.8 times greater) and fourth growing season (2.1 times greater), indicating, as described by Oakley and Garver [1] the capability of *M. falcata* for abundant forage production at low population densities in mature stands.

3. Perspectives/Implications

As pointed out by Adams [53], N.E. Hansen envisioned yellow-flowered alfalfa (*M. falcata*), introduced from the steppe of Siberia to rangelands in South Dakota, "would hold its own with the native plants found there, and is certainly a work worthwhile".

Adams expanded, "we have fallen heir not only to this vision and challenge, but also to plant materials by which they soon may be realized". It is tempting to conclude that the pattern of naturalization of yellow-flowered alfalfa on the GRNG is an example of exactly what Hansen imagined. Bolton [9] stated, "it was impossible to overestimate the value of Hansen's introductions of alfalfa" to alfalfa breeding in North America.

Keller [45] in his "Wanted: A Paragon for the Range" lauded the value of crested wheatgrass as largely responsible for restoring millions of acres of denuded farmland in the northern Great Plains and Intermountain West [46,54]. However, he pointed out that tracts of rangeland are highly variable aggregations of large numbers of irregular sites, making breeding plants for their improvement difficult. Regarding approaches for plant improvement, Keller noted rangelands in need of improvement are not represented by environments on experiment farms. Therefore, a range-plant breeding program should be conducted on rangelands. He also acknowledged that, in pastures, a grass-legume association is more productive than grass alone and deserved considerable attention.

To accomplish what Keller [45] outlined requires a complex selection screen which subjects several to perhaps many generations to fluctuating abiotic (e.g., drought) and biotic (e.g., cattle grazing, insect feeding) environmental stresses that occur at different times during a growing season, depending on the year. Clearly, populations of alfalfa that have adapted to fluctuating climatic and other environmental stresses, of which Smith's *falcata* and the GRNG population are examples, have considerable promise for immediate interseeeding of rangelands and as breeding stocks for the development of new cultivars for future rangeland and monotypic grassland improvement in the northern Great Plains. They have also demonstrated higher seed production potential in situ (GRNG) and in commercial seed production practices (Smith's *falcata*) than pure *M. falcata* populations. Therefore, they will not likely require additional direct selection for seed production to be commercially viable.

Alfalfa populations developed for improving mature stands of crested wheatgrass and/or depleted rangeland in the northern Great Plains for forage, conservation, and ecological purposes need to be: (1) Well-adapted to the climate of the region, as a result of natural selection; (2) tolerant of grazing, yet capable of producing sufficient amounts of high-quality forage to improve performance of grazing animals; (3) drought-tolerant and winterhardy; (4) able to establish and persist in monocultures of highly competitive grasses, such as crested wheatgrass, from mechanical interseeding and natural recruitment, without curtailing grazing. The commercial seed increase of Smith's *falcata* demonstrated these traits in northwestern SD [23], southwestern ND, and eastern WY [46].

This study found the GRNG population was highly variable, with a genetic variation for morphological traits. However, it also showed that environmental variation may have created genetically different subpopulations (e.g., topographical variation for physical soil characteristics and moisture-holding capacity and the associated native or introduced plant communities) based on a few morphological traits. Therefore, seed increase of the highly heterogeneous GRNG population, as described here, would likely result in rapid genetic shifts depending on where the increase occurred. Therefore, we propose conservation of this highly heterogeneous naturalized [24] cross-pollinating population should employ both in situ and ex situ strategies, similar to what might be prudent for landraces [55]. Since seed production episodes are sporadic, due to unpredictable abiotic and biotic factors, including variation in grazing time and intensity, ex situ methods for standing seed crops may be possible only once or twice during a decade. However, ex situ conservation of the population through a sampling of the soil seed bank could be done at will, as the hard seed characteristic of the population presumably provides long-term storage/viability of seeds in the soil [24]. Clearly, in situ conservation, under the past and current grazing management strategies employed by the U.S. Forest Service, appears to be a viable long-term strategy for the preservation of the genetically heterogeneous germplasm. In addition, it provides for genetic recombination for natural selection and a continuous germplasm source for timely ex situ seed collections. Rincker [56] showed that seed production in widely-spaced plants of alfalfa exceeded that of rows in an environment that was conducive to seed production.

The work of Misar et al. [46], in particular, provides instructive information regarding the value of Smith's *falcata*, and presumably the GRNG population, as well, for interseeding into crested wheatgrass, based on results from >20 year-old stands of this species at four locations in ND, SD, and WY. The degree of interseeding success with Smith's *falcata* was site-specific (e.g., amount of wheatgrass cover, soil texture) and related to cultural practices (e.g., seeding date, seeding rate, and type of herbicide). Schellenberg et al. [57] determined that killing crested wheatgrass in a strip alongside the furrow where seeds were planted was crucial for alfalfa establishment, the wider the strip the better. They also reported similar establishment success and forage yield for Rangelander (*M. media*), a creeping rooted cultivar, and Yellowhead (*M. falcata*), a taprooted cultivar.

The GRNG alfalfa population includes a subpopulation of reproductive individuals from seedlings that are established in swales that support a plant community dominated by alfalfa, crested wheatgrass, and Kentucky bluegrass. The swales were more than likely privately farmed up to the 1930s, but then federally reclaimed and reseeded to wheatgrass for permanent management, predominantly for grazing starting in the 1950s [24]. The swales also contain large soil seed banks of hard alfalfa seed, up to 30,000 viable seeds m^{-2}, compared with a virtual paucity of seeds on shoulder and summit landscape positions. Existing plants and the soil seed bank represent a gene pool, likely a result of multiple generations of genetic recombination, from which to select for establishment and persistence of alfalfa in crested wheatgrass dominated grasslands in the semiarid northern Great Plains. Correspondingly, existing plants and the associated soil seed bank at other landscape positions should provide similar sources, through natural selection, of potentially adapted germplasm.

Since improved establishment, persistence, and recruitment from natural reseeding of alfalfa interseeded in crested wheatgrass or depleted native rangeland are primary plant breeding goals in the northern Great Plains, collecting seeds from surviving alfalfa plants in natural reseeded or successful mechanically interseeded stands, preferably after exposure to several cycles of the appropriate management system, would be a logical approach for initiating breeding programs for the development of new cultivars with specific attributes for solving those particular rangeland problems/needs. Smith's *falcata* [23] and the GRNG population [24] would seem to be good base populations for those situations in the semiarid regions of the northern Great Plains. However, the magnitude of genetic variation among and within families in the GRNG population for dry matter forage production, from initial and regrowth harvests, and morphological traits revealed by this research suggests their potential for the accomplishment of other breeding goals for alfalfa in the semiarid northern Great Plains, as well. A good example was reported by Berdahl et al. [21]. A germplasm derived from open-pollinated bulk seed collected from the Smith Ranch, and two germplasms derived by bulking open-pollinated seeds from stands planted in the early 1920s at Mandan, ND had the highest survival (78%) of 10 entries, including hay- and pasture-types, after grazing by sheep for three growing seasons at Mandan [21]. These three germplasms traced to introductions of *M. falcata* by N.E. Hansen to the Dakotas during the early 1900s.

Interseeding native rangeland with alfalfa in the semiarid northern Great Plains has met with mixed success, based on general assessments made at two [58], five [59], or seven years after planting [17]. Fewer studies have been conducted with alfalfa interseeded into mature (i.e., 20- to 30-year-old) stands of crested wheatgrass in the northern Great Plains [22,57]. The only study that included post establishment stand density data at six years after planting was that by Misar et al. [22]. In addition, a recent reconnaissance (i.e., July 2020) was conducted of the four experimental sites where crested wheatgrass stands greater than 20 years of age were interseeded with the first generation increase of seeds (Wind River Seed Company) of Smith's *falcata* in 2008 [22]. This recent reconnaissance revealed that two of the experimental plantings (Newcastle, WY (43°51′11″ N, 104°12′34″ W) and Hettinger, ND (46°0′3″ N, 102°38′0″ W)) of Smith's *falcata* made in 2008 had expanded beyond the post-establishment analysis in 2014 [22], primarily as a result of notable vegetative spread and crown expansion. These successful stands were achieved under two very different forage management strategies. The Newcastle interseeding has been hayed annually during early summer; whereas, the Hettinger interseeding has been grazed with sheep annually during spring [60]. Recently, interseeding alfalfa into native grassland in the southern High Plains showed initial seedling density and short-term post establishment trends for crown density similar to those reported by Rumbaugh and Thorn [58] in the northern Great Plains. The Texas study indicated that wide row spacing (71 cm) was more desirable than narrow spacing (36 cm) for seed cost, since the two different row spacing converged for crown density within three years after planting [61].

Currently, Smith's *falcata* is providing a valuable commercial seed source for improving native rangeland and mature competitive monotypic perennial cool-season grasses pastures, such as crested wheatgrass, in the northern Great Plains. The GRNG population, derived from Smith's *falcata*, represents further natural selection for the establishment, natural recruitment, and persistence under grazing. To preserve these novel germplasms, we recommend a combined in situ and ex situ conservation strategy similar to that used for the conservation of landraces.

The expansive natural distribution of *M. falcata* in Eurasia traverses 42 to 60° N. latitude and 10 to 80° E. longitude [62,63]. In many regions, indigenous strains provide essential forage for livestock and other ungulates [41]. Recently, more than 200 PIs of *M. falcata* were evaluated at multiple widely spaced locations in diverse environments in the United States and Canada [36]. These evaluations identified promising new genetically diverse *M. falcata* germplasms for potential cultivar development for multiple purposes in North America. It is reasonable to think that the two novel germplasms that became naturalized under grazing in northwestern SD may be of interest for evaluation to improve grasslands dominated by perennial cool-season grasses in steppes and prairies in temperate climates globally.

Author Contributions: Conceptualization, A.B. and K.D.K.; methodology, A.B., J.D.B., M.D.P, and E.C.B.; writing—original draft preparation, A.B.; writing—review and editing, A.B., K.D.K. J.D.B., M.D.P., E.C.B., L.X., and Y.W.; All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported from funding by USDA-NIFA for collaborative Multi-State Research; USDA NIFA Multi-State Research Objective (NE 1010 Breeding and Genetics of Forage Crops to Improve Productivity, Quality, and Industrial Uses). We also recognize funding from US Forest Service Prairie Grasslands, Bismarck, ND, and Lemmon, SD to support seed collection on the Grand River National Grassland.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Oakley, R.W.; Garver, S. *Medicago Falcata, a Yellow-Flowered Alfalfa*; Bulletin 428; United States Department of Agriculture: Washington, DC, USA, 1917.
2. Rumbaugh, M.D. *Hansen's Contributions to Alfalfa Breeding in North America*; Bulletin 665; Agricultural Experiment Station, South Dakota State University: Brookings, SD, USA, 1979.
3. Tysdal, H.M.; Westover, H.L. Alfalfa Improvement. In *1937 Yearbook of Agriculture*; USDA, United States Government Printing Office: Washington, DC, USA, 1937; pp. 1122–1153.
4. Hume, A.N.; Joy, E.; Franzke, C. *Twenty-One Years of Crop Yields from Cottonwood Experiment Farm. South Dakota State College of Agriculture and Mechanic Arts*; Bulletin 312; Agronomy Department, South Dakota Agricultural Experiment Station: Brookings, SD, USA, 1937.
5. Hansen, N.E. *Plant Introductions*; Bulletin 224; South Dakota Agricultural Experiment Station: Brookings, SD, USA, 1927.
6. Hansen, N.E. *Cooperative Tests of Alfalfa from SIBERIA and European Russia*; Bulletin 141; South Dakota Agricultural Experiment Station: Brookings, SD, USA, 1913.
7. Adams, M.W.; Semeniuk, G. *Teton Alfalfa*; Bulletin 469; South Dakota State College Agricultural Experiment Station: Brookings, SD, USA, 1958.
8. Garver, S. *Alfalfa in South Dakota: Twenty-One Years Research at the Redfield Station*; Bulletin 383; South Dakota Agricultural Experiment Station: Brookings, SD, USA, 1946.
9. Bolton, J.L. *Alfalfa: Botany, Cultivation, and Utilization*; World Crops Books; Polunin, N., Ed.; Interscience Publishers, Inc.: New York, NY, USA, 1962.
10. Clark, K.W.; Heinrichs, D.H. *Grass-Legume Mixture Trials;* Canada Department of Agriculture: Ottawa, ON, Canada, 1957.
11. Whitman, W.C.; Carter, J.F.; Rogler, G.A. North Dakota. In *1954 Report of Observations on Dryland Grazing Alfalfas*; Graumann, H.O., Beltsville, M.D., Eds.; USDA-ARS Field Crops Research Branch: Beltsville, MD, USA, 1955; pp. 33–36.
12. Adams, M.W. Grasses and Legumes. In *Progress Report: Agricultural Research at the Range Field Station, South Dakota State College Experiment Station: Cottonwood, S.D.*; Circular 116; Agricultural Experiment Station, South Dakota State College: Brookings, SD, USA, 1955; pp. 28–41.
13. Heinrichs, D.H.; Bolton, J.L. *Rambler Alfalfa*; Publication 1030; Canada Department of Agriculture: Ottawa, ON, Canada, 1958.
14. Heinrichs, D.H. Roamer alfalfa. *Can. J. Plant Sci.* **1967**, *47*, 220–221. [CrossRef]
15. Heinrichs, D.H. Drylander alfalfa. *Can. J. Plant Sci.* **1971**, *51*, 430–432. [CrossRef]
16. Rumbaugh, M.D.; Colburn, J.D.; Semeniuk, G. Registration of Travois alfalfa. *Crop. Sci.* **1964**, *4*, 117. [CrossRef]
17. Berdahl, J.D.; Wilton, A.C.; Frank, A.B. Survival and agronomic performance of 25 alfalfa cultivars and strains interseeded into rangeland. *J. Range Manag.* **1989**, *42*, 312. [CrossRef]
18. Heinrichs, D.H. Developing creeping-rooted alfalfa for pasture. *Can. J. Agric. Sci.* **1954**, *34*, 269–280.
19. Rumbaugh, M.D.; Moore, S.R.; Colburn, J.D. *Travois: An Alfalfa for Grazing*; Bulletin 525; South Dakota State University Agricultural Experiment Station: Brookings, SD, USA, 1965.

20. Boe, A.; Bortnem, R.; Higgins, K.F.; Kruse, A.D.; Kephart, K.D.; Selman, S. *Breeding Yellow-Flowered Alfalfa for Combined Wildlife Habitat and Forage Purposes*; South Dakota Agricultural Experiment Station 727; South Dakota State University: Brookings, SD, USA, 1998.
21. Berdahl, J.D.; Wilton, A.C.; Lorenz, R.J.; Frank, A.B. Alfalfa survival and vigor in rangeland grazed by sheep. *J. Range Manag.* **1986**, *39*, 59–62. [CrossRef]
22. Misar, C.G.; Xu, L.; Gates, R.N.; Boe, A.; Johnson, P.S. Stand persistence and forage yield of 11 alfalfa (*Medicago sativa*) populations in semiarid rangeland. *Rangel. Ecol. Manag.* **2015**, *68*, 79–85. [CrossRef]
23. Smith, N.G. Yellow-blossomed alfalfa on rangeland in South Dakota. *Rangelands* **1997**, *19*, 24–25.
24. Xu, L.; Boe, A.; Johnson, P.S.; Gates, R.N.; Misar, C.G. Distribution and productivity of naturalized alfalfa in mixed-grass prairie. *Am. J. Plant Sci.* **2019**, *10*, 1030–1047. [CrossRef]
25. Townsend, C.E.; Rogler, G.A.; Wilton, A.C.; Lorenz, R.J. Registration of C-6 alfalfa germplasm. *Crop Sci.* **1979**, *19*, 298.
26. Bingham, E.T. Registration of WISFAL alfalfa (*Medicago sativa* subsp. *falcata*) tetraploid gemplasm derived from diploids. *Crop Sci.* **1993**, *33*, 217–218. [CrossRef]
27. Wilton, A.C.; Townsend, C.E.; Lorenz, R.L.; Rogler, G.A. Longevity of alfalfa seed. *Crop Sci.* **1978**, *18*, 1091–1093. [CrossRef]
28. Ries, R.A. Experimental factors and alfalfa persistence in dryland pastures and rangeland. In *Alfalfa for Dryland Grazing*; Lorenz, R.J., Ed.; Agriculture Information Bulletin No. 444; U.S. Department of Agriculture: Washington, DC, USA, 1982; pp. 4–8.
29. Heinrichs, D.H. Creeping alfalfas. *Adv. Agron.* **1963**, *15*, 317–337.
30. Rumbaugh, M.D. Origins of alfalfa cultivars used for dryland grazing. In *Alfalfa for Dryland Grazing*; Lorenz, R., Ed.; U.S. Department of Agriculture Information Bulletin No. 444; U.S. Department of Agriculture: Washington, DC, USA, 1982; pp. 15–19.
31. Rumbaugh, M.D.; Pedersen, M.W. Survival of alfalfa in five semiarid range seedings. *J. Range Manag.* **1979**, *32*, 48–51. [CrossRef]
32. Rumbaugh, M.D. Reseeding by eight alfalfa populations in a semiarid pasture. *J. Range Manag.* **1982**, *35*, 84–86. [CrossRef]
33. Pankiw, P.; Siemens, B. Anik alfalfa. *Can. J. Plant Sci.* **1976**, *56*, 203–205. [CrossRef]
34. McLeod, J.G.; Muri, R.; Jefferson, P.G.; Bittman, S.; McCartney, D. Yellowhead alfalfa. *Can. J. Plant Sci.* **2009**, *89*, 653–655. [CrossRef]
35. Peel, M.D.; Asay, K.H.; Waldron, B.L.; Jensen, K.B.; Robins, J.G.; Mott, I.W. 'Don', a diploid falcata alfalfa for Western U.S. rangelands. *J. Plant Regist.* **2009**, *3*, 115–118. [CrossRef]
36. Riday, H.; Brummer, E.C. Registration of two improved yellow-flowered alfalfa germplasms. *J. Plant Regist.* **2007**, *1*, 131–133. [CrossRef]
37. Hendrickson, J.R.; Berdahl, J.D. Survival of 16 alfalfa populations space planted into a grassland. *J. Range Manag.* **2003**, *56*, 260–265. [CrossRef]
38. Hendrickson, J.R.; Liebig, M.A.; Berdahl, J.D. Responses of *Medicago sativa* and *M. falcata* type alfalfas to different defoliation times and grass competition. *Can. J. Plant Sci.* **2008**, *88*, 61–69. [CrossRef]
39. Hansen, N.E. *The Wild Alfalfas and Clovers of SIBERIA, with a Perspective View of the Alfalfas of the World*; U.S. Department of Agriculture Bureau of Plant Industry Bulletin No. 150; U.S. Department of Agriculture: Washington, DC, USA, 1909.
40. Hume, A.N.; Champlin, M. *Comparative Yields of Hay, from Several Varieties and Strains of Alfalfa, at Brookings, Highmore, Cottonwood, and Eureka*; Bulletin 163; South Dakota Agricultural Experiment Station: Brookings, SD, USA, 1916.
41. Sinskaya, E.N. *Flora of Cultivated Plants of the U.S.S.R. XIII Perennial Leguminous Plants. Part I Translation. 1961*; Israel Program of Scientific Translations: Jerusalem, Israel; U.S. Department of Commerce: Washington, DC, USA, 1950.
42. Markegard, W.; Duckwitz, W.; Jensen, N. *Yellow-Flowered Alfalfa and Cool-Season Grass Mixtures: A Demonstration Planting in Perkins, County, South Dakota*; Final Report Demonstration Planting; Bismarck Plant Materials Center: Bismarck, ND, USA, 2018. Available online: www.blogs.nrcs.usda.gov/Intenet/FSE_PLANTMATERIALS/publications/ndpmcsr13320.pdf (accessed on 1 July 2020).
43. Derscheid, L.A. *1979 Alfalfa Variety Descriptions*; EMC 804; Cooperative Extension Service, Plant Science Department, South Dakota State University: Brookings, SD, USA, 1979.

44. Katepa-Mupondwa, F.; Singh, A.; Smith, S.R., Jr.; McCaughey, W.P. Grazing tolerance of alfalfa (*Medicago* spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (*Bromus riparius* Rehm. syn. *B. biebersteinii* Roem & Schult). *Can. J. Plant Sci.* **2002**, *82*, 337–347.
45. Keller, W. Wanted: A paragon for the range. In *Grass: The Yearbook of Agriculture*; Stefferud, A., Ed.; United States Department of Agriculture, U.S. Government Printing Office: Washington, DC, USA, 1948; pp. 347–351.
46. Misar, C.G.; Xu, L.; Gates, R.N.; Boe, A.; Johnson, P.S.; Schauer, C.S.; Rickertsen, J.R.; Stroup, W.W. Establishment and persistence of yellow-flowered alfalfa no-till interseeded into crested wheatgrass stands. *Agron. J.* **2015**, *108*, 141–150. [CrossRef]
47. Mortenson, M.C.; Schuman, G.E.; Ingram, L.J.; Nayigihugu, V.; Hess, B.W. Forage production and quality of mixed-grass rangeland interseeded with *Medicago sativa* ssp. *falcata*. *Rangel. Ecol. Manag.* **2005**, *58*, 505–513. [CrossRef]
48. Mortenson, M.C.; Schuman, G.E.; Ingram, L.J. Carbon sequestration in rangelands interseeded with yellow-flowering alfalfa (*Medicago sativa* ssp. *falcata*). *Environ. Manag.* **2004**, *33*, S475–S481. [CrossRef]
49. Anower, M.R.; Boe, A.; Auger, D.; Mott, I.W.; Peel, M.D.; Xu, L.; Kanchupati, P.; Wu, Y. Comparative drought response in eleven diverse alfalfa accessions. *J. Agron. Crop. Sci.* **2015**, *203*, 1–13. [CrossRef]
50. Kanchupati, P.; Wang, Y.; Anower, M.R.; Boe, A.; Hu, T.; Wu, J. The *CBF-Like* gene family in alfalfa: Expression analyses and identification of potential functional homologs of Arabidopsis *CBF3*. *Crop. Sci.* **2017**, *57*, 2051–2063. [CrossRef]
51. Dhakal, M.; West, C.P.; Deb, S.K.; Villabolos, C.; Kharel, G. Row spacing of alfalfa interseeded into native grass pasture influences soil-plant-water relations. *Agron. J.* **2020**, *112*, 274–287. [CrossRef]
52. Barnes, D.K. *A System for Visually Classifying Alfalfa Flower Color*; USDA-ARS Agriculture Handbook No. 424; U.S. Government Printing Office: Washington, DC, USA, 1972.
53. Adams, M.W. *Creeping Alfalfa*; South Dakota Farm and Home Research: Brookings, SD, USA, 1956; pp. 74–77.
54. Rogler, G.A.; Lorenz, R.L. Crested wheatgrass-Early history in the United States. *J. Range Manag.* **1983**, *36*, 91–93. [CrossRef]
55. Brush, S.B. In situ conservation of landraces in centers of crop diversity. *Crop. Sci.* **1995**, *35*, 346–354. [CrossRef]
56. Rincker, C.M. Alfalfa seed yields from seeded rows vs. spaced transplants. *Crop. Sci.* **1976**, *16*, 268–270. [CrossRef]
57. Schellenberg, M.P.; Waddington, J.; King, J.R. Sod-seeding alfalfa in spring into established crested wheatgrass in southwest Saskatchewan. *Can. J. Plant Sci.* **1994**, *74*, 293–301. [CrossRef]
58. Rumbaugh, M.D.; Thorn, T. Initial stands of interseeded alfalfa. *J. Range Manag.* **1965**, *18*, 258–261. [CrossRef]
59. Vigil, F.G.; Pollmann, R.; Boe, A. *Travois: An Alfalfa for Grazing*; South Dakota Agricultural Experiment Station Bulletin 526; South Dakota Agricultural Experiment Station: Brookings, SD, USA, 1982.
60. Rickertsen, J.; (North Dakota State University, Fargo, ND, USA). Personal communication, 2020.
61. Dhakal, M.; West, C.P.; Villalobos, C. Establishment and development of alfalfa interseeded into native grass mixture: Cultivar and row spacing effects. *Crop Sci.* **2019**, *59*, 2271–2279. [CrossRef]
62. Lesins, K.; Lesins, I. Diploid *Medicago falcata* L. *Can. J. Genet. Cytol.* **1964**, *6*, 152–163. [CrossRef]
63. Barnes, D.K.; Bingham, E.T.; Murphy, R.P.; Hunt, O.J.; Beard, D.F.; Skrdla, W.H.; Teuber, L.R. *Alfalfa Germplasm in the United States: Genetic Vulnerability, Use, Improvement, and Maintenance*; USDA-ARS Technical Bulletin 1571; USDA-ARS: Washington, DC, USA, 1977.