

Registration of 'Centennial' Sand Bluestem

T. L. Springer,* R. L. Wynia, and G. L. Rea

ABSTRACT

'Centennial' (Reg. No. CV-15, PI 670042) sand bluestem (*Andropogon hallii* Hack.) is a synthetic cultivar selected for greater seed germination at a low water potential and seedling establishment under field conditions. Two cycles of recurrent selection were used to develop Centennial (AB-medium Syn-2) from 'Chet' sand bluestem (AB-medium Syn-0). Cycle 1 consisted of germinating 3500 open-pollinated seeds of population AB-medium Syn-0 in a -0.8 MPa D-mannitol solution for 7 d. All germinated seeds (~250) were selected to create population 'AB-medium Syn-1'. Cycle 2 selection was similar to Cycle 1 except that population AB-medium Syn-1 was used to create population AB-medium Syn-2. Germination of seeds of Centennial was 16.3% higher than for those of Chet in a water potential of -0.8 MPa and 12.8% higher than Chet in deionized water. Seeds from Centennial had significantly greater field emergence (62.7%) than either the AB-medium Syn-1 (59.6%) or Chet sand bluestem (54.6%). Thus, percentage field emergence of sand bluestem was increased by recurrent selection for increased seed germination at a low water potential. Centennial sand bluestem was cooperatively released by the USDA-ARS and USDA-NRCS to provide forage for pasture, hay, or complementary rangeland-forage production systems and to stabilize soils by reclaiming marginal croplands in the central and southern Great Plains of the United States.

SAND BLUESTEM, *Andropogon hallii* Hack., is a warm-season grass indigenous to the Great Plains of North America (Hitchcock, 1951; McGregor et al., 1977). As its common name implies, sand bluestem prefers sandy soils and, except for its creeping rhizomes and villous racemes with grayish to pale golden hairs, it resembles big bluestem, *A. gerardii* Vit. (Hitchcock, 1951). Sand bluestem is used for pasture, prairie restoration, and wildlife habitat, and it has potential as a bioenergy crop (Riley et al., 1992; Stubbendieck and McCully, 1972; Weimer and Springer, 2007). As Harlan and Kneebone (1960, p. 8) stated, "Sand bluestem is one of the most productive native grasses on sandy soils in the southern Great Plains."

Six sand bluestem cultivars have been released since 1955. 'Woodward' was released in 1955 by the Oklahoma and Kansas Agricultural Experiment Stations and by the USDA-ARS (Harlan and Kneebone, 1960). Its superior qualities were leafiness and seed set compared with common sand bluestem. Woodward was adapted to the southern Great Plains in Plant Hardiness Zones (PHZ) 6 and 7. 'Garden' was released in 1960 by the USDA Soil Conservation Service (Hanson, 1972). Garden was a tall, leafy ecotype with good seed yields and was adapted to PHZ 4b. 'Cherry' was released in 1961 by the USDA Soil Conservation Service for its high seed production potential and to provide seed adapted to the Nebraska sandhills (Hanson, 1972). Cherry was adapted to PHZ 4b. 'Elida' was released in 1963 by the New Mexico Agricultural Experiment Station and USDA Soil Conservation Service (Hanson, 1972). Elida's superior characteristics were good foliage extending up the culms, uniformity in seed ripening, and excellent establishment and production in eastern New Mexico. Elida was adapted to PHZ 5. 'Goldstrike' was released in 1973 by the Nebraska Agricultural Experiment Station and USDA-ARS (Alderson and Sharp, 1995). Goldstrike was released for its unique color of the inflorescence which characterizes the variety and resistance to leaf rust [*Puccinia andropogonis* (Schwein.)]. Goldstrike

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5585 Guilford Rd., Madison, WI 53711 USA

*Corresponding author (tim.springer@ars.usda.gov)

T.L. Springer, USDA-ARS, Southern Plains Range Research Station, 2000 18th St., Woodward, OK 73801; R.L. Wynia, USDA-NRCS, Manhattan Plant Materials Center, 3800 S. 20th St., Manhattan, KS 66502; G.L. Rea, USDA-NRCS, James E. "Bud" Smith Plant Materials Center, 3950 FM 1292 STE 100, Knox City, TX 79529. Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply approval to the exclusion of other suitable products.

Abbreviations: DAP, days after planting; PHZ, Plant Hardiness Zone.

is adapted to PHZ 4 and 5. Lastly, 'Chet' sand bluestem was released in 2004 by the USDA-ARS and USDA-NRCS and the Oklahoma Agricultural Experiment Station (Springer et al., 2005). Chet was selected for growth and regrowth, leaf rust resistance, leafiness, seedling vigor, and plant height. Chet is adapted to PHZ 5b, 6, and 7a.

Of the six sand bluestem cultivars outlined above, only Woodward and Chet are adapted to the southern Great Plains (PHZ 6 and 7). Because breeder and certified seed of Woodward are no longer available, Chet was developed and released. Chet sand bluestem is and will continue to be a good replacement for Woodward. Although Chet has good seedling vigor, there was room for improvement in seed germination and seedling vigor. Thus, our goal was to select sand bluestem populations for increased seed germination at low water potentials, leading to improved field emergence and overall improved plant density.

Materials and Methods

'Centennial' sand bluestem (Reg. No. CV-15, PI 670042) was selected for greater seed germination and seedling establishment under field conditions. Populations AB-medium Syn-1 and Syn-2 were developed from AB-medium Syn-0 using two cycles of recurrent selection for increased seed germination at a low water potential (Springer, 2012). Population AB-medium Syn-0 was released as 'Chet' sand bluestem in 2004 (Springer et al., 2005). Chet was selected for improved growth and regrowth, disease resistance, leafiness, seedling vigor, and plant height at the Southern Plains Range Research Station, Woodward, OK (36°25' N, 99°24' W, elevation 586 m asl).

Germplasm

Chaffy seed of Chet sand bluestem (AB-medium Syn-0) was harvested with a Woodward Flail-Vac Seed Stripper (Dewald and Beisel, 1983, Ag-Renewal, Inc.), scalped with a Woodward High Intensity Seed Scalper (Dewald et al., 1983, Ag-Renewal, Inc.), and classified with a Woodward Seed-Material Classifier (Dewald et al., 1983, Ag-Renewal, Inc.). The seed harvesting and cleaning processes produced a product of about 60% chaffy pure seeds (intact spikelets each containing a caryopsis).

The chaffy seeds were further processed in the laboratory with a South Dakota Style seed blower (Seedburo, model 757) having an air valve opening of 30 mm. Seeds were blown in 3-g increments to remove all light and empty chaffy-seed material. Laboratory processing produced $98 \pm 1\%$ chaffy pure seed of population AB-medium Syn-0.

Selection

Cycle 1 of Centennial consisted of germinating 3500 open-pollinated chaffy pure seeds of population AB-medium Syn-0 in deionized water having a water potential of -0.8 MPa for 7 d (Springer, 2005). One hundred seeds were placed in 35 sterile, clear plastic boxes (11.0 by 11.0 by 3.5 cm; Tri-State Plastics, Inc.) on two layers of absorbent paper towel substrates moistened with 17 mL of a water potential solution of D-mannitol (Sigma-Aldrich Co.) at -0.8 MPa (29.2 g of D-mannitol in 0.5 kg of deionized water). The germination environment consisted of 8 h d^{-1} of fluorescent light (light: fluorescent; photosynthetic photon flux density = $9.0 \mu\text{mol m}^{-2} \text{ s}^{-1}$) at 30°C and 16 h d^{-1}

of darkness at 20°C in a germination chamber (Stultz Scientific Engineering Corp.). Seeds were considered germinated if the seedling radicle and shoot were at least 1 mm long. After 7 d, all seedlings were removed, washed with deionized water, planted into 64-cell cavity trays containing a soil mix, and maintained in the greenhouse until field planting. The resulting population was designated as AB-medium Syn-1. Plants of AB-medium Syn-1 were transplanted into a field isolation plot at the Southern Plains Range Research Station on a Carey silt loam soil (fine-silty, mixed, superactive, thermic Typic Argiustolls) spaced 1.1 m apart in a 15 by 15 grid planting. Seeds of AB-medium Syn-1 were harvested and cleaned as described above under "Germplasm."

Cycle 2 selection consisted of germinating 3500 open-pollinated pure seeds of population AB-medium Syn-1 in deionized water having a water potential of -0.8 MPa for 7 d as outlined above. After 7 d, all seedlings were removed, washed with deionized water, planted into 64-cell cavity trays containing a soil mix, and maintained in the greenhouse until field planting. Field transplanting, harvesting, and cleaning of seeds were similar to those outlined above. This population was designated as AB-medium Syn-2.

During the establishment phase of each population outlined above, plots were maintained weed-free by cultivation, and hoeing and were irrigated on an as needed basis. In the years following establishment, plots were burned in March, and atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) was applied 7 to 14 d later for weed control at $1.68 \text{ kg a.i. ha}^{-1}$ and nitrogen (N) was applied in the form of urea (46-0-0) at 70 kg N ha^{-1} in April. The plots were not irrigated following the establishment year.

Laboratory Seed Germination

In October 2004 and 2005, seed was harvested from populations AB-Medium (Syn-0, Syn-1, and Syn-2) using the seed stripper and processed (as outlined previously) to obtain a 98% chaffy, pure-seed product.

In December 2005, four samples of 50 intact sessile spikelets of each of the three sand bluestem populations were weighed and placed in sterile, clear plastic boxes (7.0 by 7.0 by 2.5 cm; Tri-State Plastics, Inc.) on two layers of absorbent paper towel substrates moistened with 7 mL of either deionized water (0 MPa) or a water-D mannitol solution at -0.8 MPa and placed in the germination chamber under conditions outlined above. The experimental design was a factorial arrangement of year of seed harvest, sand bluestem populations (AB-medium Syn-0, Syn-1, and Syn-2), and water potential treatment (0 and -0.8 MPa) in a randomized block design with four blocks, and the experiment was repeated twice. Cumulative germination counts were made daily for 7 d, with treatments (germination boxes) within a block being randomized after each daily count. Germination data were converted to percentages before analysis. The root and shoot length of three randomly selected germinated seedlings from each germination box were measured on the fourth day of each germination experiment and averaged within an experiment.

Seed germination data were analyzed using a general linear mixed model analysis of variance (PROC GLIMMIX)

with blocks within year and germination evaluations (runs) as random effects and year as a repeated measure using SAS software (Littell et al., 1996; SAS Institute, 2010). Fixed effects were sand bluestem populations (AB-medium Syn-0, Syn-1, or Syn-2), water potential treatment (0 and -0.8 MPa), and their associated interactions. The dependent variables were 50-sessile spikelet weight, 7-d cumulative seed germination, and 4-d seedling root and shoot lengths.

Field Establishment

Field testing involved planting the three populations (AB-medium Syn-0, AB-medium Syn-1, AB-medium Syn-2) in a randomized complete block design replicated four times. The plot size was 2 by 10 m, and the seeding population was equivalent to 108 pure live seeds m^{-2} . Testing was conducted at three locations: (i) USDA-ARS, Southern Plains Range Research Station, Woodward, OK, on an Eda loamy fine sand (mixed, thermic Lamellic Ustipsamments) soil; (ii) USDA-NRCS, Manhattan Plant Materials Center, near Manhattan, KS ($39^{\circ}12' N$, $96^{\circ}35' W$, elevation 325 m asl), on a Haynie silt loam (coarse-silty, mixed, superactive, calcareous, mesic Mollic Udifluvents) soil; and (iii) USDA-NRCS, James E. "Bud" Smith Plant Materials Center, near Knox City, TX ($33^{\circ}35' N$, $100^{\circ}02' W$, elevation 503 m asl), on an Altus fine sandy loam (fine-loamy, mixed, superactive, thermic Pachic Argiustolls) soil. Seedling counts (plants m^{-2}) were determined using the frequency grid method of Vogel and Masters (2001) at 30, 60, and 90 d after planting (DAP). Count data were used to calculate the percentage field emergence and plant density. Plantings were made on or near 1 May in 2008 to 2010, and a pre-emergence application of atrazine [2-chloro-4-ethylamino-6-isopropylamino-s-triazine] for weed control was applied at 2.2 kg ha^{-1} .

Plant density data were analyzed using a general linear mixed model analysis of variance (PROC GLMMIX) with block, location, year of planting, and their interactions as random effects and number of DAP as a repeated measure (Littell et al., 1996; SAS Institute, Inc., 2010). Location and year of planting were classified into random effects to broaden the inference of the experiment (Gbur et al., 2012). In this experiment, fixed effects were sand bluestem populations (AB-medium Syn-0, Syn-1, or Syn-2), DAP (30, 60, and 90), and their interactions. Comparisons of means were made using an adjusted Tukey's procedure at $P \leq 0.05$ (SAS Institute, 2010).

Field emergence data were analyzed using a general linear mixed model analysis of variance (PROC GLMMIX) with block, location, year of planting, and their interactions as random effects and number of DAP as a repeated measure (Littell et al., 1996; SAS Institute, 2010). Location and year of planting were classified into random effects to broaden the inference of the experiment (Gbur et al., 2012).

Characteristics

Centennial sand bluestem was cooperatively released by the USDA-ARS and USDA-NRCS on 21 Oct. 2013 to provide forage for pasture, hay, or complementary rangeland-forage production systems and to stabilize soils by reclaiming marginal croplands in the central and southern Great Plains of the United States. The superior characteristics of Centennial are greater seed germination and seedling establishment under field conditions. Centennial was tested under the experimental designation AB-medium Syn-2.

Laboratory Seed Germination

Germination of seed from AB-medium Syn-1 was 7.3% higher than that of AB-medium Syn-0, and AB-medium Syn-2 was 9.0% higher than that of AB-medium Syn-1 in a water potential of -0.8 MPa (Table 1). After two selection cycles, germination of seed from generation Syn-2 was 16.3% higher than those of generation Syn-0 in a water potential of -0.8 MPa ($P < 0.01$, Table 1).

Germination of seed in deionized water or a water potential of 0 MPa between the generations Syn-0 and Syn-1 was not different (0 MPa, $P > 0.30$, Table 1). Germination of seed from the generation Syn-2 was 6.6% higher than that of generation Syn-1 in deionized water (0 MPa, $P < 0.25$, Table 1). After two selection cycles, germination of seeds in deionized water (0 MPa) from generation Syn-2 was 12.8% higher than generation Syn-0 ($P < 0.05$, Table 1).

Selecting seed that germinated at a water potential of -0.8 MPa resulted in a synthetic population (AB-medium Syn-2) that averaged a $7.8 \pm 0.6\%$ increase in seed germination with each selection cycle. Although further increases in germination percentage may be possible in additional generations (Syn-3, etc.), there may be appreciable inbreeding during selection in small populations that could result in inbreeding depression if the trait is affected by such (Falconer 1981). Law and Anderson (1940) reported a 60 to 71% reduction in leaf area and loss of

Table 1. Least square means \pm SE for sand bluestem generations (AB-medium Syn-0, AB medium Syn-1, and AB-medium Syn-2), at two water potential treatments (-0.8 and 0 MPa) for dependent variables 7-d seed germination and 50-spikelet weight. AB-medium Syn-0 was released as 'Chet' sand bluestem in 2004 and AB-medium Syn-2 was released as 'Centennial' sand bluestem in 2013.

Water potential	Main effects Cultivar, generation†	Variable	
		7-d seed germination	50-spikelet weight
MPa		%	mg
-0.8	AB-medium Syn-0 (Chet)	21.8 ± 13 a‡	232 ± 19 a
	AB-medium Syn-1	29.1 ± 13 b	222 ± 19 a
	AB-medium Syn-2 (Centennial)	38.1 ± 13 c	258 ± 19 b
0	AB-medium Syn-0 (Chet)	49.6 ± 20 a	234 ± 23 a
	AB-medium Syn-1	55.7 ± 20 ab	221 ± 23 a
	AB-medium Syn-2 (Centennial)	62.3 ± 20 b	252 ± 23 b

† Syn-0, base population; Syn-1, first generation after selection; Syn-2, second generation after selection.

‡ Mean \pm SE followed by the same letter within water potential treatment are not significantly different ($P \leq 0.05$, F-test).

plant vigor for inbred lines of big bluestem compared with their open-pollinated sibs. They also reported similar reductions for numbers of culms per plant, maximum height, basal area, and seed set in inbred lines.

Variation in 50-sessile spikelet weight for both water potential treatments was attributable only to generation ($P < 0.05$). When the statistical model was modified to include the effects of water potential treatment, no water potential treatment or its associated interactions with generation occurred ($P > 0.67$ for water potential treatment; and $P > 0.69$ for the interaction of generation and water potential treatment). Thus, the 50-sessile spikelet weights among the three bluestem populations and water potential treatments were similar. When means from water potential treatments were pooled, Syn-0 seed (233 ± 4 mg) was equal in weight to Syn-1 seed (223 ± 4 mg), and Syn-0 and Syn-1 seed weighed significantly less ($P < 0.05$) than Syn-2 seed (255 ± 4 mg).

After two selection cycles at a negative water potential, spikelet weight and associated caryopsis weight in sand bluestem were increased. Harlan and Ahring (1960) reported that a caryopsis weight of sand bluestem is 0.54 times that of the spikelet. Thus, caryopsis weight can be calculated from the 50-sessile spikelet weight data reported herein by dividing 50-sessile spikelet weight by 50 and multiplying the quotient by 0.54. Kneebone and Cremer (1955) found that seed (caryopses) size affected the seedling vigor of sand bluestem but not seed germination. In their experiment, they used caryopses that averaged >1.80 mg per caryopsis. Springer (1991) observed similar findings for big bluestem for caryopses averaging >1.55 mg. Both studies revealed that caryopsis weight had minimal effect on seed germination. However, the ability of a seed to germinate at reduced water potentials as described herein is physiological in nature. The Syn-0 and Syn-1 generations, with similar spikelet weights, germinated differently at a water potential of -0.8 MPa, with seed germination significantly greater for Syn-1 compared with Syn-0 (Table 1). Likewise, the germination of Syn-2 seed was significantly greater than that of seed of the Syn-1 generation. Syn-2, however, had greater mean spikelet weight than either Syn-0 or Syn-1. If Kneebone and Cremer's (1955) and Springer's (1991) hypotheses that caryopsis weight has little or no influence on seed germination is correct, then the significant increase in seed germination from generation Syn-0 to generation Syn-1 is the result of changes in the physiological processes of seed germination.

Field Establishment

Seed from population AB-medium Syn-2 had significantly greater field emergence (62.7%) than either the AB-medium Syn-1 (59.6%) or AB-medium Syn-0 (54.6%) generations, and the AB-medium Syn-1 generation had significantly greater emergence than generation AB-medium Syn-0 (Table 2).

Percentage field emergence of sand bluestem varied with generation of selection ($P < 0.01$), and number of DAP ($P < 0.05$). Sand bluestem populations (AB-medium Syn-0, Syn-1, and Syn-2) did not interact with number of DAP ($P > 0.47$). Thus, percentage field emergence of sand bluestem was increased by recurrent selection for increased seed germination at a low water potential.

At 30 DAP, field emergence averaged $51.8 \pm 5.3\%$. This was significantly lower than at 90 DAP, which averaged $65.3 \pm 5.3\%$ ($P < 0.05$; Table 2). Field emergence at 30 and 60 DAP did not differ ($P = 0.05$), nor did percentage emergence differ at 60 and 90 DAP ($P = 0.05$). Thus, sand bluestem field emergence was greatest at approximately 60 DAP.

The plant density of sand bluestem plots varied only with generation of selection ($P < 0.01$) and number of DAP ($P < 0.05$). Generation AB-medium Syn-2 had significantly greater plant density compared with either generation AB-medium Syn-1 or AB-medium Syn-0 (Table 2), and generation AB-medium Syn-1 had significantly greater plant density compared with generation AB-medium Syn-0 (Table 2).

Launchbaugh (1966) reported that plantings with ≥ 10 seedlings m^{-2} could be classified as good; thus, the plant densities reported herein are considered good. Springer and Gillen (2007) reported the optimum plant density for sand bluestem at Woodward, OK, was 10.8 plants m^{-2} . This density provided the best forage yield with the highest forage quality. Rapid germination and early establishment of seedlings is important to the success of field plantings when moisture and temperatures are optimum (Shaidae et al., 1969). Selecting for seed that germinated at lower water potentials resulted in plant populations with larger caryopses and higher seed germination (Springer, 2012). Seed from selected populations also produced greater field emergence, resulting in greater plant populations (Springer et al., 2012). Thus, Centennial sand bluestem was selected for its higher seed germination at a low water potential and improved seedling establishment under field conditions.

Availability

Centennial is a stable, random mating population improved for increased seed germination and seedling emergence. It is adapted to USDA Plant Hardiness Zones 5b, 6, and 7a of the Central and Southern Great Plains of the United States. Four classes of seed (breeder, foundation, registered, and certified) are recognized for Centennial sand bluestem. Breeder seed will be produced and maintained by USDA-ARS, Southern Plains Range Research Station at Woodward, OK, for a period of 10 years. Foundation seed will be produced and maintained by

Table 2. Least square means \pm SE for sand bluestem generations (AB-medium Syn-0, AB medium Syn-1, and AB-medium Syn-2), and for number of days after planting (DAP) for dependent variables field emergence and plant density. AB-medium Syn-0 was released as 'Chet' sand bluestem in 2004 and AB-medium Syn-2 was released as 'Centennial' sand bluestem in 2013.

Main effects	Field emergence	Plant density
	%	plants m^{-2}
Cultivar, generation		
AB-medium Syn-0 (Chet)	54.6 ± 4.7 a†	14.4 ± 1.2 a
AB-medium Syn-1	59.6 ± 4.7 b	15.7 ± 1.2 b
AB-medium Syn-2 (Centennial)	62.7 ± 4.7 c	16.6 ± 1.2 c
DAP		
30	51.8 ± 5.3 a	13.7 ± 1.4 a
60	59.8 ± 5.3 ab	15.8 ± 1.4 ab
90	65.2 ± 5.3 b	17.2 ± 1.4 b

† Dependent variable means followed by the same letter within main independent effect are not significantly different ($P \leq 0.05$, Tukey's test).

the USDA–ARS, Southern Plains Range Research Station at Woodward, OK, the USDA–NRCS, Manhattan Plant Material Center at Manhattan, KS, and the USDA–NRCS James E. “Bud” Smith Plant Materials Center at Knox City, TX, for a period of 10 years. Certified seed production of Centennial will be limited to a single generation and can only be marketed as Centennial. Certified seed production will also be limited to USDA Hardiness Zone 5b, 6, or 7a in its known adaptation range in the Central and Southern Great Plains region. Seed of Centennial has been deposited in the USDA–ARS National Plant Germplasm System (NPGS). Appropriate recognition is requested if this release contributes to the development of new breeding lines or cultivars.

Centennial was named to signify the first 100 yr (1913–2013) of USDA agricultural research at Woodward, OK. Selection and testing of Centennial was accomplished through a cooperative effort of USDA–ARS and USDA–NRCS.

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