3-4-2013

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Published March 4, 2013

GENETIC STOCK

Registration of N614, A_3N615, N616, and N617 Shattercane Genetic Stocks with Cytoplasmic or Nuclear Male Sterility and Juicy or Dry Midribs

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ABSTRACT

Four shattercane [*Sorghum bicolor* subsp. *drummondii* (Nees ex Steud.) de Wet ex Davidse] genetic stocks—N614 (Reg. No. GS-652, PI 665684), A_3N615 (Reg. No. GS-651, PI 665683), N616 (Reg. No. GS-653, PI 665685), and N617 (Reg. No. GS-654, PI 665686)—with A_3 cytoplasmic male sterility or the nuclear male sterility gene ms_3 containing either juicy (*dd*) or dry (*DD*) culms were developed jointly by the USDA-ARS; the Iowa Agricultural and Home Economics Experiment Station, College of Agriculture and Life Sciences, Iowa State University; and the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska. The stocks were released in July 2011. The source material for these genetic stocks was isolated from an archetypical shattercane population found near Lincoln, NE. Release of these genetic stocks makes available shattercane lines with both A_3 cytoplasmic male sterility, and ms_3 genetic (nuclear) male sterility to facilitate crossing. These genetic stocks also contain juicy (*dd*) or dry (*DD*) culms, a visible genetic marker to facilitate screening progeny resulting from crosses. The genetic stocks have immediate application for basic research involving gene flow from cultivated sorghum [*Sorghum bicolor* (L.) Moench] to shattercane and on the fitness of offspring resulting from such crosses.

The weed shattercane [*Sorghum bicolor* subsp. *drummondii* (Nees ex Steud.) de Wet ex Davidse] can have a major economic impact on agriculture. In highly infested fields, yield reductions for maize and soybean can be as much as 85% (Fellows and Roeth, 1992; Hans and Johnson, 2002), however shattercane can currently be controlled by cultural and mechanical methods and through the use of herbicides (Curran and Lingenfelter, 1999).

The recent advancements in technologies to develop herbicide resistance for cultivated sorghum have resulted in patent applications filed for sorghum with resistance to several herbicides: acetolactate synthase (ALS) inhibitors (Tuinstra and Al-Khatib, 2008), acetyl-CoA carboxylase (ACCase) inhibitors (Tuinstra and Al-Khatib, 2010), and dinitroanilines (Clement, 2010). Advances in plant transformation technologies have also made possible the development of sorghum with new nutrition-enhancing traits (Zhao, 2007), and these technologies also hold promise for development of insect- (Girijashankar et al., 2005) and disease-resistance traits (Indra Arulselvi et al., 2010; Kosambo-Ayoo et al., 2011).

Shattercane and cultivated sorghum are both species of *Sorghum bicolor* and are expected to be fully interfertile; hence, the genes or transgenes conferring potentially advantageous traits, such as resistance to herbicides, diseases, or insects, to cultivated sorghum are expected to be rapidly introgressed into shattercane populations. Previous introgressions of genes from cultivated sorghum into its weedy relatives shattercane and Johnsongrass (*Sorghum halepense* L. Pers.) have been well documented (Morrell et al., 2005). Several factors influence the gene-flow rate, including the dispersal kernel containing the new gene, the frequency of introductions, and the fitness of the individual carrying the new gene in the recipient population (Andow and Zwahlen, 2006). The dispersal kernel is affected by the proximity of the pollen parent to the recipient plant, mechanisms of pollen dispersal, size of the pollen cloud, synchrony of flowering, reproductive compatibility between parents, and production of viable seed (Halsey et al., 2005; Auer, 2008; Craig et al., 2008; Sosnoskie et al., 2009). To predict outcomes from gene flow

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Published in the Journal of Plant Registrations.
doi: 10.3198/jpr2012.09.0033crgs
Received 18 Sept. 2012. Registration by CSSA.

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between cultivated sorghum and shattercane populations, it is desirable to have shattercane lines with male-sterility-inducing systems to evaluate the hybrid progeny and a readily visible marker-gene system to study pollen flow. Experiments that monitor pollen flow use a pollen source containing the dominant or codominant genetic marker at a central location and surveillance plants containing the recessive genetic marker at various distances from this pollen source. For example, Jhala and coworkers used such an experimental design to determine the rate of outcrossing at given distances from the pollen source (Jhala et al., 2011). The release of the four shattercane genetic stocks—N614 (Reg. No. GS-652, PI 6656834) A_3N615 (Reg. No. GS-651, PI 665683), N616 (Reg. No. GS-653, PI 665685), and N617 (Reg. No. GS-654, PI 665686)—with cytoplasmic (A_3) and nuclear male sterility (*ms3*) and/or dry (white midribs; *DD*) or juicy (green midribs; *dd*) culms provide needed tools to facilitate such research and to develop management strategies.

Materials and Methods

The source material for genetic stocks N614, A_3N615, N616, N617 was a roadside collection of an archetypical shattercane population made near Lincoln, NE in 2004. The population consisted of approximately 20 individuals. This shattercane population was segregating for juicy culms (*dd*) and dry culms (*D*_). N614 and A_3N615 were developed concurrently; A_3N615, by crossing the original shattercane selection (one of the 20 individuals) to the A_3 cytoplasm source A_3N243 sudangrass [*Sorghum bicolor* subsp. *drummondii* (Steud.) de Wet ex Davidse], followed by six backcrosses to the shattercane line while advancing the N614 selected line through concurrent generations of self-pollination. N616 and N617 were developed via two additional selections from the source population. Those two selections were crossed to a source of the nuclear male-sterility gene ms_3, NP35 sudangrass, followed by six generations of backcrossing to their respective shattercane line while advancing the selected line through concurrent generations of self-pollination. For N616 and N617, male sterility was evaluated in the generation following self-pollination of the final backcross generation (Table 1). To avoid seed loss due to shattering, seeds of these genetic stocks were collected before shattering and the rachis was covered by a pollination bag.

N614, A_3N615, N616, N617 were evaluated in studies conducted in Nebraska and Iowa in 2009. The Nebraska experiment was conducted at the University of Nebraska Field Laboratory, Ithaca, NE. Plots consisted of single 6.6-m rows spaced 76 cm apart, which were replicated five times in a randomized block design. Each plot consisted of 120 seeds per row (240,000 seed ha^{-1}) delivered by a cone planter. The experiment was planted 21 May 2009. Nitrogen fertilizer was applied at 112 kg ha^{-1} before planting. Atrazine [6-chloro-*n*-ethyl-*N*′(1-methylethyl)-1,3,5-triazine-2,4,diamine] was applied at 1.12 kg ha^{-1} immediately after planting, followed by an application of quinclorac (3,7-dicholoro-8-quinolinecarboxylic acid) and atrazine at 0.37 kg ha^{-1} and 0.56 kg ha^{-1}, respectively, approximately 14 d postemergence. Supplemental irrigation (3.8 cm) was applied via overhead sprinklers on 6 and 29 August 2009. The number of seedlings emerged per plot were counted and the average seedling height recorded 23 June. Days to 50% anthesis was estimated and recorded as the lines approached maturity. At anthesis, male-sterile and male-fertile plants were scored visually based on the ability of their anthers to shed pollen from 10 representative plants per plot. On 19 August the following traits were measured on 10 representative plants in each plot: midrib color (white

Table 1. Comparisons of days to 50% anthesis, ratio of male-sterile to fertile plants, and midrib color for shattercane genetic stocks and sudangrasses.

Entry	Days to 50% anthesis†	Pairwise comparisons‡			Sterile: fertile ratio†	Pairwise comparisons‡			Midrib color§
		N614	Greenleaf	Piper		N614	Greenleaf	Piper	
					Ithaca, NE				
N616	79	ns	*	*	0.25	*	*	*	Green
N617	79	ns	*	*	0.34	*	*	*	White
A_3N615	79	ns	*	*	∞				White
N614	79				0				White
Greenleaf	83				0				Green
Piper	81				0				White
					Ames, IA				
N616	85	ns	ns	*	0.21	*	*	*	Green
N617	85	ns	ns	*	0.25	*	*	*	White
A_3N615	86	ns	ns	*	∞				White
N614	85				0				White
Greenleaf	86				0				Green
Piper	83				0				White
					Combined locations				
N616	82	ns	*	ns					
N617	82	ns	ns	*					
A_3N615	82	ns	*	ns					
N614	82								
Greenleaf	84								
Piper	82								

*Significant at $P \leq 0.05$.

†Least squares means.

‡Single-degree-of-freedom tests made between controls and A_3N615, N616, and N617 with the LSMEANS/DIFF statement; ns, not significant.

§Determined visually.

or green), height (to top of panicle), diameter of stem at the first elongated internode above the soil, width of the first leaf below the flag leaf at its widest point, and number of tillers per plant.

The IA experiment was conducted at the Iowa State University Department of Agronomy Station, Ames, IA. Plots consisted of single 6.9-m rows spaced 76 cm apart, which were replicated five times in a randomized block design. Plots were hand seeded to 120 seeds per row (240,000 seeds ha^{-1}); planting was delayed until 21 June due to heavy rainfall. Nitrogen fertilizer was applied at 56 kg ha^{-1}. No herbicides were used and weed control was accomplished by hand hoeing. The number of seedlings emerged per plot and the seedling height were not recorded. All other traits were measured on 15 and 16 September as described above.

N614, a direct selection from the source shattercane population and considered to be representative of that population, was used for comparison in experiments. 'Greenleaf' (PI 659692; Karper, 1955) and 'Piper' (PI 658665; Smith et al., 1973) sudangrasses were also used as controls for comparison and to provide a basis for estimating relative values to readers. Experiments at both locations were randomized complete blocks and data were analyzed using the PROC MIXED procedure of SAS/STAT (SAS Institute, 2008). Replications were considered random variables. Least square means for A_3N615, N616, and N617 and control lines were generated and single-degree-of-freedom tests were made between controls and A_3N615, N616, and N617 using the LSMEANS/DIFF statement at $P \leq 0.05$.

Characteristics

Four shattercane genetic stocks (N614, A_3N615, N616, and N617) were developed to study gene flow between shattercane and cultivated sorghum. These stocks were grown in two environments (Ithaca, NE and Ames, IA) in 2009 along with two sudangrasses, Greenleaf and Piper, for comparison. N614 was grown as a representative control for shattercane. The numbers of seedlings per plot were scored approximately 4 wk after planting at Ithaca, NE location (Table 2). The number of seedlings per plot for A_3N615, N617, and N614, ranging from 85 to 94 seedlings from 120 seeds planted, which were significantly higher than the 56 and 61 seedlings per plot for the sudangrasses Piper and Greenleaf. For N616, there were 65 seedlings per plot, which was not significantly different from Piper or Greenleaf (Table 2). Together these results indicated that these stocks have good seed establishment. Four-weeks after planting, all four shattercane stocks had statistically similar seedling heights, ranging from 21 to 23 cm and all were significantly shorter than Greenleaf (28 cm) and Piper (30 cm) (Table 2). All four stocks reached 50% anthesis at 82 d, which was based on the combined means from the two locations (Table 1).

To facilitate crossing, these genetic stocks contain either A_3 cytoplasmic male sterility (A_3N615), or *ms3* genetic (nuclear) male sterility (N616 and N617). N616 and N617 are maintained as segregating populations for *ms3* through crossing the male-sterile plants with fertile shattercane plants within the population. The progeny from heterozygous plants *Ms3*/*ms3* segregated with the expected 1:3 male-sterile:male-fertile ratio for both stocks at both locations (Table 1). No fertile plants were observed at either location for A_3N615, which is consistent with A_3 cytoplasmic male sterility (Table 1). All plants were fertile for N614 and sudangrasses across both environments (Table 1). A_3N615 is maintained through crossing with N614 as the recurrent pollen parent. In addition, N614, N616, and N617 restore fertility in A_1 cytoplasm (data not shown).

All four genetic stocks have brown caryopses, tightly clasping black glumes, and awns and are shattering. In addition, these four stocks also have the purple wound response, *P* (Doggett 1988). The four genetic stocks have either juicy (*dd*; N616) or dry (*DD*; N614, A_3N615 and N617) culms to use as a visible genetic marker, which is easily scored by observing the leaf midrib phenotype in progeny from these genetic stocks. Recessive juicy culms (*dd*) cause leaf midribs to appear green, whereas dominant dry culms (*DD*) cause midribs to appear white (Doggett 1988), results that were consistently observed at both locations (Table 1). The easily visible genetic marker of leaf midrib color has application for tracking gene flow, by indicating pollen parent of the progeny.

At anthesis, the plants were scored for height, stem diameter, leaf width, and number of tillers. For the four genetic stocks, plant height ranged from 212 to 225 cm, based on means across both environments (Table 3). N617, the tallest, was significantly taller than N614, and the other two stocks were not significantly different from N614. Stem diameters for these stocks ranged from 10.1 to 12.3 mm based on measurements at both locations, which were all significantly greater than the diameters of the sudangrasses Greenleaf and Piper, 9.0 and 8.9 mm, respectively (Table 3). N616 had the largest diameter, which was significantly greater than that of N614, and the two other stocks were

Table 2. Comparisons of number of seedlings per plot and seedling height for shattercane genetic stocks and sudangrasses.

Entry	Seedlings per plot†	Pairwise comparisons‡			Seedling height†	Pairwise comparisons‡		
		N614	Greenleaf	Piper		N614	Greenleaf	Piper
	no.				cm			
N616	65	*	ns	ns	21	ns	*	*
N617	85	ns	*	*	23	ns	*	*
A_3N615	86	ns	*	*	22	ns	*	*
N614	94				21			
Greenleaf	61				28			
Piper	56				30			

*Significant at $P \leq 0.05$.

†Least squares means.

‡Single-degree-of-freedom tests made between controls and A_3N615, N616, and N617 with the LSMEANS/DIFF statement; ns, not significant.

similar to N614. Leaf width ranged from 44 to 50 mm, which was similar to N614 for all three stocks based on both locations (Table 3). The leaves of the genetic stocks were significantly wider than those of Greenleaf or Piper, which were 28 and 33 mm, respectively. The number of tillers per plant ranged from 0.99 to 1.56 for the four genetic stocks based on combined locations (Table 3). N616 had the greatest number of tillers for these shattercane stocks at 1.56, which was significantly greater than for N614, and the other two genetic stocks were similar to N614. All four genetic stocks had significantly fewer tillers than either Greenleaf or Piper, which had 5.43 and 5.23, respectively, based on combined locations (Table 3). Overall, the plant architecture of these shattercane genetic stocks is distinct from that of sudangrass, the source of the male sterility. These stocks have broad leaves, thick main stalks, and few tillers, in contrast with the sudangrasses, which have narrow leaves, thinner stalks, and multiple tillers.

Conclusion

The release of these genetic stocks makes shattercane lines with both A_3 cytoplasmic male sterility and ms_3 genetic (nuclear) male sterility available to facilitate crossing or monitor pollen flow. Homozygous stocks for juicy (*dd*) or dry (*DD*) culms, easily observed, visible genetic marker, will provide a means to follow the paternity in progeny from crosses involving these genetic stocks. The genetic stocks have immediate application for basic research involving gene flow from cultivated sorghum [*Sorghum bicolor* (L.) Moench] to shattercane and the fitness of progeny resulting from such crosses.

Availability

Seed of these genetic stocks will be maintained and distributed by the USDA-ARS, Grain, Forage, and Bioenergy Research Unit, Department of Agronomy, University of Nebraska, Lincoln, Nebraska 68583-0937, and will be provided without cost to each applicant on written request. Seeds of these released genetic stocks have been deposited in the National Plant Germplasm System, where it will be immediately available for research

Table 3. Comparisons of height, stem diameter, leaf width and number of tillers at maturity for shattercane genetic stocks and sudangrasses.

Entry	Height at maturity†	Pairwise comparison‡			Stem diameter†	Pairwise comparison‡			Leaf width†	Pairwise comparison‡			Tillers†	Pairwise comparison‡		
		N614	Greenleaf	Piper		N614	Greenleaf	Piper		N614	Greenleaf	Piper		N614	Greenleaf	Piper
	cm				mm				mm				no.			
Ithaca, NE																
N616	236	ns	*	*	14.4	*	*	*	46	ns	*	*	2.70	*	*	*
N617	246	ns	*	*	12.1	ns	*	*	43	ns	*	*	2.22	ns	*	*
A_3N615	235	ns	*	*	12.8	ns	*	*	46	ns	*	*	1.58	ns	*	*
N614	240				13.1				47				1.82			
Greenleaf	218				10.6				29				6.20			
Piper	257				10.5				32				6.34			
Ames, IA																
N616	197	ns	*	ns	10.2	*	*	*	51	ns	*	*	0.42	ns	*	*
N617	203	*	*	ns	8.2	ns	ns	ns	46	*	*	*	0.33	ns	*	*
A_3N615	189	ns	*	ns	8.5	ns	*	*	53	ns	*	*	0.44	ns	*	*
N614	187				8				51				0.16			
Greenleaf	174				7.4				27				4.66			
Piper	202				7.2				35				4.12			
Combined locations																
N616	217	ns	*	*	12.3	*	*	*	48	ns	*	*	1.56	*	*	*
N617	225	*	*	ns	10.1	ns	*	*	44	ns	*	*	1.28	ns	*	*
A_3N615	212	ns	*	*	10.7	ns	*	*	50	ns	*	*	1.01	ns	*	*
N614	214				10.6				49				0.99			
Greenleaf	196				9				28				5.43			
Piper	229				8.9				33				5.23			

*Significant at $P \leq 0.05$.

†Least squares means.

‡Single-degree-of-freedom tests made between controls and A_3N615, N616, and N617 with the LSMEANS/DIFF statement; ns, not significant.

purposes, including development and commercialization of new varieties and cultivars. It is requested that appropriate recognition be made if this germplasm contributes to the development of a new breeding line, variety, or cultivar.

Acknowledgments

We thank Patrick O'Neill for his technical assistance with statistical analyses of data presented in this manuscript. We also thank Dr. Jeffrey D. Wolt, BIGMAP and Agronomy Department, Iowa State University for facilitating Material Transfer and Research Agreements between USDA-ARS and Iowa State University, and Dave Starret, manager of Iowa Agricultural and Home Economics Experiment Station for providing land and logistical support. The USDA prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. USDA is an equal opportunity provider and employer.

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