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CULTIVAR

Registration of 'Silver River' Sweetclover

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Abstract

'Silver River' (Reg. No. CV-6, PI 678795) is a rust-resistant cultivar of white-flowered, annual sweetclover (*Melilotus albus* Medik.) adapted to southern and central Texas. Sweetclover rust (*Uromyces striatus* Schroet.) causes a range of plant disease symptoms, including leaf drop, reduced seed and forage yield, and premature plant death. The breeding and evaluation of Silver River for rust resistance was conducted at Beeville, TX, under severe epiphytotics of sweetclover rust. Two cycles of mass selection at Beeville were used to improve the rust resistance of a sweetclover PI line from Uruguay. The original PI population had 21% rust-resistant plants. Silver River averaged 91% resistant plants at Beeville in 2014 and 2015, compared with 'Hubam', which had a 2-yr average of 7% resistance. Silver River is similar to Hubam in forage yield and maturity. This new cultivar will improve the reliability of annual sweetclover in cattle grazing systems and wildlife supplemental forage plantings in southern and central Texas.

Cultivated sweetclover exists as two species of the genus *Melilotus* Adans. Yellow-flowered sweetclover [*M. officinalis* (L.) Lam.] is biennial in growth habit with strong cold tolerance and is well adapted to northern climates. White-flowered sweetclover (*M. albus* Medik., syn. *M. alba* Desr.) has both biennial and annual forms and is useful as a forage and cover crop from southern Texas to Canada (Smith and Gorz, 1965). These two species of *Melilotus* and seven additional, primarily biennial species are native to Eurasia, specifically the area from Central Europe east to Tibet (Isely, 1954). Both species of cultivated sweetclover were introduced into the eastern United States prior to the mid-1700s and were important forage and cover crops on calcareous soils in Alabama and Mississippi by1856 (Westgate and Vinall, 1916; Duggar, 1925).

In Texas, a combination of disease and insect problems, confounded by potential for summer drought, severely limits the use of biennial sweetclover (Coffey et al., 1957). Additionally, biennial sweetclover produces less forage in the establishment year and has a later maturity relative to annual types (Trew and Hoveland, 1955). Annual white-flowered sweetclover is usually fall planted and, if allowed to mature, will produce seed in the following late spring to midsummer. Therefore, annual sweetclover can escape the damage caused by diseases such as cotton root rot [*Phymatotrichopsis omnivore* (Duggar) Hennebert] and root-boring insects. All traits considered, the annual growth form of sweetclover is better adapted to Texas agriculture than the biennial form.

Annual white-flowered sweetclover has been used for pasture, hay, and soil improvement and is very well adapted to the blackland and prairie soils of the Southern Great Plains that extend through central Texas (Trew and Hoveland, 1955). Sweetclover was used widely in Texas prior to the 1950s, when inexpensive nitrogen fertilizer reduced the use of all forage legumes. Animal health concerns related to dicoumarol toxicity also contributed to the decline of sweetclover as a hay crop (Gorz and Haskins, 1969). Dicoumarol is a blood-thinning agent formed from the natural compound coumarin (*o*-hydroxycinnamic acid β-D-glucoside) by fungal metabolism in moldy sweetclover hay or

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Abbreviations: DAP, days after planting; IAES, Iowa Agricultural Experiment Station; PVP, Plant Variety Protection; RCBD, randomized complete block design.

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spoiled sweetclover silage. Livestock that ingest sweetclover hay or silage containing dicoumarol may die due to uncontrolled internal or external bleeding. Dicoumarol toxicity is not a concern unless some form of stored forage (hay, silage, haylage, etc.) has a high sweetclover content and fungal mold is present. Cattle or other ruminant animals grazing sweetclover are not at risk for these health concerns (Smith and Gorz, 1965).

'Hubam' annual sweetclover (Wilkins, 1922) was an immediate success after its discovery by H.D. Hughes at the Iowa Agricultural Experiment Station (IAES) almost 100 yr ago. This new annual sweetclover was found growing wild in areas previously planted to biennial white sweetclover. After several preliminary trials and reports, >45,000 seed samples of Hubam were distributed by IAES in 1920. Sweetclover rust (*Uromyces striatus* Schroet.; Stuteville, 2002) was noted to be a problem on Hubam sweetclover in southern and central Texas in the late 1990s and early 2000s (G.W. Evers and W.R. Ocumpaugh, personal communication, 2000). Wilting, leaf drop, premature plant death, and orange uredinia on leaves were symptoms noted on rust-infected sweetclover. A breeding program was initiated in 2003 to develop rust-resistant annual sweetclover.

'Silver River' (Reg. No. CV-6, PI 678795) is a rust-resistant cultivar of white-flowered, annual sweetclover adapted to southern and central Texas. The name Silver River was chosen based on the collection of PI line 404798 near the Rio de la Plata in Uruguay. This sweetclover population, collected in 1975 by Langford and Burson (USDA-ARS, 2016a), with variation for rust resistance is the parental population of Silver River. Sebastian Cabot explored this region in 1526 and was the first to use the name Rio de la Plata, "River of Silver." The Cabot expedition of 1526 departed from Sanlucar de Barrameda, Spain (Murphy and Coye, 2013). It is very likely that various grass and legume hays from southern Spain, including sweetclover, were used as animal fodder for this and many other European explorations of South America. Naturalization of these forage legumes near the Rio de la Plata is a logical extension of their introduction by European expeditions to the New World. Both annual and biennial white-flowered sweetclover were widely used in world agriculture in the early 1900s and could have been introduced to Uruguay through commercial seed sales or scientific seed exchanges.

Methods

In October 2003, sweetclover seedlings from the core PI subset (USDA-ARS, 2016b) of *M. albus* were transplanted at the Texas A&M AgriLife Research Station, Beeville, TX, for rust evaluation. All sweetclover seedlings were inoculated at planting with alfalfa (*Medicago sativa* L.) and sweetclover inoculant containing *Sinorhizobium meliloti*. This study included 40 plants each of 24 PI lines, arranged in unreplicated rows. The concept of using subsets (core collections) of large PI collections as an evaluation tool was first described by Brown (1989) and expanded by Beuselinck and Steiner (1992).

Sweetclover rust was noted at Beeville on 17 May 2004, and the 24 PI lines were rated for rust reaction (data not reported). All but four PI lines were completely susceptible to rust, and the lines that did show plant-to-plant variation for rust infection were all biennial types and did not flower in 2004.

In fall 2004, a second experiment was designed to further evaluate the rust resistance of the sweetclover lines identified as variable in rust reaction in the previous year. The entries included were PI 109523, PI 372725, PI 108646, PI 404798, PI 404799, and Hubam. The PI line 404798 was included because it was collected in Uruguay on the same collection expedition as PI line 404799. Seedlings were started in the greenhouse at the Texas A&M AgriLife Research and Extension Center at Overton, TX, in September 2004 and transplanted to the field in Beeville in early November 2004. Seedlings were inoculated at planting using alfalfa and sweetclover inoculant. The five lines were space planted in rows (plants 1 m apart and rows 2 m apart) and arranged in a randomized complete block design (RCBD) with four replications. There were 20 individual plants per line in each replication, with the exception of Hubam, which had five plants per replication. Phosphorus and potassium fertilizer was applied according to soil test, and weeds were controlled using plastic mesh as a weed barrier.

In 2005, rust was noted on sweetclover at Beeville on 5 May. Notes on plant growth and rust reaction were taken on 26 May 2005 (Table 1). Rust reaction was scored using the standard rating scale developed for alfalfa, but with a minor modification (Stuteville, 1991). Rust reaction ratings used were as follows: resistant (R1) = no rust uredinia present on leaves or stems, moderately susceptible (S3) = low density of rust uredina present, susceptible (S4) = rust uredinia present, and highly susceptible (S5) = high density of rust uredinia present on all leaves. The resistant (R2) designation used in alfalfa was not used in these studies. The PI lines 109523, 404799, 372725, and 108646 did not flower in 2005 and were classified as biennials in this study, confirming results from the previous year. Both PI 404798 and Hubam were in full bloom or past full bloom on 27 May, confirming their classification as annuals.

Rust reaction rating for each reaction class was calculated for each entry in each replication by a ratio of plants rated R1 to total plants evaluated. The data were transformed using the inverse sine function (Steele and Torrie, 1960) and an ANOVA calculated using the GLM procedure in SAS (SAS Institute, 2016). Rust resistance (R1) of each sweetclover entry ranged from 78 to 0 (Table 1). Twenty-four percent of the PI 404798 plants were resistant (R1) to rust. The other four PI lines were higher ($P > 0.05$) in rust resistance but were less useful in our breeding program due to their biennial form. Both Smith (1927) and Clarke

Table 1. Rust reaction ratings of sweetclover entries at Beeville, TX, in 2005.

Entry	Rust reaction rating†			
	R1	S3	S4	S5
	%			
Hubam	0c‡	35ab	59a	6b
PI 108646	64ab	24ab	12b	0b
PI 109523	78a	11b	11b	0b
PI 372725	53b	25ab	22b	0b
PI 404798	24c	54a	22b	0b
PI 404799	51b	22ab	11b	16a

† R1, resistant, no rust uredina present; S3, moderately susceptible, low density of rust uredina present; S4, susceptible, rust uredina present; S5, highly susceptible, high density of rust uredina present.

‡ Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's protected LSD.

(1935) concluded that sweetclover growth form was dependent on a single locus with the alleles *A* and *a*, with annual expressing complete dominance. The recessive allele *a*, conditioning the biennial form when homozygous, would require progeny testing to eliminate from a breeding population. All Hubam plants in 2005 were rust infected, with ratings of S3, S4, or S5, and showed symptoms of disease stress, including early leaf drop and poor vigor. The four other PI lines evaluated in this study exhibited different patterns of rust reaction and could be useful in future breeding efforts.

Four elite plants of PI 404798, rated R1 for rust resistance; annual growth habit and excellent forage potential were selected and open-pollinated seed was harvested in mid-June 2005. Mass selection with no pollination control was used due to the timing of the rust epiphytotic, the onset of flowering, and the intent to harvest as much seed as possible from the elite selections.

The four half-sib line selections from 2005 were grown at Beeville in 2006. One line was eliminated on the basis of low vigor and low forage ratings. The three remaining lines were isolated from other sweetclovers but were allowed to intercross. Open-pollinated seed from three half-sib lines was bulked to produce TX-SC-RR. The TX-SC-RR experimental sweetclover line was grown in isolation at Uvalde, TX, in 2007 to 2008, and a seed increase was made. Good spring forage production was noted under limited irrigation (T.D.A Forbes, personal communication, 2008). Seed was produced and reseeding was noted in fall 2008. Seed was harvested from this planting in 2009 and 2010 and designated TX-SC-RR breeder seed. Germination tests (AOSA, 2010) were conducted on the 2009 and 2010 seed, and the hardseed content was 37 and 44% , respectively. This level of hardseed content indicates the high potential of Silver River for natural reseeding. No comparative data on reseeding were collected in this series of studies.

Data Collection

Silver River was evaluated in four separate trials for forage production, and two of these same trials were used to document rust resistance. In all four trials, seedlings were inoculated with alfalfa and clover inoculant containing *Sinorhizobium meliloti*. A growth-rate study that included Hubam and Silver River sweetclover was conducted at Overton in 2010 to 2011. Each entry was seeded at 14 kg ha^{-1} in 1.2-m × 8.5-m plots and arranged in a RCBD with three replications. Planting date was 19 Oct. 2010. Subplots (1.2 × 1.2 m) were sampled over time (14 April = 177 d after planting [DAP], 5 May = 198 DAP, 26 May = 219 DAP, 16 June = 240 DAP) to evaluate flowering, shoot height, and forage dry matter yield. The number of plants within subplots ranged from 16 to 28. The stage of floral development was rated using a similar system to the numerical stage ratings developed for alfalfa by Kalu and Fick (1981).

Six experimental lines of sweetclover from the Texas A&M AgriLife Research sweetclover breeding program were compared with Hubam sweetclover in 2010 to 2011 at Beeville. Entries were planted on 19 Oct. 2010 in 1.2-m × 4.5-m plots with four replications, arranged in a RCBD. The seeding rate was 12 kg ha^{-1}. All entries were harvested on 25 Apr. 2011 and dry forage yield was calculated. Additional harvests were not made due to drought conditions.

Hubam and Silver River sweetclover were planted at Texas A&M Agrilife Research at Beeville in March 2014. The two entries were planted in 6-m rows at 0.5 g seed m^{-1} arranged in a completely random design with three replications. Notes on plant growth and rust reaction were taken on 1 July 2014. Seventy-five plants per row were scored for rust reaction as follows: resistant (R1) = no rust uredinia present on leaves or stems, susceptible (S) = rust uredinia present. Rust resistance rating was calculated for each entry in each replication by a ratio of plants rated R1 to total plants evaluated. The data were transformed using the inverse sine function (Steele and Torrie, 1960), and an ANOVA was calculated using the GLM procedure in SAS. A forage harvest was taken from each row plot on 2 July 2014. A 1-m subsample was harvested from each row, leaving stubble of 4 cm to simulate a hay harvest. Each sample was dried, and dry matter forage yield was calculated for each plot. An ANOVA was calculated using the GLM procedure in SAS. This study was repeated at Beeville in 2015. Silver River experimental and Hubam sweetclover were planted in 6-m rows 30 Apr. 2015 with four replications arranged in a completely random design. Rust was noted on the sweetclover on 15 July 2015 and evaluated on 20 July 2015. A 1-m subsample was harvested from each row plot on 4 Aug. 2015. Each sample was dried, and dry matter forage yield was calculated for each plot. The rust evaluations, data transformations, and analysis were the same as in 2014.

Characteristics

Rust Resistance

In 2014, rust was noted at Beeville on Hubam sweetclover on 17 June. Diseased plants were submitted to the Texas Plant Disease Diagnostic Laboratory. Rust was identified as *Uromyces striatus* on the basis of morphological characteristics of urediniospores (globoid with two to five equatorial pores, 18–25 ×19—23 μm). Silver River (88%) had a greater (P < 0.01) rust resistance rating than Hubam (12%). In 2015, rust was noted on sweetclover at Beeville on 15 July , and entries were evaluated on 20 July. Silver River (94%) had a greater (P < 0.01) rust resistance rating than Hubam (3%).

Forage Yield, Growth Rate, and Flowering Comparisons

Dry forage yield at Overton from 177 to 240 DAP is shown for both sweetclover lines in Table 2. Forage growth was rapid for both Hubam and Silver River during this late-spring period (mid-April–mid-June). Hubam showed a moderate yield advantage (P < 0.01) over Silver River at the last harvest in mid-June. Both lines demonstrated excellent forage growth in this study, with final forage yields >7000 kg ha^{-1}. Plant heights tripled from mid-April to mid-June for both sweetclover lines (Table 2). Plant heights in April started at about 30 cm and increased to almost 90 cm by mid-June. Hubam was about 10 cm taller (P < 0.01) than Silver River in the June measurement. No differences (P > 0.05) in floral development were detected between Hubam and Silver River in this study. In mid-April and early May, most plants of both lines were vegetative. Early flowering was evident by late May, and by mid-June, both Hubam and Silver River were in full bloom.

Table 2. Dry forage yield, shoot height, and morphological stage of sweetclover entries at Overton, TX, in 2011.

	Days after planting			
	177	198	219	240
Entry	Forage yield			
	kg ha^{-1}			
Silver River	2189a†	2921a	7848a	7259a
Hubam	1711a	2228a	6877a	8636b
	Shoot height			
	cm			
Silver River	33.3a	32.6a	66.2a	79.1a
Hubam	26.2a	36.2a	69.1a	88.7b
	Morphological stage‡			
Silver River	2.2a	2.7a	5.8 a	6.1a
Hubam	2.0a	2.3a	5.5 a	6.4a

† Means within a column and data category followed by the same letter are not significantly different according LSD (0.05).

‡ Stage 2: late vegetative, no buds, flowers or seed pods; Stage 3: early bud, one or two nodes with buds, no flowers or seedpods; Stage 4: late bud, three or more nodes with buds, no flowers or seed pods; Stage 5: early flowering, one node with one open flower, no seed pods; Stage 6: late flowering, two or more nodes with open flowers, no seed pods; Stage 7: early seed pod, one to three nodes with green seed pods.

In one harvest at Beeville in spring 2011, Hubam produced more ($P < 0.01$) dry forage than Silver River. These early-season forage yields were 1402 and 1662 kg ha^{-1} for Silver River and Hubam, respectively. No harvests were made after April 2011 due to record drought conditions.

A forage harvest for the 2014 Beeville study was taken on 2 July. The average forage dry matter yields of Hubam and Silver River sweetclover were 7449 and 7945 kg ha^{-1}, respectively. The forage yields of these two entries were not different ($P = 0.72$) according to the ANOVA. The 2015 Beeville sweetclover study was harvested on 4 August. The average forage dry matter yields of Hubam and Silver River sweetclover were 4002 and 2971 kg ha^{-1}, respectively. The forage yields of these two entries were not different ($P = 0.24$) according to the ANOVA.

Conclusions

Silver River annual sweetclover has similar growth and flowering traits to Hubam, but with the addition of excellent resistance to sweetclover rust. The addition of this disease resistance trait extends reliable forage production of this plant into areas of southern and central Texas where sweetclover rust can be a severe problem. Silver River will be a valuable addition to cover crop and wildlife seed mixtures and to improved pastures in southern and central Texas and other regions of the United States where annual sweetclover is adapted.

Availability

Foundation seed of Silver River will be maintained by the Texas Foundation Seed Service, Texas A&M AgriLife Research, 11914 Hwy 70 South, Vernon, TX 76384. Application will be made for US Plant Variety Protection (PVP). Seed of Silver River has been deposited in the National Center for Genetic Resources Preservation and will be available for distribution to the public after the expiration of PVP.

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