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Breeders Work Group

Developing Legumes for use in Forage and Biomass Production Systems

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Introduction

Identification and development of introduced or native legumes with forage, biomass or nitrogen producing potential continues to be a priority need (USDA ARS legume workshop, Chicago, IL, 2004). Traditional forage legumes, such as white clover, red clover, and alfalfa continue to be important forage crops and may provide direct biomass production potential or utility as a companion crop and nitrogen source for grass based biomass production systems. Numerous plant introductions of both native and introduced legume species are available for evaluation. However, most applied breeding programs do not have the resources to systematically evaluate large numbers of these species for their agronomic or biomass potential. The legume program at El Reno, Oklahoma, has focused primarily on alfalfa, white clover and bundleflower with lesser emphasis on red clover and *Trifolium rubens*.

Grazon P+D Tolerant White Clover Research

This project was initiated in response to a request from the Louisiana Cattleman's Association for research and development of a Grazon tolerant white clover for use in pastures. This research was funded in part by the Louisiana Cattleman's Association.

Phase I of the project was to identify plants that demonstrated tolerance to the herbicide Grazon P+D. The parent population was 1964 LA S-1 breeder's seed. Thirty-three white clover plants were identified as having possible tolerance to Grazon P+D and transplanted to pots for maintenance.

Plants that had survived Grazon P+D exposure in tissue culture were moved to El Reno, Oklahoma on July 15, 2003. During the 2003/2004 season, plants were maintained in the greenhouse and propagated by stolons to increase the number of plants. On 17 March, 2004, four randomly assigned replications of each surviving plant were established in an isolated nursery at the USDA-ARS-Grazinglands Research Center. Cross-pollinated seed was harvested separately from each plant at El Reno between June 7 and June 30, 2004.

Seed from each of these plants was used to start progeny plants during the 2004/2005 greenhouse season. A total of 1800 plants were grown in the greenhouse. Nine hundred of these plants were sent to Winnsboro, Louisiana, for establishment and evaluation and the remainder was transplanted at El Reno. Three replications with 2 blocks per replication were established at each location. After establishment, one block of each replication at each location was treated with Grazon P+D and evaluated for survival. There were no surviving plants at Winnsboro but after three spray events there were surviving plants at El Reno. Survival ranged from a high of 6.5 (GR94) to a low of 0.5 plants per row (Osceola). Mean survival of sprayed plants was 3.6 plants per row compared to the unsprayed survival rate of 9.4 plants per row. At Winnsboro, although all plants treated with Grazon P+D exhibited effects from the treatment, part of the lack of survival may have been due to extremely dry conditions. The non-sprayed plants were

exhibiting drought symptoms soon after treatment and failed to survive. On January 23 and 25, 2006, surviving plants from the El Reno location were transferred from the field trial to the greenhouse. The control cultivars, LA-S1 and Osceola, had survival rates of 20 and 6.7%, respectively. The best selected line had a survival rate of 46.7% after being sprayed with Grazon P+D at three separate intervals. A field nursery of the plants was established on April 27, 2006. Due to the extremely dry and hot summer at El Reno no seed was produced in 2006. However, with irrigation plants have survived well and were flowering profusely by April 2007. Seed will be obtained from individual plants and bulked to produce a synthetic population for further evaluation.

Evaluation of methodology for production of alfalfa synthetic populations

Alfalfa yields increased slowly rate for many years. Performance of synthetic populations has been known to decrease with subsequent generations of seed increase and is presumable the result of inbreeding. This study was designed to determine the degree of self pollination among selected clones and the relative impact of this self pollination on population performance.

Two alfalfa populations were developed over a period of 10 years beginning with 8 surviving plants selected in 1993 from a large alfalfa trial at Winnsboro, Louisiana. After several cycles of crossing and full sib evaluations at several locations in Louisiana, 31 clones were selected – 15 from population A and 16 from population B – for development of synthetic populations and production of progeny families for future evaluation.

To produce synthetic seed, 32 replications of all 31 clones were established at El Reno, OK, on 17 – 18 March 2004. Seed was harvested from individual plants for four replications in 2005 and 2006. The remaining seed was bulked. During 2004/2005 all 31 clones were grown in the greenhouse and the relative seed set from self pollination was determined for each clone.

Four replications with 10 plants per row were established on 15 May 2006 for half-sib progeny evaluation of each of the 31 parent clones. Self progeny was included from 10 of the 31 parent clones. These latter clones were selected on basis of viable self seed production. Populations for evaluation were formed by taking equal amounts of seed from clones based on self seed production, field seed production, and a height and weight index. These populations are being compared to a control synthetic derived from bulked seed of all 31 clones. Four replications of each population were seeded in 1 meter rows on 25 Sep 2006.

The result of analysis of the first year's seed production in the field revealed no correlation between germination and seed yield. It was anticipated that clones with a greater capacity for producing self-pollinated seed would also produce more seed per clone in the field. However, this was not the case and a comparison of clonal field seed production with the level of observed self pollination and seed set from greenhouse evaluation did not demonstrate any correlation between the two. The percentage of seed contribution was based entirely on in-field seed production which was highly variable. All progeny rows were harvested on 1 August and 14 September to determine yield. There was no difference among entries for plant survival and there was no harvest by entry difference for plant weight. The range in mean weight per plant varied from a high of 181.4 g (HS209) to a low of 61.8 (S115). Most of the self progeny lines did not perform well. However, S204 was not different from the top yielding half-sib line (158.4 g). Evaluation of these lines will continue in 2007 and harvest of synthetic populations will be initiated in spring of 2007.

Bundleflower evaluation and selection for companion cropping with switchgrass

On 6 May 2004, 1000 space plants, derived from a common source of Illinois bundleflower and from seed collected along the Pease River near Vernon, Texas, were transplanted to a filed nursery at El Reno, Oklahoma. Plants were spaced 2' on center within rows ands rows were 2' on center.

Seed was harvested from selected plants beginning on 2 August 2004. Seed was harvested at weekly intervals until 26 August. Ten plants were selected at each harvest date resulting in 50 plants representing 5 maturity ranges. Plants were visually selected for color, upright growth habit and overall vigor. Selections from Pease River were the latest maturing and comprised the last group harvested.

On 9 June 2005, replicated progeny rows from the 50 plants identified in 2004, were transplanted into two replications of 10 plants each. Plants were 2' on center within rows and rows were 2' on center. On 20 July and 2 August, 2006, Plants were evaluated for maturity, height, and survival. Thirteen lines were selected and seed was harvested and bulked by row and family. Seed from the tallest line, BF50 (Pease River) was used to establish a companion planting with switchgrass in April 2007. Depending upon results from this study, seed may be increased from this line for further evaluation.

Trifolium rubens evaluation

Ten accessions of *Trifolium rubens* (L.) were evaluated for morphological, reproductive, and agronomic diversity. Available seed, provided by the Regional Plant Introduction Station, Pullman, WA, ranged from 30 to 100 seed per accession. Seedlings from each accession were established at El Reno, OK, on 5 May 2004. Establishment year plant survival (95%) and two year survival on 5 April 2006 (83.5%) were not different among accessions. Differences were observed among accessions for all vegetative characters including height, vigor, growth habit and leaf characteristics. Dry matter yield per plant averaged 67.1 g and ranged from 48.6 to 86.6 g (542923 and 325507, respectively). Neutral detergent fiber varied among accessions, averaging 492 g kg^{-1} and ranging from 469 (255396) to 505 g kg^{-1} (542847). Harvest dry matter, crude protein, and acid detergent fiber were not different. Flowering was determinate and flowering date, percent plants in flower, and seed production varied among accessions. Seed per plant ranged from 0 to 0.6g per plant in the establishment year (accessions 255396 and 314123 respectively) and from 0 to 27.5 seed per flower in 2005 (accessions 255396 and 314123, respectively). Overall survival and the useful variation observed indicate agronomic potential for this species.

Superior plants were identified from 2 accessions and seed increased for evaluation of replicated progeny rows at 3 locations. Four replications of 10 plants per replication were established in April and May of 2007.