Selection of Turf Type and Seed Production in Inland Saltgrass (*Distichlis spicata*)

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1. *Determine turf performance of 7 elite CSU-USGA lines, 7 elite University of Arizona lines, 7 Great Basin lines.*
2. *Determine seed production of 7 elite CSU-USGA lines.*
3. *Evaluate Kopec collection and Northern Great Plains collection.*
4. *Determine the relative chromosome number of elite clones.*
5. *Study the viability and germination requirements of inland saltgrass seed.*

In the evaluation of selected lines for turf performance the Great Basin lines from Utah showed winterkill and are being replaced by selected lines derived from seed collected from Modoc, California. The clones A138 & A55 had the highest shoot densities and overall turf appearance while the CSU-USGA had the lowest shoot densities with the exception of C10 & C8. The 20 inch deep boxes have contained the clones after 3 years despite the report that saltgrass rhizomes and roots have been reported to reach 8 foot in depth.

Seed production of the 7 elite CSU-USGA lines was observed to be high as compared to last year. The application of a burn treatment to ½ of the plots during the last 2 years did not appear to result in differences from those plots not burned. Although seed production was high in all lines but accurate assessment was not possible as seed shattered.

The evaluation of the 190 clones in our germplasm nursery demonstrated that saltgrass is not a poor seed producer as indicated in the literature as many genotypes had good seed production. Flower production was high in 2000 during a drought when buffalograss, blue grama, crested wheatgrass and bermudagrass plots were browning and only saltgrass remained green. All single crosses among 15 females and 11 males selected for high number of racemes, apparent rust resistance and short height were made. These crosses will be evaluated in the coming years.

We are continuing to make chromosome counts for new collections and accessions selected for parents in the breeding program. The pattern of broad regional separation of plants with chromosome numbers of $2n=4x=38$ and $2n=4x=40$ reported previously remains unchanged with additional counts from plants collected in Colorado, Utah, Kansas, and Arizona. It is interesting to note that although plants with ca. 74 chromosomes occur at a frequency of greater than 10% in our collections from regions of both 38- and 40-chromosome plants, within-region variations of these tetraploid numbers, except for B chromosomes and one intermediate (56 chromosomes), have not been observed.

A pilot study was conducted to investigate the relationship of leaf blade angle to the region of origin of the plants as delimited by the distribution of tetraploid chromosome numbers of either 38 or 40. Saltgrass plants with 40 chromosomes and polyploids found in the same region have been generally observed to have more horizontally-oriented leaves than plants with 38 chromosomes or polyploids within the same region which are more verticle. Although leaf angle varies within these regions there appears to be a general relationship that will be investigated further in the future.

An evaluation of mechanically scarified seed as compared to hand scarifed seed showed no differences in germination but improved germination as compared to nonscarifed seed in laboratory tests. Field tests were mixed with a low vigor seed source having greater germination of nonscarifed seed while high vigor seed showing similar results as laboratory tests. It is possible that the low vigor seed had greater disease in the field when excessive moisture was observed during the early days of seed germination.

Annual Report of Selection of Turf Type And Seed Production in Inland Saltgrass

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This report will present progress according to the objectives as presented in the original proposal.

OBJECTIVES 1: Determining turf performance of 7 elite CSU-USGA clones, and 13 University of Arizona clones, and Salt Lake seed source. The Salt Lake seed source showed some winterkill at Ft. Collins, and is being replaced by a Modoc, California seed source. Lines A138 (a parent who has previously been shown to be a turf type by greenhouse work) and A55 show the highest shoot densities and best overall turf appearance. The elite 7 (C strains selected by Robin Cuany) with the exception of C10 and C8, show the lowest shoot densities. The South half of the plots were mowed at 50 cm and the north half at 75 cm with no differences in densities observed between the 2 heights as compared to differences in 1999. Regrowth varies between genotypes. Mowing injury(where reel blade did not make a clean cut) was observed in all plots and was variable. Short genotypes exhibited minimal injury. The 20 inch deep boxes contained clones even after 3 years, which is surprising, since communication (Julie Etra, Western Botanical Services) is that rhizomes and roots reach 8 foot down.

OBJECTIVE 2: Determining the range of stress tolerance (drought, salinity) present in inland saltgrass. Information on this will be forthcoming directly from University of Arizona.

OBJECTIVE 3: Determining seed production of 7 elite CSU-USGA lines. Compared to last year, seed production was high. Half the plots were burned fall 1999, and the results with the control were variable. It may be that the burn treatment applied to part of one plant may stimulate the entire plant to flower, overriding the control part. The high rainfall in 1999(over 20 inches) shattered seed, making it impossible to determine relative seed set. This is contrary to communication with Comstock Seed, Reno, Nevada, where they collect seed in wet years. This is 2 out of 2 years where high seed production followed fall burning, yet a burn study on potted plants preceding vernalization showed no effect. Burning is used in cool season grasses to increase vernalization(personal communication, Bob Shearman, University of Nebraska), and floral induction. Nitrogen may also play a role, by increasing the number of growing points in the fall. Saltgrass appears to have a high vegetative response to nitrogen, an effect Dr. Qian is studying. Vernalization exeriments as noted in the semi-annual report were negative. This completes this study.

OBJECTIVE 4: Evaluate Colorado(includes Kopec), Northern Great Plains, Great Basin and Northern California collections. A germplasm nursery of 190 clones, replicated twice, was established in 1998 at the HRC, Fort Collins. An additional 15 clones from a northern California seed accession were established in 1999. The elite 7 selected by Robin Cuany from 100 accessions in the 1980s, along with the Colorado accessions, show the most vigor, followed by the Northern Great Plains, Great Basin, and Northern California. Flower production was much higher in 2000 than in 1999, even though we were in a drought from September 1999, through August 2000(about 4 inches of precipitation with normal at 10 inches). At a time when buffalograss,
blue grama, crested wheatgrass, and bermudagrass plots were browning from drought, only saltgrass remained green.

Contrary to the literature which reports saltgrass as a poor seed producer which mainly reproduces through rhizomes, the nursery shows many genotypes with good seed production. Clearly there are environmental effects which trigger flowering, but vernalization experiments conducted in winter 1999-2000 had no positive results. In this nursery, the southern half of each plant was burned in October of 1999, with mixed treatment results on flowering in 2000. However, many genotypes flowered heavily (over 100 culms per sq ft.). All single crosses possible were made between the 15 females and 11 males selected for high number of racemes, apparent rust resistance, and short height. In addition, all seeded females were harvested from open pollination in 1999 and 2000.

A database (including 1999 and 2000 individual plant digital photos) is being formed from measured traits in order to analyze and publish correlated response to selection for high number of racemes.

OBJECTIVE 5: Evaluation of seed germination and seedling vigor of all crosses. This part of the research is in the planning stage and will proceed this fall and winter.

OBJECTIVE 6: Evaluate RAPD as a means of identifying unique genotypes of saltgrass. This part of the work will proceed this winter.

OBJECTIVE 7: Determine the relative chromosome number of elite clones.

This work has been done by Mr. Scott Reid as part of his MS degree and he has summarized that on the following pages.

Chromosome counts and cytogenetic analysis of the saltgrass germplasm collection.

We are continuing to make chromosome counts for new collections and accessions selected for parents in the breeding program. The pattern of broad regional separation of plants with chromosome numbers of 2n=4x=38 and 2n=4x=40 reported previously remains unchanged with additional counts from plants collected in Colorado, Utah, Kansas, and Arizona. The one unique plant we have examined, originating in the otherwise 38-chromosome region of northern Colorado, has 56 chromosomes (figure 1). This plant may have originated as the result of a naturally occurring cross between a 38-chrosome and a ca. 74-chromosome individual, both of which have been identified as occurring in the area from which the 56-chromosome plant was collected. The chromosome number in this plant represents the number that would be obtained from the combination of gametes obtained by a regular reduction (halving) in meiosis from plants of these two different ploidy levels. It is also possible that the origin was strictly from within one ploidy level of plants. Fusion of an unreduced gamete with a nominally normal gamete among 38-chromosome plants, with the loss of one chromosome during gamete formation or elimination of one chromosome during embryogenesis, is a possible mechanism of origin from within the tetraploids. For the 56-chromosome plant to have originated among 74-chromosome plants, at least one, if not both, of the gametes would have to be very reduced in number, but still with a viable chromosome complement. The chance formation of two such gametes in two nearby polyploids flowering at the same time would seem to be an event of low probability. In number, the 56-chromosome plant is essentially a hexaploid compared to the 2n=4x=28 plants that represent the tetraploid saltgrass of the region. However, it is probably best to consider this plant an "intermediate" form (similar to those in buffalograss recently desvribed by Dr. Paul Johnson), since the composition of full genome sets in unknown and it may be genetically as much a hyper-pentaploid as a hypo-hexaploid. Multivalent associations among chromosomes are commonly observed in meiosis I in the ca 74 chromosome types. Figure 2 illustrates a representative meiotic figure observed at diakinesis in AZ 104, one of the higher-order plolyploids. Many of the chromosomes are involved in quadrivalent associations. The effect on viable gamete formation is unknown at this time, but we have some very limited circumstantial evidence from plants collected by Dr. Kopec in Utah that they may be from breeding populations. Three of four plants collected in St. George, Utah form a summer golf course, all with different phenotypes in our greenhouse, have about 74 chromosomes. This may be an anomaly due to small sample size, but may also indicate either a breeding population of the result of some factor favoring production of unreduced gametes resulting in polyploids. It is interesting to note that although plants with ca. 74 chromosomes occur at a frequency of greater than 10% in our collections from regions of both 38- and 40-chromosme plants, within-region variations of these tetraploid numbers, except for B chromosomes and the one intermediate, have not been observed. Whatever mechanism gave rise to the 38-chromosome type (and the possible involvement of higher-order ployploids), it would seem that the current octaploids in each group probably arise form within each group. The current hypothesis is that the higher-order polyploids from the 38-chromosome plants compared to the 40-chromosome plants or their polyploids.

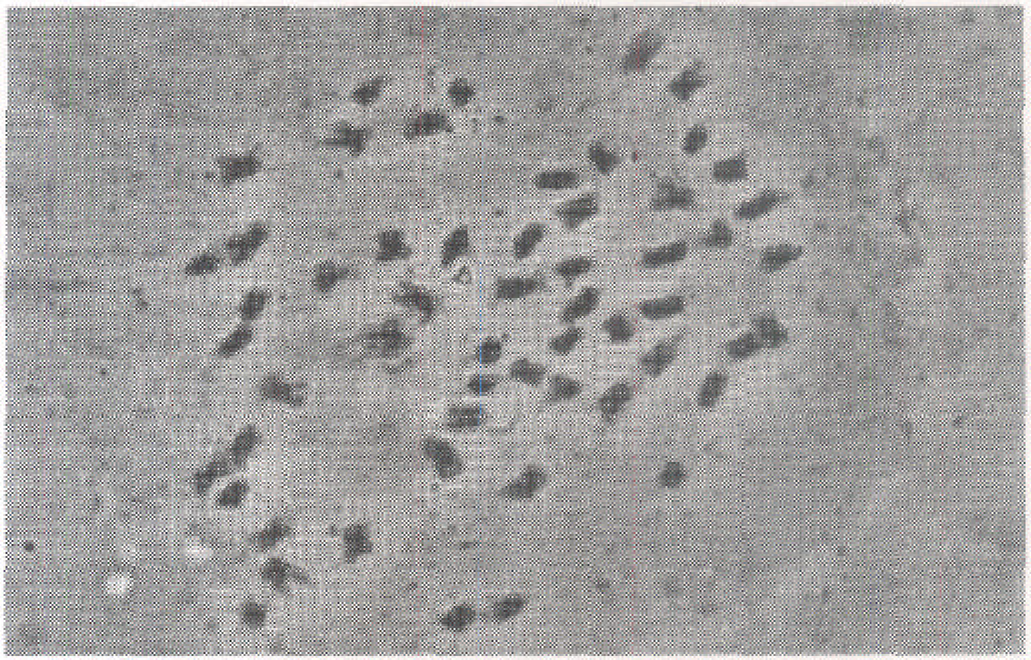

Figure 1. Mitotic metaphase in accession AZ 27, a 56-chromosome *Distichlis spicata* clone.

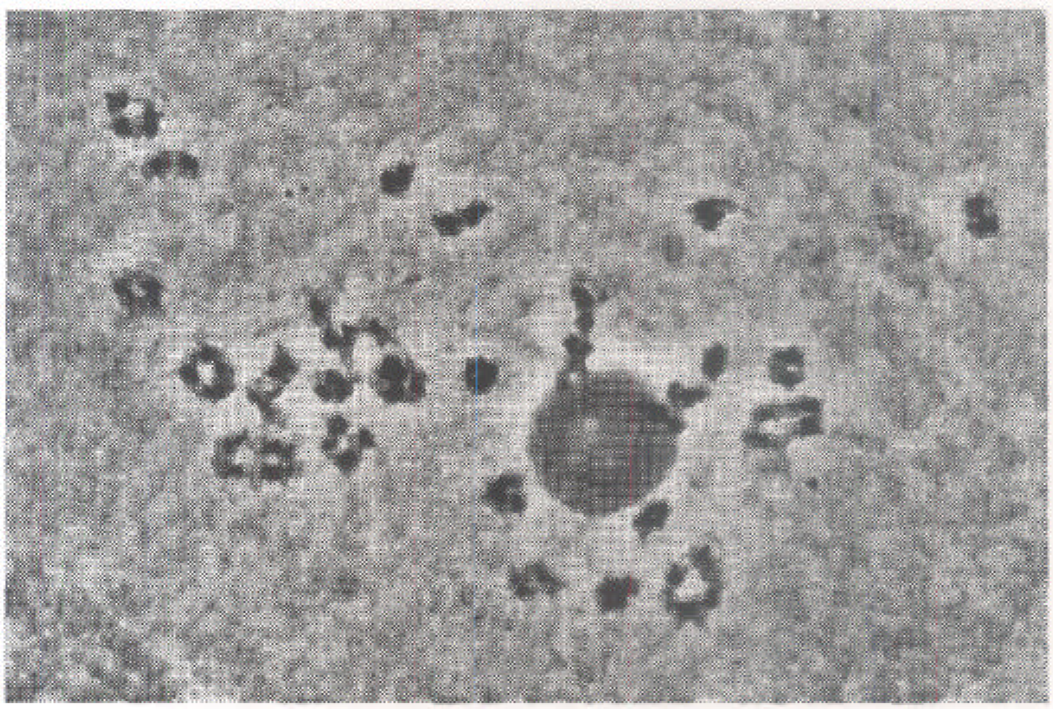

Figure 2. Diakinesis in AZ 104 with 13 quadrivalents and 11 bivalents formed.

A pilot study was conducted to investigate the relationship of leaf blade angle (the declination of the leaf blade away from the vertical axis of the culm) to the region of origin of the plants as delimited by the distribution of tetraploid chromosome numbers of either 38 or 40. Plants with a small angle of declination (more vertical leaf orientation) have a very different appearance than those with a larger angle of declination (more horizontal leaf orientation), that, along with other factors involved in the composite canopy architecture, can affect perception of turf quality. Saltgrass plants with 40 chromosomes (and polyploids from the 40-chromosome regions) have been observed to generally have more horizontal-oriented leaves than plants with 38 chromosomes (or the polyploids originating from the 38-chromosome regions). Although leaf orientation is a variable characteristic among plants within both chromosome groups, the overall group differences suggested a relationship. Leaf angles of 35 accessions were measured. Included were fifteen 38-chromosome plants and one 74 chromosome plant from the 38-chromosome region, ten 40-chromosome plants and one ca.74-chromosome plant from the 40-chromosome region, and eight plants grown from seed obtained from three different 38- x 40-chromosome crosses. Measurements were made beginning with the first fully expanded leaf expanded leaf using culms with at least five leaves. A total of 427 measurements were included in the analysis. All plants were growing in the greenhouse under similar conditions and had grown unmowed for approximately the same number of weeks. Analysis of variance was calculated for leaf angle with individual accessions as the independent variable, and with group (plants originating from the 38-chromosome region, plants originating from the 40-chromosome region, and 38 x 40 crosses) as the independent variable. Results for the three groups are illustrated in figure 3 and for the individual plants in figure 4.

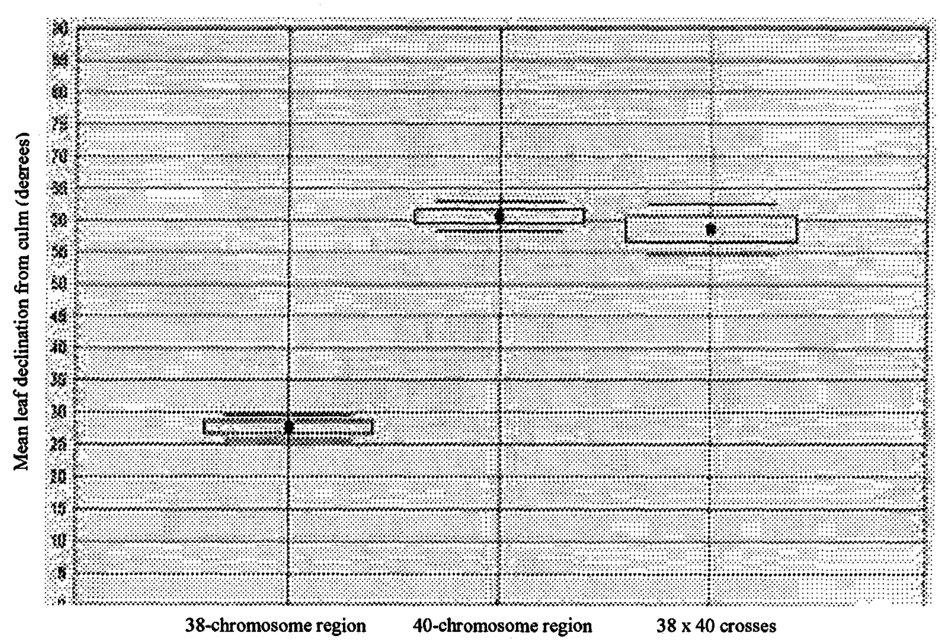

Figure 3. Mean leaf for all plants within each of the three groups. Plants from the 38-chromosome region are significantly different from the other two groups (more vertical leaf orientation). Plants from the 40-chromosome region are not significantly different from the crosses. Boxes around means are ± SE; bars represent 95% confidence intervals for the means.

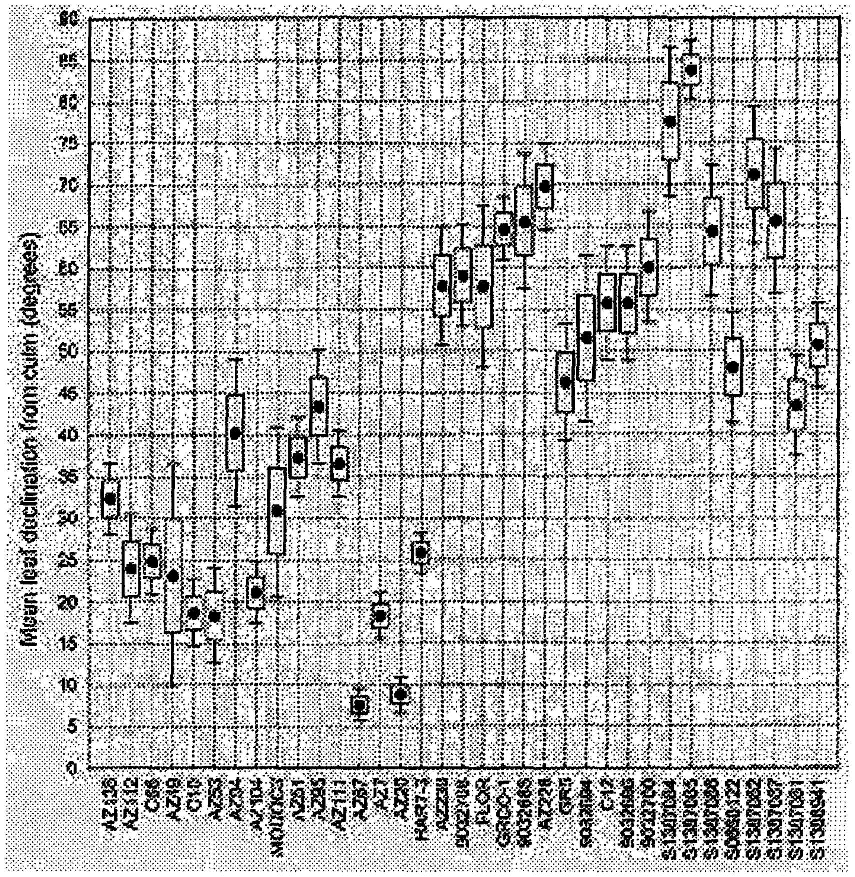

Figure 4. Mean leaf angles for individual plants. Accessions AZ 138 through HAR 7-10 are from the 38-chromosome region, AZ 230 through 9032700 are from the 40-chromosome region, and S1387084 through S1386941 are 38- x 40-chromosome crosses. Accessions AZ 104 and 9032700, from different regions, both have ca. 74 chromosomes but are representative of the means for each group as a whole. Significant differences (Tukey's HSD for unequal n; p=0.05) are found within each group as well as among individuals in different groups. Individuals in the intermediate band from approximately 35 degrees to55 degrees were not significantly different. Boxes around means are ± SE; bars represent 95% confidence intervals for the means.

Although not all plans from the different populations are significantly different for leaf angle, the two chromosome groups as a whole are significantly different. In this study, plants in the 38-chromosome region vary increasingly and plants from the 40-chromosome region vary decreasingly to a line of demarcation at about 45 degrees. Mean leaf angles of the crosses were quite variable, but were in the range of about 45 degrees or greater, and as a group they were not statistically significantly different from the 40-chromosome group. It is not known to what extent this characteristic might be environmentally labile. Plants from both chromosome groups with greater variability in either direction would not be unexpected. This study provides some evidence that canopy architecture is affected by chromosome complement. Only three 40-chromosome plants from northern Utah and western Colorado were included in this study. Plants from our limited collections in this area tend to be tall, with relatively long leaf blades, and are more variable in appearance for the leaf angle characteristic as the culms elongate (narrow angle on young culms, increasing as internodes elongate) than our other 40-chromosome accessions. This also seems to occur more in the 38-chromosome plants compared to the other 40-chromosome plants. Selecting plants at similar stages of development was considered important for consistent results within the clones and to avoid bias in the comparisons among clones. We also have one 40-chromosome plant with narrow leaf angles, very compressed internodes, and short, fine leaves that was not included in the study. This plant is quite variable in appearance under different conditions in our greenhouse, and selection of a comparable form for the study was uncertain. This plant is probably and outlier from is chromosome group, even after taking into account the observed variability, but our collections are too limited in the 40-chromosome regions to fully describe the variability with confidence.

OBJECTIVE 8: Study the viability and germination requirements of inland saltgrass seed.

Ms. Judy Harrington has completed her study of scarification and temperature influences on germination of several seed lots and has defended her thesis. A copy of her thesis will be sent the USGA under separate cover.

Mr. Remi Bonnart has continued his comparison of hand and machine scarification on germination of 2 seed lots, hereafter termed 'Granite', from the Salt Lake area and 'Modoc', from northern California. Analysis of laboratory germination studies indicated that scarified seed had significantly greater overall germination with both seed lots as compared to nonscarified seed. Although not statistically significant, there appeared to be a slight increase in germination of machine scarified seed as compared to hand scarified seed. The 'Modoc' seed lot had considerably lower rates of gemination as compared to 'Granite'. This was no doubt related to the observation that the 'Modoc' seed had originally arrived with a much higher percentage of moisture and appeared to have not been cleaned and stored with the care of the 'Granite' seed lot. Field data from a comparison of the same treatments had interesting results. In general, 'Granite' seed germinated more quickly in the field with scarification as compared to nonscarified. However, after approximately 45 days there appeared to be no difference among the treatments. 'Modoc' seed behaved differently with nonscarified seed showing a greater germination rate than scarified seed. We believe that might be the result of low vigor of 'Modoc' seed combined with excess moisture during the early days of germination resulting in damping-off which was observed in the field. Recognizing that low vigor was likely a factor, a series of laboratory experiments was done comparing machine scarified 'Modoc' and 'Granite' seed. 'Granite' seed showed significantly greater vigor as determined by early germination. This would support the idea of the impact of damping-off as a problem in the germination of 'Modoc' in the field under excessive moisture, an environmental situation likely to encourage greater disease problems.

OBJECTIVE 9: Evaluate seed priming as a possible method by which germination can be improved. The planning of this research has been done and will be started in early November.

There has been considerable research initiated with saltgrass with a new graduate student, Mohamed A. Shahba, coadvised by Drs. Qian and Hughes. He has initiated studies on the influence of nitrogen on the establishment of saltgrass via plugs. The first years data shows substantial differences although that data is still being analyzed. Furthermore, he is also evaluating freezing tolerance and assessing the relationship between nonstructural carbohydrates and freezing tolerance. Initial studies indicate that selected lines appear to have greater hardiness than selected lines of buffalograss used in a comparison. Furthermore, there appears to be some correlation in relative hardiness with nonstructural carbohydrates. This data is being analyzed.

Proposed Research Schedule for the Coming Year

OBJECTIVE 1: Parents will be moved into the box study to study herbicide, nitrogen and mowing effects. We don't anticipate to see injury, but this will allow screening of any undesirables.

OBJECTIVE 2: This will be forth coming directly from the University of Arizona.

OBJECTIVE 3: This objective has been completed.

OBJECTIVE 4: The accession nursery will be used for studying mowing injury.

OBJECTIVE 5: Full sib analysis of parents will begin to enable us to pick the best combination of parents for best results. Seedlings will be germinated in the greenhouse and will be moved into the field for progeny evaluation. This will include 100 individuals per cross replicated twice. This would be establishment year, so limited data will be taken. In addition, 15 randomly selected females will have 10 progeny of each grown out in order to cmpare genetic gain of seed production from selecting for high raceme numbers. In addition, we will bring potted plants in at different times in the fall to determine vernalization requirements. We also, make some plantings to enable us to study vacuum and windrow harvesting of seeds.

OBJECTIVE 6: We will finally begin this work in January.

OBJECTIVE 7: We will continue to evaluate chromosome number of elite clones and new accessions. However, we plan to summarize the data thus for completion of Mr. Scott

Reid's thesis and a paper for submission for publication. After completion of the paper, we hope the study the leaf blade angle in greater detail to see if this supports our ideas on the population dynamics of saltgrass.

OBJECTIVE 8: We hope to complete a short report to publish on Mr. Remi Bonnart's research. If time and space allows we do hope to try another field evaluation comparing scarified and nonscarified seed.

OBJECTIVE 9: We will be evaluating various treatments for stimulating germination of saltgrass. We will use filter paper soaked in polyethylene glycol as an osmoticum for priming of seeds as well as "matric priming" with the addition of GA3 and various other compounds

followed by a dry down and subsequent laboratory germination test. We hope thereby to establish best procedures to stimulate rapid germination of saltgrass seed.

ADDITIONAL WORK; GOLF COURSE: We are in discussion to establish some of the saltgrass selections at a golf course with high soil salinity and irrigation water which is of poor quality. In this way we hope to be able to evaluate relative turf quality under golf course conditions.

ADDITIONAL WORK; EVALUATION OF FREEZING TOLERANCE: The data collected from the last two seasons will be analyzed and results will be summarized as part of Mr. Mohamed Shahla's dissertation.

ADDITIONAL WORK; INFLUENCE OF NITROGEN ON ESTABLISHMENT OF SALTGRASS: Data from the past season will be analyzed. We will establish a second planting for data collection as well. Furthermore, we will take a second years data next season from the initial planting.