COMPARISON OF SCREENING TECHNIQUES FOR TRAITS RELATING TO DROUGHT TOLERANCE IN GRAIN SORGHUM

by

WILLIAM JOSEPH MAJERUS

B.S., University of Minnesota, 1983

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1987

Approved by:

Major Professor

ACKNOWLEDGEMENTS

I sincerely thank Dr. Paula Bramel-Cox, my advisor, for her guidance, encouragement and friendship during my graduate work. The knowledge I have gained in plant breeding is due in a large part to her leadership and persistence. I'm very fortunate to have been a graduate student under her direction. I am grateful to Drs. Mary Beth Kirkham and Stan Cox, who served on my Advisory Committee, for their councel. I would like to thank all my fellow graduate students in the department and on the sorghum project for their friendship and assistance during my stay at K-state. Special thanks are due to the memory of Dr. Dan M. Rodgers, my first advisor, for providing me the opportunity to attend graduate studies at Kansas State University.

My time at Kansas State University was very precious to myself and my family. We thank all of you, faculty members, secretaries, and graduate students for making our stay an enjoyable and memorable one.

TABLE OF CONTENTS

Page

Abstract..... 7

Introduction..... 8

Literature Review..... 10

Materials and Methods..... 21

Results and Discussion..... 30

Summary..... 59

Literature cited..... 61

Appendix..... 66

LIST OF TABLES

Page

Table 1. Description of female and male parents used in this study to compare screening techniques for drought tolerance..... 25

Table 2. Description of traits measured on test plots during weekly intervals and throughout the growing season..... 26

Table 3. Analysis of variance with sources of variation and degrees of freedom for the combined year analysis over 1985 and 1986..... 29

Table 4. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in seven sample dates in irrigated and dryland treatments in 1985 and 1986..... 42

Table 5. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 72 days after planting in 1985..... 43

Table 6. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 80 days after planting in 1985..... 44

Table 7. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 42 days after planting in 1986..... 45

Table 8. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 48 days after planting in 1986..... 46

Table 9. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatment at 54 days after planting in 1986..... 47

Table 10. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 61 days after planting in 1986..... 48

Table 11. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT),blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 68 days after planting in 1986..... 49

Table 12. Analysis of variance for blackening pressure (P3) and membrane strength (ECLEAK) of three female lines, five male lines and their fifteen hybrids in irrigated and dryland water treatment in 1985 and 1986, separately..... 50

Table 13. Parental lines and hybrids mean date of flag leaf expansion of three female lines and five male lines in irrigated and dryland water treatments in 1985 and 1986, separately..... 51

Table 14. Mean parental lines and hybrids for blackening pressure (P3) of three female lines and five male lines in irrigated and dryland water treatments in 1985 and 1986, separately. 52

Table 15. Mean parental lines and hybrids for membrane strength (ECLEAK) of three female lines and five male lines in irrigated and dryland water treatments in 1985 and 1986, separately..... 53

Table 16. Correlation coefficients between grain yield (GYLD), seed number (SNB), seed weight (SW), flowering date (BLM), physiological maturity (PM), plant height (HEIGHT), and the ratio of number of green leaves versus plant growth stage at date of flag leaf expansion (GREFLAG) versus response traits blackening pressure (P3), cellular strength (ECLEAK) in irrigated and dryland water treatments in 1985 and 1986, separately..... 54

Table 17. Ranked correlation coefficients of cellular membrane strength (ECLEAK) for each year and water treatment. Cellular membrane strength under irrigated treatment in 1985 (EC85I), cellular membrane strength under dryland treatment in 1985 (EC85D), cellular membrane strength under irrigated treatment in 1986 (EC86I), cellular membrane strength under dryland treatment in 1986 (EC86D)..... 55

Table 18. Ranked correlation coefficients of blackening pressure (P3) for each year and water treatment. Blackening pressure under irrigated treatment in 1985 (P385I), blackening pressure under dryland treatment in 1985 (P385D), blackening pressure under irrigated treatment in 1986 (P386I), blackening pressure under dryland treatment in 1986 (P386D)..... 56

LIST OF FIGURES

Figure 1. The effect of plant growth stage on blackening pressure on 28 entries under irrigated and dryland treatments over sampling dates recorded in days after planting in 1986..... 57

Figure 2. The effect of plant growth stage on percent injury by the osmotic potential of polyethylene glycol on 28 sorghum entries under irrigated and dryland treatments over sampling dates recorded in days after planting in 1986..... 58

ABSTRACT

Although several screening techniques involving various physiological and morphological traits have proven effective, implementing these screening methods in a breeding program have been hypothesized to be both labor and time intensive. In this study the objectives were to compare certain physiological and/or morphological measurements taken prior to anthesis on the bases of their ability to discriminate between genotypes under stress and their inheritance. Eight sorghum (Sorghum bicolor (L.) Moench) parent lines, ranging from susceptible to tolerant in reaction to water stress, were mated in a 3 line x 5 tester factorial design. The eight parents, 15 F_1's,and 5 commercial checks were tested in a randomized complete block design with a split-plot arrangement under 2 water treatments. Stomatal conductance, leaf temperature, transpirational rate, leaf water potential, and cellular membrane strength were measured during weekly intervals from approximately 40 days after planting until anthesis. Significant entry mean differences were found for leaf water potential and cellular membrane strength just prior to flag leaf stage of development. No significant parent vs progeny correlations, GCA or SCA effects and heterosis were found for these two plant traits. Both traits were significantly correlated with senescence at this pre-anthesis stage.

INTRODUCTION

Grain sorghum (Sorghum bicolor L. Moench) is one of the leading cereal grains in Africa and a major crop in the United States, India, Pakistan, and China. It is best adapted to warm conditions and is very tolerant of drought and heat stresses (Martin, 1930). Grain sorghum ranks fourth in production among the world's cereals. World production in 1986 was nearly 72.4 million metric tons of grain from 48.9 million hectares (USDA, 1986). In the United States, grain sorghum production has reached 28.3 million metric tons from 6.7 million hectares (USDA, 1986). The state of Kansas produced 7.4 million metric tons from 1.7 million hectares in 1985 (Kansas Farm Facts, 1985).

Cultivated grain sorghum is hypothesized to have its origin among the wild sorghums of the Ethiopian area of Eastern Africa (Dogget, 1965). The greatest variability in both cultivated and wild sorghums has been found in the northeastern quadrant of Africa (Dogget, 1965). De Wet and Harlan (1971), Brown (1965) and Murdock (1959) found sorghum was the dominant crop in regions which received between 500 to 1500 millimeters annual precipitation. The introduction of grain sorghum to the Americas was initiated with the slave trade approximately 135 years ago (Dogget, 1965). Its evolution in semi-arid regions of Africa has resulted in a cereal crop well adapted to the Great Plains

of the United States (Martin, 1930).

Many physiological and morphological studies on various crop plants [Dalton (1967), Garrity et al. (1984), Levitt (1972), Henzell et al. (1975), Jordan and Miller (1980), Sharkey and Badger (1982), Stout and Simpson (1978), Sullivan and Blum (1970),Ogren and Oquist (1985), Wright and Smith (1983), Waring and Cleary (1967), Weatherley (1970) and Wright et al. (1983)] have contributed to the understanding of these crop plants' response to less than optimal growth conditions. Many of these authors have suggested the use of a screening technique based upon these plant responses be used by plant breeders to improve the selection of drought tolerant hybrids. None of these papers has discussed the actual use of an indirect criterion based upon the measurement of a differential plant response. Thus the two objectives of this study were to:

1) determine whether certain physiological and/or morphological measurements taken once before anthesis could be used to discriminate between various sorghum genotypes' response to water stress.

2) estimate heterosis, evaluate the general combining and specific combining ability effects, and correlate line and topcross performance for those response variables.

LITERATURE REVIEW

Agricultural drought has been defined as "a climatic excursion involving a shortage of precipitation sufficient to adversely affect crop production or range productivity" (Saarinen, 1966; Hershfield et al., 1973; World Meteorological Organization, 1975). Inadequate precipitation, relative to evapotranspiration, coupled with uneven seasonal rainfall distribution define a condition of drought (Rosenberg, 1979). These conditions occur frequently in the Great Plains of North America as well as other semi-arid and sub-humid regions of the world.

Quizenberry (1981) suggested that plant breeders with responsibility for the development of drought tolerant sorghum varieties or hybrids take an interdisciplinary approach toward germplasm evaluation. This approach must include an indirect selection criteria based upon a thorough understanding of the plant physiology of drought stress tolerance. Levitt (1972) classified various plant responses under water stressed conditions into three basic categories: drought escape, drought tolerance at high leaf water potential, and drought tolerance at low leaf water potential. Drought escape is defined as the ability of a plant to grow and complete its life cycle prior to serious soil and plant water deficits. Drought tolerance at high leaf water potential is defined as the ability to tolerate

water deficits during growth periods by maintaining high leaf water potentials. For example, the plant may possess some morphological characteristic such as a deep, well developed root system which can maintain the plant at a high leaf water potential by extracting plentiful, deep soil water. This has been equated with some forms of avoidance. Drought tolerance at low tissue water potential is defined as the ability to tolerate water deficits as low tissue water potentials develop. The plant has the ability to extract soil water at lower potentials.

Drought escape, through early maturity, has been a useful avenue used by grain sorghum grown under limited water supply (Blum, 1970). Quizenberry (1981) criticized the description of maturity as a true resistance mechanism. If total crop production is expected to be made on existing stored soil moisture, then early maturing varieties have an advantage. However, maturity has been negatively correlated with yield under conditions of adequate moisture (Dalton, 1967).

The maintenance of sufficient leaf water potential under periods of water stress is vital to the processes of photosynthesis and transpiration (Sharkey and Badger, 1982; Ogren and Oquist, 1985). O'Toole and Moya (1978) visually scored the leaf water potential of 17 diverse genotypes of rice. They found substantial differences in the capability

of rice cultivars to maintain high leaf water potentials when exposed to dry-season field screening conditions. Drought tolerance at high leaf water potential was maintained by the reduction of water loss in the leaves via stomatal regulation, leaf senescence and /or leaf rolling. Maintenance of water uptake into the plant by the roots has been shown to be another method of supporting high leaf water potentials during periods of drought (Jordan and Miller, 1980).

Henzell et al. (1975) reported that a reduction in water loss by increased stomatal resistance was found in 22 sorghum hybrids. As the hybrids were progressively moisture stressed, stomatal sensitivity and leaf water potential varied significantly among the entries. In general, the behavior of an F_1 hybrid was more similar to that of the sensitive parent where sensitive referred to rapid stomatal closure when plants were water stressed. Their results suggested that stomatal sensitivity was an important element of sorghum tolerance to drought, and there was some genetic variation within these 22 hybrids.

Garrity et al. (1984) compared three sorghum hybrids (RS626, NB505 and NC+55X) exposed to similar environmental conditions. Their results demonstrated that stomatal resistance was sensitive to small reductions in leaf water potential during the vegetative period. During the

reproductive period, the guard cells became nearly insensitive to reductions in leaf water potential and the stoma remained open at lower leaf water potentials.

Leaf area reduction to minimize water loss either through leaf rolling (Begg, 1980), leaf senescence (Henzell et al., 1975) or both have been widely reported in the Graminae family. Reduction in leaf area was the result of leaf rolling (Begg, 1980) and leaf senescence (Henzell et al., 1975). Stout and Simpson (1978) reported that sorghum cultivars M35 and NK300 suffered a much larger decrease in leaf area through senescence when grown under non-irrigated than irrigated conditions. They concluded that leaf senescence was an important mechanism for avoiding low leaf water potential by decreasing the absolute amount of water required per plant. Garrity et al. (1984) found that leaf rolling in three sorghum hybrids resulted in transpiration control under drought during the entire reproductive and grain-filling period.

Liver-Munoz et al. (1986) concluded that drought stress was most detrimental to sorghum yields when it occurred during initiation of pistil and stamen primordia based upon the observation of 144 sorghum entries. Any reduction in grain yield was directly proportional to the reduction in seed number.

Increased root depth and root density preserved a high

water potential in sorghum leaves by maintaining water uptake during periods of severe water stress (Jordan and Miller, 1980). Wright and Smith (1983) concluded that the sorghum hybrid Dekalb E57 possessed a higher root mass and longer root length than TX-671. These two factors resulted in a more effective exploitation of stored soil water at a greater depth by E-57 than TX-671 and thus E57 was able to maintain a higher rate of water uptake.

Drought tolerance at low leaf water potential depends upon a maintenance of leaf water turgor and a prevention of leaf cell desiccation (Jordan and Monk, 1980). Maintenance of turgor can be accomplished by either the active accumulation of solutes in the cell and/or an increase in cellular membrane elasticity (Wright et al., 1983; Weatherley, 1970). Wright et al. (1983) reported that the sorghum hybrid Dekalb E57 was able to maintain stomatal opening at a lower leaf water potential than TX-671 due to its greater osmoregulating capacity. In E57 osmotic potential declined due to active solute accumulation, and thus a higher turgor pressure at lower leaf water potentials was maintained. Weatherley (1970) demonstrated that an increase in elasticity of castor bean cell walls could also lower the osmotic potential of the cell at a given leaf water potential, thereby increasing the turgor pressure at that water potential, with no increase in net solute

content. Sullivan and Blum (1970) used a desiccation tolerance test to compare 5 grain sorghum lines to the Zea mays L.'Conico'. Conico corn was chosen as a drought resistant corn from Mexico. When plants were wilted to the point where only 50 % of the leaf tissue would recover turgidity, sorghum recovered from greater leaf water desiccation levels than Conico corn.

Gardner et al. (1981) studied the relationship of mid-day leaf water potential and mid-day crop temperatures between water stressed and nonstressed sorghum. They used the hybrid RS626. Large differences in leaf water potential occurred between stressed and nonstressed plants. Generally, a more negative leaf water potential in the stressed plants indicated a reduced transpiration rate. Under such conditions, the difference in temperature between stressed and nonstressed plants increased. Such an increase was observed until a temperature difference of about 4° Celsius. Beyond this point, transpiration from the stressed plants may have been restricted sufficiently to permit the leaf water potential of the stressed plants to increase slightly. This increase caused a decrease in the difference between leaf water potential of the stressed and nonstressed plants.

Measurements of leaf water potentials have been useful to quantify water stress levels and determine plant

responses to drought (Waring and Cleary, 1967). The Scholander pressure chamber (Scholander et al., 1964) is the most widely accepted method for determining the leaf water potential of plants.

The pressure chamber measures the hydrostatic pressure which exists in the vascular system prior to the cutting of the growing plant sample. This is measured by determining at which external gas pressure the system starts to exude liquid. A leafy shoot, with exposed xylem at the cut end, is fitted through a rubber compression gland in the top of a pressure chamber. Then the gas pressure is increased in the chamber until a small and spotty exudation is observed; at a critical point, the cellular fluids rapidly exude with vigorous bubbling (Scholander et al., 1964). Field measurements with the pressure chamber have been easily obtained (Waring and Cleary, 1967), but the compressed gas canisters, which are necessary for the pressure chamber operation, are bulky, hard to handle, and present a safety hazard (Cox and Hughes, 1982). To alleviate these inherent problems with the pressure chamber when measuring leaf water potential, Campbell Scientific developed the J-14 leaf press (Campbell and Brewster, 1980). The mechanical and operational simplicity, ruggedness, rapid measurements, and low cost of the J-14 leaf press enhance its utility for determination of leaf water potential (Shayo-Ngowi and

Campbell, 1980; Hicks et al., 1986; Bristow et al., 1981; Cox and Hughes, 1982). The J-14 measurement of leaf water potential, blackening pressure, have been correlated with leaf water potential measurements of the Scholander pressure chamber, with r=0.91 (Jones and Carable, 1980).

Sullivan and Ross (1979) developed a procedure to quantify desiccation tolerance in sorghum via cellular membrane integrity. Leaf samples were exposed to an artificial desiccation stress level (-18 bars) created by polyethylene glycol. Tolerance was quantified by the amount of electrolyte leakage from damaged cells. Sullivan and Ross (1979) compared two sorghum genotypes, M35-1 and RS626, using this desiccation test. They found M35-1 significantly higher desiccation tolerant than RS626.

Blum and Ebercon (1981) found younger leaf tissue in wheat was more tolerant than older tissue to the drought tolerance test described by Sullivan and Ross (1979). The difference in percent injury between the two cultivars, Lakhish and Inbar, was the greatest during the jointing stage. Upon flowering differences were the smallest, as leaves became most susceptible to injury by PEG. Blum and Ebercon (1976) found similar results in sorghum. Younger sorghum leaf tissues were more tolerant to drought than older tissue. They also suggested sampling procedures for genotypic comparisons should take careful account of

differences in morphological growth stage, could cause apparent genotypic not reflecting differences in true drought tolerance.

Attempts have been made in various crops to integrate various physiological and morphological phenomenon into specific traits that could be evaluated by plant breeders to select among a large number of genotypes (O'Toole et al., 1984; Seropian and Planchon, 1984; Peacock et al.,1985). O'Toole et al. (1984) concluded that seven measurements, namely, leaf water potential, stomatal resistance, transpiration rate, canopy minus air temperature, crop water stress index, photosynthetic rate, visual leaf rolling score, could be utilized to quantify a specific rice (Oryza sativa L.) genotype's response to drought stress. Crop water stress index is calculated from the line relating the difference between canopy temperature and air temperature and air vapor pressure deficit (Idso et al. 1979). Simply stated, crop water stress index is the difference between crops under well watered and stressed conditions. It is a form of indexing a crop's response to drought stress. Crop water stress index was negatively correlated with daily photosynthetic rate (r=-0.84). O'Toole et al.,(1984) concluded that only crop water stress index and leaf rolling scores had potential utility as rapid, nondestructive, nondisruptive measurement of crop

water stress in rice and which selection could be based upon.

Seropian and Planchon (1984) compared the response of six wheat (Triticum aestivum L.) genotypes to water stress when grown in a growth chamber. Leaf water potential, photosynthesis, stomatal resistance, and transpiration were measured during the period of stress. Stress was applied by terminating watering at the sixth leaf stage and the above measurements were taken as the seventh, eighth, and ninth leaf stage. The study concluded that these five response variables could be measured on genetically diverse genotypes and used as a selection criteria for drought tolerance in a breeding program.

Peacock et al. (1984) compared screening techniques for drought tolerance in grain sorghum. They evaluated 226 sorghum lines' response to water deficits based upon relative leaf water content, leaf water potential, stomatal conductance, and leaf temperature. After a critical level of stress was attained (about 56 days after planting), the resistant lines demonstrated higher plant water status than did the susceptible lines, as measured by relative leaf water content and leaf water potential under both soil and atmospheric water stress. They also reported that relative leaf water content was a more sensitive measurement than either leaf water potential or stomatal conductance. They

concluded that plant breeders should consider screening procedures based on physiological phenomenon such as relative leaf water content to assist them in their selection process for drought stress tolerance.

Although several screening techniques involving physiological and morphological traits have been accomplished in field screening programs (O'Toole et al., 1984; Peacock et al., 1985; Seropian and Planchon, 1984; Liver-Munoz et al.,1986), only limited resources or time have been placed on drought tolerance as a primary breeding objective (Rosenow and Clark, 1981).

MATERIALS AND METHODS

The parental materials in this study consisted of eight grain sorghum inbred lines selected under drought conditions during the summer of 1983 at the Kansas State University Agronomy Research Farm, Manhattan, Kansas. Table 1 lists the eight inbred lines selected, and their phenotypic response to drought conditions.

The eight parent lines were mated in a 3 line x 5 tester factorial design in the winter of 1985 at the Pioneer Hi-Bred International Inc. winter nursery near Kingston, Jamaica and in the summer of 1985 at the Kansas State University Research Farm. The experimental material used in subsequent evaluations included the eight parents, 15 F_1 hybrids, and five commercial grain sorghum hybrids (Pioneer 8493, Cargill 60, Dekalb 39Y, NC+ 174, and Garst 5511).

Field Preparation

The experiment was conducted at the Southwest Branch Agricultural Experimental Station located eight kilometers east of Garden City, Kansas in 1985 and 1986. The plots were located on an Ulysses silt loam, Aridic Haplustoll, fine-silty, mixed, mesic soil. The previous rotation of the plot area was maize followed by grain sorghum. Plant stubble was incorporated by discing in the spring each year. Preplant irrigation application consisted of 250

millimeters of water applied during April for both years. A preplant application of 138 kg/ha elemental nitrogen was applied. After planting, Propachlor /Atrazine, [2-chloro-N-isopropylacetanilide(2-chloro-N-(lmethylethyl)-N-phenylacetamide]/[2-chloro-4-(ethylamino)-6-(isopropylamino)-S-triazine], (9.35 liters/hectare) wasapplied for weed control. Additional weed control was accomplished by hand.

Plots were planted May 25 and June 13 in 1985 and 1986, respectively. Each plot consisted of four six meter rows with seventy-six centimeters between rows. Plant population under both water treatments was approximately 129,160 plants/hectare. All physiological and morphological measurements were taken from the center two rows of the four row plots. Irrigated plots received 100 additional millimeters of water on 39, 55, 81, and 101 days after planting in 1985. In 1986 supplemental water was applied at 23 days, 34 days, and 82 days after planting.

Physiological and Morphological Measurements

Leaf water potential was measured using a Campbell and Brewster hydraulic press (Campbell Equipment Co., Logan, UT). Leaf diffusive resistance, stomatal conductance, leaf temperature, and transpiration rate were measured on the abaxial leaf surface in the middle of the upper fully expanded leaf with a steady state porometer (Model LI-1600, Licor Inc., Lincoln, NE) using a one cm^2 aperture. A

desiccation tolerance test described in Sullivan and Ross (1979) was conducted. Twenty 7mm leaf-disc samples were cut, divided into a control and a treated test tube and washed. In 1985, the treated set of ten leaf discs was soaked in 20ml of 43% polyethylene glycol 600 wt/volume with a water potential of -18 bars and in 1986 polyethylene glycol 8000 wt/volume with a water potential of -18 bars was used. Polyethylene glycol 8000 molecular weight, which has a much larger molecular size than polyethylene glycol 600, was used in 1986. The other ten leaf discs were soaked in deionized water as a control. The two sets were soaked for 24 hours at 10°C, the 20ml of polyethylene glycol and deionized water were poured off, and all samples were washed thoroughly with deionized water. Thirty ml of deionized water were added to each test tube and soaked at to 10°C for 24 hours. Tubes were allowed to warm to 25° C. Conductivity measurements of the water was taken with a YSI model 32 conductance meter (Cleveland, Ohio). Then the discs were autoclaved at 100°C for fifteen minutes, cooled to 25° C, and the conductivity of the solution was measured a second time. Percent desiccation injury was calculated as described by Sullivan and Blum (1970):

$$\text{Percent injury} = 1-[1-(T_1/T_2)/1-(C_1/C_2)]X100,$$

where T and C refer to treatment and control samples, respectively, and the subscripts 1 and 2 refer to initial

and final conductivities, respectively.

Table 2 describes the traits measured during the growing season. Physiological measurements were taken on fully expanded leaves which best characterized the entire plot canopy. All measurements were taken during a 4 hour period encompassing solar noon (Bennett 1978). All developmental stages were recorded weekly. These plant stages were determined as described by Vanderlip (1979). Plant height (HT) was measured from the soil surface to the top of the main culm panicle. Grain yield (GYLD) was determined in 1985 by hand harvesting one meter from the middle of each center two rows of the 4 row plots. In 1986, grain yield was measured using a self propelled plot combine on the entire center two rows of the four row plot. Stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) were taken during weekly intervals. Grain yield (GYLD), seed weight (SW), seed number (SNB), bloom date (BLM), physiological maturity (PM), plant height (HT) and the ratio of number of green leaves to total number of leaves at flag leaf expansion (GREFLAG) were once measured during the growing season.

Table 1. Description of female and male parents used in this study to compare screening techniques for drought tolerance.

Inbred lines	Phenotypic response **
A-lines (female)	
A Dwarf Redlan	susceptible
A SC35-6	preflowering susceptible postflowering tolerant
A KS-9	tolerant
R-testers (male)	
R IA25	very susceptible
R KS14	susceptible to average tolerance
R 81EON86	above average tolerance
R IA28	tolerant
R SC118	very tolerant

** Dr. Dan M. Rodgers' description of grain sorghum phenotypic responses to dry conditions during the summer of 1983.

Table 2. Description of traits measured on test plots during weekly intervals and throughout the growing season.

Trait	Abbreviations	Description
Stomatal conductance	CD	molar flux density/water vapor partial pressure gradient/ atmospheres(cm s^{-1})
Transpiration rate	TR	mass of water evaporated into cuvette/(time interval in which given mass of H_2O evaporated into cuvette) (aperture area entered cuvette (mg cm^{-1} s^{-1})
Leaf temperature	LT	temperature of leaf at point of attachment (degree celsius)
Blackening pressure	P3	pressure required to blacken leaf interveinal area and rapidly exude leaf water from cut edges (psi)
Membrane strength	ECLEAK	percent cellular membrane breakage when exposed to polyethylene glycol for 24 hours
Grain yield	GYLD	total grain yield per plot (kilograms/hectare)
Seed weight	SW	average weight of 300 seed sample (kilograms)
Seed number	SNB	GYLD/(SW/300)
Bloom	BLM	50% of panicles on the main culm in a plot had 50% of stigmas exposed
Physiological maturity	PM	50% of main heads per plot had 50% of their seeds in black layer stage
Green leaf ratio at flag	GREFLG	calculated as number of green leaves on main culm at flag leaf expansion/ total number of leaves produced
Plant height	HT	plant height of main culm from soil surface to top of panicle (cm)

Statistical Analysis

The study was conducted in a randomized complete block design with a split-split plot arrangement. Whole plots consisted of the two water treatments, dryland and irrigated. The subplots and sub-subplots consisted of the entries and sample dates, respectively. Whole, subplot and sub-subplot treatments were randomly assigned. The overall experimental model for each year was:

$$O_{ijkl}=U + R_i + T_j + E_k + D_l + TE_{jk} + TD_{jl} + ED_{kl} + TED_{jkl} + e_{ijkl}$$

O_{ijkl} represents an individual observation in the l^{th} sample date, k^{th} entry, j^{th} treatment, and i^{th} replicate. The symbol U represents the overall population mean while the symbols, R, T, E, D, TE, TD, ED, TED, and e represent the effect due to replicate, treatment, entry, sample date, treatment x entry, treatment x sample date, entry x sample date, treatment x entry x sample date and experimental error, respectively. The combined analysis of variance with the sources of variation, degrees of freedom, and expected mean squares are found in table 4. In this model, year, water treatment, entry, and sample date were fixed effects. Replicate was considered random. The experimental error term used to test significance of the year, water treatment, and water treatment x year mean squares were treatment x replication within year (error A). The entry source of variation and its interactions with treatment, year, and

treatment x year were tested using the pooled entry x treatment x replication within year and entry x replication within year mean squares (error B). Separate analyses of variance were calculated for individual sample dates. Homogeneity of error variances and entry variances was tested. Significant entry sums of squares were divided into the listed orthogonal comparisons (table 4). The average mid-parent heterosis of hybrids was estimated by the following method:

$$\text{Heterosis} = \frac{\bar{X}\ \text{hybrids} - (\bar{X}\ \text{males} + \bar{X}\ \text{females})/2}{(\bar{X}\ \text{males} + \bar{X}\ \text{females})/2}$$

The general combining ability and specific combining ability effects were estimated from respective means for significant males, females, and males x females for each response variable within water treatments.

Spearman (1904) and Pearson (1902) correlation coefficients were estimated between all variables described in table 2. Parental mean versus progeny mean correlations were also calculated for stomatal conductance (CD), transpirational rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK).

Table 3. Analysis of variance with sources of variation and degrees of freedom for the combined year analysis over 1985 and 1986.

Sources of variation	d.f.	Expected Mean Squares
YEAR	1	$\sigma^2_{ea}+r(TY)+ry(T)+t(R(y))+r(Y)$
REP(YEAR)	4	$\sigma^2_{ea}+r(TY)+ry(T)+t(R(y))$
TRT	1	$\sigma^2_{ea}+r(TY)+ry(T)$
TRT*YEAR	1	$\sigma^2_{ea}+r(TY)$
ERROR A		σ^2_{ea}
TRT*REP(YEAR)	4	
ENT	27	$\sigma^2_{eb}+r(ETY)+rt(EY)+ey(ET)+rty(E)$
PARENT	7	
HYBRID	14	
MALE	2	
FEMALE	4	
MALE*FEMALE	8	
CHECK	4	
PARENT VS HYBRID	1	
HYBRID VS CHECK	1	
ENT*TRT	27	$\sigma^2_{eb}+r(ETY)+rt(EY)+ey(ET)$
ENT*YEAR	27	$\sigma^2_{eb}+r(ETY)+rt(EY)$
ENT*TRT*YEAR	27	$\sigma^2_{eb}+r(ETY)$
ERROR B		σ^2_{eb}
ENT*TRT*REP(YEAR)	108	
ENT*REP(YEAR)	108	

RESULTS AND DISCUSSION

Record rainfall in the summers of 1985 and 1986 at the Garden City Experimental Research Station hampered efforts to compare screening techniques for drought tolerance in grain sorghum. The mean precipitation for the summer months at the Garden City Experimental Research Station is 120 millimeters (Meteorological Library, Dept. of Physics, KSU, 1985-1986). In the summers of 1985 and 1986 a total of 320 millimeters and 297 millimeters of rainfall, respectively, accumulated from planting to physiological maturity.

The results and discussion section is presented in two parts. The first section discusses the development of an indirect selection criterion based upon the overall combined analyses over sample dates and years for various physiological plant traits, an examination of each sample date, and the partitioning of entry mean squares for each trait. The second section addresses the question of inheritance of this indirect criterion based upon heterosis, GCA and SCA effects, and correlations for physiological plant traits measured on the 28 sorghum entries in 1985 and 1986.

Screening technique

Two of the five physiological measurements, CD and ECLEAK, had significantly different means in the two years (Table 4). The year mean differences for ECLEAK were related

to the use of different molecular weights of polyethylene glycol (600 mwt and 8000mwt) in 1985 and 1986, respectively. Polyethylene glycol 600 molecular weight was recommended for this screening technique by Sullivan and Ross (1979). The polyethylene glycol (PEG) 600 , unlike the higher weight PEG 8000, may have penetrated the leaf cellular membrane and entered the leaf cells, thereby minimizing the function of PEG in this test, i.e., to create an osmotic gradient forcing cellular fluids out of the leaf tissue.

Water treatment means differed significantly only for P3 (Table 4). Lack of a significant difference for ECLEAK in the two water treatments indicated that membrane strength was not altered by stress at the levels found in the dryland treatment. Cellular membranes have been reported to "harden" under water stress (Levitt, 1972). Hardening is defined as the physiological process in which plants exposed to moderate stress conditions develop tolerance to that stress. Due to the abundant rainfall and resultant lack of stress, these entries did not produce a "hardening" response to dryland conditions.

Entry mean differences were significant for both P3 and ECLEAK. A significant interaction implies that two or more main effects, when observed at different levels provide a response that is not additive. Thus, in this study, any significant interaction with the entries would indicate that

the 28 entries did not respond similarly in different water treatments, years, or both, and that these factors would need to be taken into account when selecting an indirect technique to measure drought tolerance. The only interaction of significance was entry x year for P3 and ECLEAK.

Error variances were tested for homogeneity over sample dates and were not significantly different (P=.05 or .01). Thus the significant entry mean differences for P3 and ECLEAK over sample dates and years was statistically sound.

One of the main objectives of this study was to develop a rapid method of indirectly evaluating drought tolerance in segregating breeding populations. The rapidity of this technique depends upon the identification of a measurement of a physiological response which when measured once during the growing season will accurately portray a genotype's response at any other time during its life cycle. The selection of the particular sampling date and indirect screen method was based upon that individual date within year which best explained the most variability for entry and or treatment mean differences shown to be significant in the combined analysis (Table 4.) In 1985 planting was approximately 19 days earlier than planting in 1986. Growth stages of each entry were observed at each sampling date to be similar (plus or minus 3 days).

Separate analysis of each sample date was conducted for P3 and ECLEAK, which demonstrated significant entry and/or treatment mean differences in the combined analysis. In 1985, at 72 days after planting, water treatments were nonsignificant for all traits (Table 5). No entry mean differences were observed for traits measured. Significant entry and treatment mean differences were found for P3 and ECLEAK at 80 days after planting in 1985 (Table 6). CD, TR, and LT failed to express any significant entry and treatment differences. This would imply that significant entry differences to be found for P3 and ECLEAK would be at a sampling date closest to anthesis which occurred at approximately 57 days after planting.

In 1986, 42 days after planting significant entry and treatment mean differences were observed for P3 and ECLEAK (Table 7). Forty-eight days after planting in 1986 there were significant treatment mean differences observed for LT and significant entry differences for P3 and ECLEAK (Table 8). Significant entry differences were shown on 54 days after planting for all traits except LT (Table 9). Sixty-one days after planting in 1986, there were significant entry mean differences for CD, TR and ECLEAK (Table 10). At 68 days after planting in 1986 significant entry mean differences were found for P3 and ECLEAK (Table 11). In 1986 as in 1985 CD, TR, and LT showed no consistent significant

entry differences. Throughout each sampling date in 1986 significant entry mean differences were found for P3 and ECLEAK. These results for 1986 imply that entry mean differences for P3 and ECLEAK can be determined throughout the growing season.

Peacock et al. (1985) showed that significant differences among sorghum genotypes for leaf water potential were detected later in the growing season. Blum and Ebercon (1981) found that injury to wheat leaf tissues exposed to PEG significantly increased as sampling later plant growth stages of development. Plotting P3 and ECLEAK means over days after planting in 1986 shows that as the plant developed, entry means increased for P3 and ECLEAK until they began to decrease approximately 61 to 68 days after planting in 1986. Blum and Ebercon (1976) suggested that growth stages could be used to indicate sample periods for genotype comparison in sorghum. When growth stages of development were compared to sampling dates in this study, the flag leaf expansion stage was found to coincide within 61 days after planting in 1986 and 73 days after planting in 1985. Therefore, as the flag leaf appeared, a decrease in leaf water potential and cellular membrane stength was found. It was at this point that the greatest range of variability could be found (Peacock et al., 1985) (Fig. 1 and 2). These results would suggest that the best period

to detect the most variablity among genotypes for leaf water potential and cellular membrane strength would be at the flag leaf stage. This information would allow the breeder to identify genotypes' leaf water potential and cellular membrane stength once during their life cycle and predict with some accuracy their response to drought. This would add to breeders' ability to make pre-pollinating decisions to maximize their breeding efforts.

Inheritance study

Two dates, 80 days after planting and 68 days after planting, were used for further analysis to represent growing conditions in 1985 and 1986, respectively. These dates were used to determine the inheritance of leaf water potential and cellular membrane strength. Table 12 lists the partitioning of the entry means for P3 and ECLEAK under both water treatments, separately in 1985 and 1986. When each year-treatment combination was examined separately, no significant entry differences were observed in 1985 under irrigated or dryland treatments for either P3 or ECLEAK. In 1986 under irrigated conditions, significant entry mean differences for ECLEAK were found. Significant entry means under dryland conditions for P3 were also present in 1986. Significant parental mean differences were found for P3 under dryland and ECLEAK under irrigated conditions in 1986. No significant hybrid differences or parental versus hybrid

mean differences were found.

Check hybrids were selected on the basis of their range of past yield performance under drought conditions (Kansas Sorghum Performance Test, 1984). It should be noted that no significant differences between check means were found in these environments and water treatments. Either there was no genetic variation for these traits among the checks or more obvious hypothesis was that no significant stress contributed to no significant differences among checks in both years. As reported above, the greatest variability among genotypes for cellular membrane strength and leaf water potential occurred at flag leaf expansion. Table 13 shows the number of days to flag leaf expansion for parental lines and hybrids under irrigated and dryland water treatments in 1985 and 1986, respectively. The range of days to flag leaf expansion was approximately 20 days under both irrigated and dryland conditions in 1985. Similarily, 24 days under irrigated and 18 days under dryland conditions in 1986. Some of the observed variation for P3 and ECLEAK could have been generated by measuring at various growth stages on 80 days after planting and 68 days after planting in 1985 and 1986, respectively. In 1985 flag leaf expansion encompassed the 80 day sample. However, the 68 day sample in 1986 was up to 22 days after flag leaf expansion for all entries except for one parent, KS 14

(Table 13). By sampling after flag leaf expansion parents showed significance for P3 and ECLEAK under dryland and irrigated conditions in 1986 (Table 12). This suggests the importance of sampling all entries after flag leaf expansion when detecting any significant differences for P3 and ECLEAK. It may be hypothesized that environmental stress conditions were needed to show significant differences among hybrids for P3 and ECLEAK. Under the abundant rainfall conditions of 1986 only parents demonstrated any differences (Table 12) due in part, to the diversity among inbreds (Table 2).

Tables 14 and 15 show the mean values of parents and hybrids under both irrigated and dryland conditions. P3 and ECLEAK values shown in these tables tended to coincide with the visual appraisal by Rodgers (Table 2). For example in table 15, SC-118, visually observed to be tolerant to stress, showed a relative high P3 value in relation to the other inbreds. Conversely DWF REDLAN,drought susceptible, showed a low value in relations to the other inbreds for P3. Large values for P3 signify a genotype's better ability to extract water from the soil in relation to the other genotype; such as in the example of SC-118 and DWF REDLAN. SC-118 was able to extract water from the soil at a lower potential then DWF REDLAN. This discovery may seem unimportant given the abundant rainfall conditions in 1985

and 1986, but such findings suggest distinctions among tolerant and susceptible genotypes may be apparent under any environmental condition. The hybrid mean comparison for P3 values did not correspond with those found based upon inbred mean comparisons. For example, hybrids with drought susceptible DWF REDLAN as a parent were found to extract water from the soil at lower potentials then hybrids with drought tolerant SC-118 as a parent under dryland conditions. This was contrary to what has been found when comparing DWF REDLAN and SC-118 inbred means for P3 under dryland conditions. An explanation for these conflicting results could be the effect of abundant rainfall conditions throughout both years. It is hypothesized that stress conditions were needed to demonstrate hybrid differences for P3. Similar results were found to exist among inbred and hybrid means for ECLEAK as seen for P3 (Table 15). ECLEAK is an estimate of percent injury from PEG that various genotypes experienced at 68 days after planting and 80 days after planting. Genotypes such as the drought tolerant SC-118 showed less injury to PEG or more cellular membrane strength than the drought susceptible DWF REDLAN. Therefore inbred values for P3 and ECLEAK tended to show similar differences among genotypes as visually observed by Rodgers under drought stress in 1983. These results loosely suggest that leaf water potential and cellular membrane strength

could be used before anthesis to differentiate among diverse genotypes.

Parents and hybrids correlation coefficients were reported in table 16. GREFLAG was calculated as the ratio of the number of green leaves on the main culm at flag leaf expansion to total number of leaves produced. Rosenow (1981) referred to stay-green as the ability of the plant to retain green leaves and stem in the presence of a drought stress. In the strictest sense, Rosenow has referred to post-flowering stay-green ability of genotypes. However in this study we will broaden the definition to include drought stress conditions before flowering. Without stress, only the senescence of the lower leaves were determined. As leaf water potential decreased, indicated by a larger P3 value, the genotypes increased their capability to extract soil water and senescence of leaves decreased. But under climatic conditions found in 1985 and 1986, soil water was obviously in abundance so genotypes senescence ability and not its defensive response to drought, as calculated by stay-green, was expressed. As cellular membrane strength increased, as measured by a smaller ECLEAK value, senescence increased; maintaining cell stuctural and functional constitutions, i.e. photosynthetic and transpirational processes which increase accumulation of plant biomass.

Rank correlation coefficients (Spearman 1904) of

parental and hybrid entry means for P3 and ECLEAK in each year and water treatment were reported in tables 17 and 18. ECLEAK measured in 1986 under irrigated conditions were significantly correlated with ECLEAK under both dryland in 1986 and under irrigated conditions in 1985 (Table 17). This indicated that rank entry means for ECLEAK under irrigated conditions in both 1985 and 1986 were similar. Therefore, irrigated conditions from year to year did not affect the results for cellular membrane strength. The presence of a significant rank correlation between dryland 1986 and irrigated 1986 for ECLEAK indicated selection for ECLEAK would be independent fo the environmental conditions in which the screening was conducted. One must hasten to point out there was very little difference between water treatments in 1985 and 1986 due to excessive rainfall in both years. P3 measured under dryland in 1986 was significantly correlated with P3 under irrigated in 1986 (Table 18). Correlations with a more drought-stressed environment could well be lower for both traits. The results presented above are encouraging in spite of the excessive moisture which plagued this study for two consecutive years. Conducting this or a similar study under drier conditions would increase our insight into the evaluation and selection of specific screening techniques for drought tolerance in grain sorghum. Additional research is needed to understand

more fully the interaction between stay-green, maturity, leaf water potential, and cellular membrane strength. The use of random lines and accurate measurement of flag leaves at equivalent growth stages would further test the credibility of such a indirect screening technique for drought tolerance in grain sorghum.

Table 4. Combined analysis of variance mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in seven sample dates in irrigated and dryland treatments in 1985 and 1986.

sources	d.f.	CD	TR	LT	P3	ECLEAK
YEAR	1	14429.38**	1814.08	549.63	53269.63	432311.08**
TRT	1	239.85	12.95	161.28	61289.70*	1546.81
TRT*YEAR	1	557.06	1.74	5.75	1233.70	2.40
ENT	27	8.98	6.65	0.79	964.96**	327.48**
ENT*TRT	27	17.04	3.85	1.10	408.15	129.00
ENT*YEAR	27	22.51	3.66	1.42	595.81*	167.30*
ENT*TRT*YEAR	27	42.26	2.78	1.39	308.63	106.48
C.V.%		139.14	47.34	4.32	10.08	17.15

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 5. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 72 days after planting in 1985.

source	d.f.	CD	TR	LT	P3	ECLEAK
REP	2	169.40	190.60	82.90*	1027.80	215.50
TRT	1	14.30	7.17	15.40	1024.10	1538.20
ENT	27	10.30	3.94	1.64	445.20	166.85
ENT*TRT	27	11.90	5.33	1.89	501.44	179.30
C.V.		78.60	33.69	4.11	8.98	12.88

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 6. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 80 days after planting in 1985.

source	d.f.	CD	TR	LT	P3	ECLEAK
REP	2	0.46	186.75	40.95	2924.25*	466.00
TRT	1	0.00	2.36	7.88	38857.00**	4502.00*
ENT	27	0.00	1.09	0.48	311.25*	619.90*
ENT*TRT	27	0.00	0.58	0.56	118.70	58.74
C.V.		71.20	29.81	1.92	6.92	10.00

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 7. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 42 days after planting in 1986.

source	d.f.	CD	TR	LT	P3	ECLEAK
REP	2	0.02	4.86	20.68	19038.50	3766.50
TRT	1	0.01	3.73	100.00*	16205.30	964.30
ENT	27	0.00	0.84	0.98	350.40	263.63**
ENT*TRT	27	0.00	0.68	0.75	277.50	308.85**
C.V.		216.45	234.02	3.30	9.57	31.38

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 8. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 48 days after planting in 1986.

source	d.f.	CD	TR	LT	P3	ECLEAK
REP	2	0.03	5.92	21.59	6324.50	1894.00
TRT	1	0.02	7.84	103.07*	2672.00	2.63
ENT	27	0.00	0.72	0.83	406.15**	284.92**
ENT*TRT	27	0.00	0.69	0.67	156.56	78.51
C.V.		260.92	276.40	3.22	7.00	23.30

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 9. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 54 days after planting in 1986.

source	d.f.	CD	TR	LT	P3	ECLEAK
REP	2	0.89	239.50	61.70	44947.00	1029.30
TRT	1	0.00	6.26	0.17	952.30	11.67
ENT	27	0.05**	10.75**	0.44	558.60*	176.89*
ENT*TRT	27	0.01	3.34	0.29	252.67	87.32
C.V.		13.79	14.90	1.66	7.51	21.89

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 10. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT), blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 61 days after planting in 1986.

source	d.f.	CD	TR	LT	P3	ECLEAK
REP	2	0.69	8.54	211.96	2243.30	51.50
TRT	1	0.21	4.29	27.09	20703.00	152.80
ENT	27	0.05**	6.66*	0.62	675.10	116.33**
ENT*TRT	27	0.01	3.50	0.50	601.50	71.63
C.V.		19.85	22.46	2.46	10.11	13.55

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 11. Mean squares for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT),blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries in irrigated and dryland treatments at 68 days after planting in 1986.

source	d.f.	CD	TR	LT	P3	ECLEAK
REP	2	0.82	1007.80	72.04	39567.00	918.10
TRT	1	0.91	312.70	3.67	17918.00	768.70*
ENT	27	0.05	7.79	0.51	736.25*	110.30**
ENT*TRT	27	0.03	6.70	0.41	386.50	29.77
C.V.		18.36	19.24	2.21	7.98	11.18

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 12. Analysis of variance for blackening pressure (P3) and membrane strength (ECLEAK) of three female lines, five male lines and their fifteen hybrids in irrigated and dryland water treatments in 1985 and 1986, separately.

		1985				1986			
		Irrigated		Dryland		Irrigated		Dryland	
		P3	ECLEAK	P3	ECLEAK	P3	ECLEAK	P3	ECLEAK
REP	2	1339.51**	292.73**	1674.11**	40.82	26283.03**	548.93**	16616.00**	377.32**
ENT	27	252.67	48.51	177.29	72.23	395.71	60.39**	727.07**	79.68*
PARENT	7	296.27	98.31	287.94	49.14	687.52*	132.71**	1007.14*	164.52
HYBRIDS	14	170.07	28.08	178.57	95.24	303.41	44.78	593.41	43.32
MALE	4	53.61	44.14	112.50	92.75	475.83	62.76	707.52	69.76
FEMALE	2	62.22	7.48	60.00	119.54	53.89	65.01	442.22	107.77*
F X M	8	255.28	2.71	241.25	90.36	279.58	30.73	574.17	13.98
CHECK	4	244.16	14.09	52.51	23.71	185.02	15.82	919.17	17.15
P VS H	1	15.22	162.22*	6.11	157.39	773.05	5.66	324.83	247.21
H VS C	1	1175.55*	46.19	61.25	64.76	3.47	12.36	108.89	14.29
ERROR									
POOLED	54	199.77	31.86	130.89	101.93	239.83	27.72	298.47	48.14
C.V.		8.28	6.11	5.69	12.32	7.35	10.16	7.47	14.61

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 13. Parental lines and hybrids mean date of flag leaf expansion of three female lines and five male lines in irrigated and dryland water treatments in 1985 and 1986, separately.

	DATE OF FLAG LEAF EXPANSION (days after planting)+			
1985	Irrigated		Dryland	
FEMALE	INBRED	F_1	INBRED	F_1
SC35-6	86	76	85	78
KS-9	86	79	86	80
DWF REDLAN	79	79	79	80
MALE				
IA 25	73	74	69	74
KS 14	81	80	81	81
81EON86	70	80	71	82
IA 28	77	79	75	84
SC118	66	76	66	74
1986				
FEMALE				
SC35-6	55	60	55	58
KS-9	63	56	58	55
DWF REDLAN	55	54	51	55
MALE				
IA 25	52	52	55	52
KS 14	70	60	69	58
81EON86	51	54	57	54
IA 28	65	57	63	56
SC118	46	50	52	52

+ Planting in 1985 was 19 days earilier than in 1986.

Table 14. Mean parental lines and hybrids for blackening pressure (P3) of three female lines and five male lines in irrigated and dryland water treatments in 1985 and 1986, separately.

	P3 (psi)			
1985	Irrigated		Dryland	
FEMALE	INBRED	F_1	INBRED	F_1
SC35-6	160.00	168.67	191.67	201.67
KS-9	163.33	167.33	191.67	199.67
DWF REDLAN	158.33	171.33	190.00	208.61
MALE				
IA 25	183.33	168.89	211.67	198.33
KS 14	156.67	170.56	196.67	198.22
81EON46	171.67	170.00	215.00	203.33
IA 28	178.33	165.00	206.67	201.67
SC118	173.33	171.11	205.00	206.67
1986				
FEMALE				
SC35-6	211.67	210.67	221.67	226.67
KS-9	185.00	213.00	206.67	228.00
DWF REDLAN	205.00	214.00	226.67	236.67
MALE				
IA 25	193.33	215.00	230.00	223.89
KS 14	198.33	203.33	243.33	222.22
81EON86	213.33	216.11	233.33	227.78
IA 28	201.67	206.67	255.00	243.89
SC118	235.00	221.11	263.33	234.45

Table 15. Mean parental lines and hybrids for membrane strength (ECLEAK) of three female lines and five male lines in irrigated and dryland water treatments in 1985 and 1986, separately.

	ECLEAK (%)			
	Irrigated		Dryland	
FEMALE	INBRED	F_1	INBRED	F_1
1985				
SC35-6	91.54	92.73	85.69	77.63
KS-9	97.55	93.95	81.49	80.83
DWF REDLAN	92.71	93.89	90.38	49.32
MALE				
IA 25	81.77	93.89	84.13	81.27
KS 14	87.00	91.32	79.24	83.36
81EON86	92.21	91.33	86.14	81.64
IA 28	83.50	93.82	77.92	75.01
SC118	96.31	96.36	84.97	80.57
1986				
FEMALE				
SC35-6	51.44	51.33	44.67	45.31
KS-9	55.63	54.46	49.04	50.06
DWF REDLAN	50.49	50.52	44.82	48.84
MALE				
IA 25	58.16	54.77	50.16	48.49
KS 14	45.74	49.24	38.91	46.67
81EON86	57.87	53.46	56.27	52.78
IA 28	38.63	53.78	33.58	45.45
SC118	54.11	49.30	38.02	49.62

Table 16. Correlation coefficients between grain yield (GYLD), seed number (SNB), seed weight (SW), flowering date (BLM), physiological maturity (PM), plant height (HT), and the ratio of number of green leaves verses plant growth stage at date of flag leaf expansion (GREFLAG) versus response traits blackening pressure (P3), cellular strength (ECLEAK) in irrigated and dryland water treatments in 1985 and 1986, separately.

	1985				1986			
	irrigated		dryland		irrigated		dryland	
trait	P3	ECLEAK	P3	ECLEAK	P3	ECLEAK	P3	ECLEAK
GYLD	0.15*	-0.05	0.14	-0.02	0.02	-0.04	-0.08	0.11*
SNB	0.06	-0.02	0.05	0.00	0.06	-0.08	-0.03	0.08
SW	0.12	-0.04	0.13	-0.02	-0.08	0.10	-0.14**	0.07
BLM	-0.11	0.02	-0.26**	0.00	0.05	-0.09	0.19**	-0.17**
PM	-0.12	0.03	-0.28**	0.00	0.00	0.05	0.11*	-0.10*
HT	0.01	-0.01	-0.03	0.01	-0.07	0.02	-0.05	0.07
GREFLAG	0.12	-0.83**	-0.30**	-0.74**	-0.50*	-0.37**	-0.29*	-0.44**

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 17. Ranked correlation coefficients of cellular membrane strength (ECLEAK) for each year and water treatment. Cellular membrane strength under irrigated treatment in 1985 (EC85I), cellular membrane strength under dryland treatment in 1985 (EC85D), cellular membrane strength under irrigated treatment in 1986 (EC86I), cellular membrane strength under dryland treatments in 1986 (EC86D).

	EC85I	EC85D	EC86I	EC86D
EC85I	1.00	0.15	0.25*	-0.03
EC85D		1.00	0.12	-0.10
EC86I			1.00	0.51**
EC86D				1.00

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Table 18. Ranked correlation coefficients of blackening pressure (P3) for each year and water treatment. Blackening pressure under irrigated treatment in 1985 (P385I), blackening pressure under dryland treatment in 1985 (P385D), blackening pressure under irrigated treatment in 1986 (P386I), blackening pressure under dryland treatment in 1986 (P386D).

	P385I	P385D	P386I	P386D
P385I	1.00	0.32	0.02	-0.17
P385D		1.00	-0.01	-0.15
P386I			1.00	0.57**
P386D				1.00

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.

Figure 1. The effect of plant growth stage on blackening pressure on 28 entries under irrigated and dryland treatments over sampling dates recorded in days after planting in 1986.

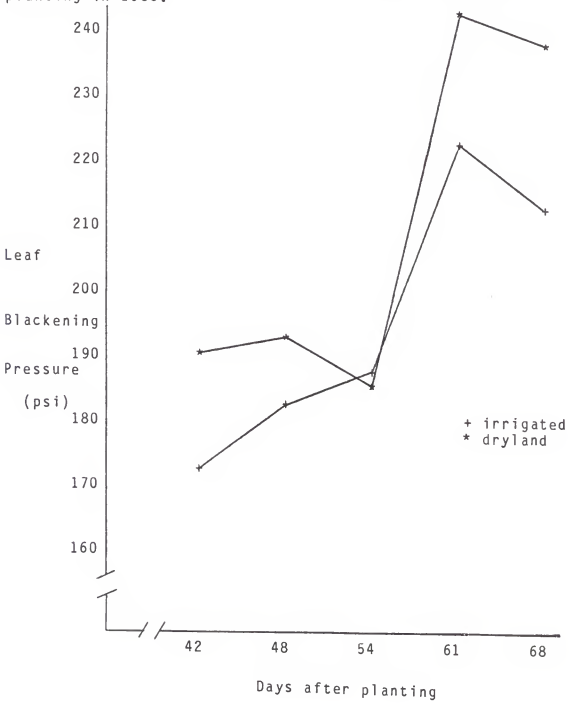
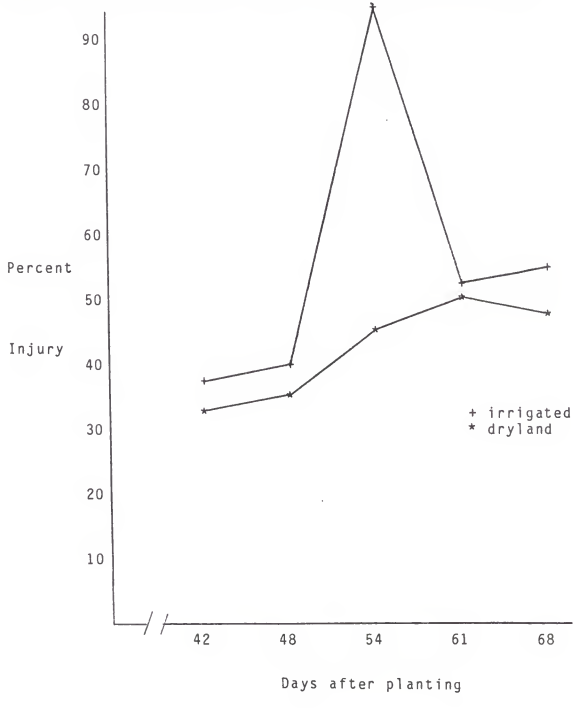


Figure 2. The effect of plant growth stage on percent injury by the osmotic potential of polyethylene glycol on 28 sorghum entries under irrigated and dryland treatments over sampling dates recorded in days after planting in 1986.



SUMMARY

Eight parental sorghum inbreds, known to range in phenotypic response to drought from susceptible to very tolerant, were mated in a 3 line x 5 tester factorial design. The experimental material included eight parents, 15 F_1 hybrids,and 5 commercial grain sorghum hybrids planted in a randomized complete block design with a split-plot arrangment under two water treatments. Stomatal conductance, leaf temperature, transpirational rate, leaf water potential, and cellular membrane strength were measured during weekly intervals from approximately 40 days after planting until anthesis.

The results indicated that significant entry mean differences for leaf water potential and cellular membrane strength were not detected until sorghum entries reached the flag leaf stage of development. No significant heterosis was expressed among genotypes under irrigated or dryland conditions in either 1985 or 1986. No significant GCA or SCA effects were expressed in these sorghum entries for leaf water potential and cellular membrane strength under both irrigated and dryland conditions. The evidence suggested as leaf water potential and cellular membrane strength increased senescence ability decreased. There was no general relationship between parental lines and their topcross performance for leaf water potential and cellular

membrane strength. Cellular membrane strength measurements of sorghum entries in 1986 under irrigated conditions were ranked similarly with those under irrigated in 1985 and dryland conditions in 1986. Leaf water potential measured on sorghum entries under dryland conditions was ranked with those under irrigated in 1986.

LITERATURE CITED

Begg, J. E. 1980. Morphological adaptations of leaves to water stress. p. 33-42. In: Adaptation of Plants to Water and High Temperature Stress. N. C. Turner and P. J. Kramer (eds.), Wiley-Interscience, New York.

Bennett, R. 1978. Sun angles for design. Robert Bennett, Bala Cynwyd, Pennsylvania. 76 pp.

Blum, A. 1970. Effect of plant density and growth duration on grain sorghum yield under limited water supply. Agron.J.62:333-336.

Blum, A.and A. Ebercon. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21:43-47.

Blum, A. and A. Ebercon. 1976. Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. Crop Sci. 16:428-431.

Bristow, K. L., W. H. van Zyl, and J. M. de Jager. 1981. Measurement of leaf water potential using the J14 press. J. Exp. Bot. 32:851-854.

Brown, L. 1965. Africa, a natural history. Random House. New York.

Campbell, G. S. and S. F. Brewster. 1975. Leaf water potential, matric potential and soil water content measured with a simple hydraulic press. Paper presented at the Western Regional Res. Proj. W-67: Quantifcation of water-soil plant relations for efficient water use. Honolulu, Hawaii, Jan. 1975.

Cox, J. R. and H. G. Hughes. 1982. Leaf water potentials of perennial grasses--leaf press and pressure chamber evaluation. J.Range Management 35(1):5-6.

Dalton, L. G. 1967. A positive regression of yield on maturity in sorghum. Crop Sci. 7:271.

de Wet, J. M. and J. R. Harlan. 1971. The origin and domesticationof Sorghum bicolor. Economic Botany 25:128-135.

Doggett, H. 1965. The development of cultivated sorghums. p. 50-69. In:Crop Plant Evolution, Sir Joseph Hutchinson (ed.), Cambridge University Press, London.

Gardner, B. R., B. L. Blad, R. E. Maurer, and D. G. Watts. 1981. Relationship between crop temperature and the physiological and phenological development of differentially irrigated corn. Agron.J. 73:743-747.

Garrity, D. P., C. Y. Sullivan, and D. G. Watts. 1984. Changes in grain sorghum stomatal and photosynthetic response to moisture stress across growth stages. Crop Sci. 24:441-446.

Henzell, R. G., K. J. McGee, C. H. M. van Bavel and K. F. Schertz. 1975. Method for screening sorghum genotypes for stomatal sensitivity to water deficits. Crop Sci. 15:516-518.

Hershfield, D. M., D. L. Brakensiek and G. H. Comer. 1973. Some measures of agricultural drought. In: Proc. second Int'l. symp. on hydrology-floods and droughts. Water Resources Publ. p.491-502, Ft. Collins, CO.

Hicks, S. K., R. J. Lascano, C. W. Wendt, and A. B. Onken. 1986. Use of a hydraulic press for estimation of leaf water potential in grain sorghum. Agron.J. 78:749-751.

Idso, S. B., J. L. Hatfield, R. D. Jackson, and R. J. Reginato. 1979. Grain yield prediction: extending the stress-degree-day approach to accommodate climatic variablity. Remote Sensing Environment 8:267-272.

Jones, C. A. and A. Carable. 1980. Estimation of leaf water potential in tropical grasses with the Campbell-Brewster hydraulic press. Trop. Agric. 57:305-307.

Jordan, W. R. and R. L. Monk. 1980. Enhancement of drought resistance of sorghum: Progress and limitations.p. 185-204. In Proc., 35th Annual Corn and Sorghum Res. Conf., Am. Seed Trade Assn., Chicago, IL.

Jordan, W. R. and F. R. Miller. 1980. Genetic variability in sorghum root systems: Implications for drought tolerance. p. 383-399. In Adaptation of Plants to Water and High Temperature Stress. N. C. Turner and P. J. Kramer (eds.), Wiley-Interscience, New York.

Kansas Farm Facts (1985) 68th Annual Report. Crop and Livestock Reporting Service. Kansas State Board of Agriculture. p. 22-29.

Kansas Sorghum Performance Tests. 1984. Report of Progress Agricultural Experimental Station. Kansas State University, Manhattan, Kansas. p. 27-29.

Levitt, J. 1972. Responses of Plants to Environmental Stresses. Academic Press, New York.

Liver-Munoz, M., A. M. Dhote, P. K. Verma, V. Gonzalez-Hernandez, L. E. Mendoza-Onofre, V. B. Ogunleta, J. R. Rice and J. D. Eastin. 1986. Sorghum growth stages. Pres. for the Chinese Sorghum Conference, Aug. 20-24, 1986. University of Nebraska, Dept. of Agronomy. Lincoln, N.E.

Martin, J. H. 1930. The comparative drought resistance of corn and sorghum. Agron.J. 22:993-1003.

Murdock, G. P. 1959. Staple subsistence crops of Africa. Geogr. Rev. 50:521-540.

O'Toole, J. C. and T. B. Moya. 1978. Genotypic variation in maintenance of leaf water potential in rice. Crop Sci 18: 873-876.

O'Toole, J. C., N. C. Turner, O. P. Namuco, M. Dingkuhn, and K. A. Gomez. 1984. Comparison of some crop water stress measurement methods. Crop Sci. 24:1121-1128.

Ogren E. and G. Oquist. 1985. Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. Planta 166:380-388.

Peacock, J. M., S. N. Azam-Ali, and R. B. Matthews. 1985. An approach to screening for resistance to water and heat stress in sorghum [Sorghum bicolor (L.) Moench]. Paper presented at an International Arid Lands Research and Development Conference "Arid Lands: Today and Tomorrow", 20-25, October 1985.

Pearson, K. and A. Lee. 1902. On the laws of inheritance in man. I: Inheritance of physical characters. Biometrika 2:357.

Quizenberry J. E. 1981. Breeding for drought resistance and plant water use efficiency. p.193-209. In: Breeding Plants for Less Favorable Environments, M.N. Christiansen and C. F. Lewis (eds.), Wiley-Interscience, New York.

Rosenberg, N. J. 1979. Drought in the Great Plains: Research on impacts and strategies. p.1-17. Water Resources Publications. Littleton, Colorado.

Rosenow, D.T. and L. E. Clark. 1981. Drought tolerance in sorghum. p. 19-31. In: Proc., 36th Annual Corn and Sorghum Res. Conf., Am. Seed Trade Assn., Chicago,IL.

Saarinen, T. F. 1966. Perception of the hazard on the Great Plains. Dept of Geography, University of Chicago, Res. Paper 106:183.

Scholander, P.F., H. T. Hammel, E. A. Hemmingsen, and E. D. Bradstreet. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. Natl. Sci. USA 52:119-125.

Seropian, C. and C. Planchon. 1984. Physiological responses of six bread wheat and durum wheat genotypes to water stress. Euphytica 33:757-767.

Sharkey, T. D. and M. R. Badger. 1982. Effects of water on photosynthetic electron transport, photophosphorylation, and metabolite levels of Xanthium strumarium mesophyll cells. Planta 156:199-206.

Shayo-Ngowi, A. and G. S. Campbell. 1980. Measurement of matric potential in plant tissue with ahydraulic press. Agron.J. 72:567-568.

Spearman, C. 1904. Phoof and measurement of association between two things. Am. J. Psych.15:72-103.

Stout, D. G. and G. M. Simpson. 1978. Drought resistance of Sorghum bicolor. I. Drought avoidance mechanisms related to leaf water status. Can.J. Plant Sci. 58:213-224.

Sullivan, C. Y. and W. M. Ross. 1979. Selecting for drought and heat resistance in grain sorghum. p. 263-281. In: Stress Physiology in Crop Plants, H. Mussell and R. C. Staples (eds.), Wiley-Interscience, New York.

Sullivan, C. Y. and A. Blum. 1970. Drought and heat resistance of sorghum and corn. p. 55-66. In: Proc., 25th Annual Corn and Sorghum Res. Conf., Am. Seed Trade Assn., Chicago, IL.

United States Department of Agriculture. 1986. World crop production. Foreign Agriculture Circular. July 14, 1986. p. 13. Washington D. C.

Vanderlip R. L. 1979. How a sorghum plant develops. Cooperative Extension Service, Kansas State University, Manhattan, Kansas.

Waring, R. H. and B. D. Cleary. 1967. Plant moisture stress: evaluation by pressure bomb. Science 155:1253-1254.

Weatherley, P. E. 1970. Some aspects of water relations. Adv. Bot. Res.3:171-206.

World Meteorological Organization. 1975. Drought and agriculture. p.128. In Tech. Note 138. World Meteorological Organization No. 392, Geneva.

Wright, G. C. and R. C. Smith. 1983. Differences between two grain sorghum genotypes in adaptation to drought stress. II root water uptake and water use. Aust.J. Agric. Res. 34:615-626.

Wright, G. C. and R. C. Smith and J. M. Morgan. 1983. Differences between two grain sorghum genotypes in adaptation to drought stress. III physiological responses. Aust.J. Agric. Res. 34:627-636.

APENDIX

Table A. Number of observations, mean, variance, standard error of the mean, minimun, and maximun values for grain yield (GYLD), seed number (SNB), seed weight (SW), flowering date (BLM), physiological maturity (PM), plant height (HEIGHT), and the ratio of number of green leaves versus plant growth stage at date of flag leaf expansion (GREFLAG) measured throughout the growing season in 1985 and 1986.

traits	1985 mean	1986 mean
GYLD (kg/ha)	17132.53	7605.82
SNB (no.)	106.00	333.33
SW (kg)	6046.60	7143.69
BLM (days)	79.77	65.85
PM (days)	113.94	102.61
HT (cm)	118.41	122.50
GREFLAG (leaves no.)	0.90	0.61

Table B. Number of observations, mean, variance, standard error of the mean, minimun, and maximun values for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT),blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries measured seventy-two and eighty days after planting in 1985.

traits	72 day mean	80 day mean
CD ($cm\ s^{1}$)	4.63	11.90
TR ($mg\ cm^{-1}\ s^{-1}$)	6.43	2.78
LT (^{o}C)	33.49	30.41
P3 (psi)	192.29	185.80
ECLEAK (%)	84.79	87.08

Table C. Number of observations, mean, variance, standard error of the mean, minimun, and maximun values for stomatal conductance (CD), transpiration rate (TR), leaf temperature (LT),blackening pressure (P3), and membrane strength (ECLEAK) from the twenty-eight entries measured 42, 48, 54, 61, and 68 days after planting in 1986.

traits	42 day mean	48 day mean	54 day mean	61 day mean	68 day mean
CD ($cm\ s^{1}$)	Ø.Ø2	Ø.Ø2	Ø.78	Ø.67	1.Ø6
TR ($mg\ cm^{-1}\ s^{-1}$)	Ø.38	Ø.31	13.52	8.54	14.Ø2
LT (^{o}C)	29.41	29.4Ø	31.43	3Ø.33	31.62
P3 (psi)	181.84	19Ø.ØØ	192.26	234.91	22Ø.83
ECLEAK (%)	34.58	38.2Ø	46.Ø9	49.ØØ	49.61

COMPARISON OF SCREENING TECHNIQUES FOR TRAITS RELATING TO DROUGHT TOLERANCE IN GRAIN SORGHUM

by

WILLIAM JOSEPH MAJERUS

B.S., University of Minnesota, 1983

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1987

ABSTRACT

Although several screening techniques involving various physiological and morphological traits have proven effective, implementing these screening methods in a breeding program have been hypothesized to be both labor and time intensive. In this study the objectives were to compare certain physiological and/or morphological measurements taken prior to anthesis on the bases of their ability to discriminate between genotypes under stress and their inheritance. Eight sorghum (Sorghum bicolor (L.) Moench) parent lines, ranging from susceptible to tolerant in reaction to water stress, were mated in a 3 line x 5 tester factorial design. The eight parents, 15 F_1's,and 5 commercial checks were tested in a randomized complete block design with a split-plot arrangement under 2 water treatments. Stomatal conductance, leaf temperature, transpirational rate, leaf water potential, and cellular membrane strength were measured during weekly intervals from approximately 40 days after planting until anthesis. Significant entry mean differences for leaf water potential and cellular membrane strength were detected upon flag leaf stage of development. No significant parent vs progeny correlations, GCA or SCA effects and heterosis were found for these two plant traits. Both traits were significantly correlated with senescence at this pre-anthesis stage.