DEVELOPMENT OF A SCREENING TEST FOR WATER-USE EFFICIENCY IN GRAIN SORGHUM

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IN GRAIN SORGHUM

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CHAPTER I

INTRODUCTION

There is considerable conflict between traditional and modern lines of research on the usefulness of selections obtained under controlled greenhouse or growth chamber conditions (43). Many researchers feel that selections obtained in this manner are superior under the same environmental conditions only, and the advantages disappear if the plants are placed in different environments. Even if this is true, the fact remains that such selections may carry genes that improve performance under adverse environments, but remain masked under favorable growing conditions.

The most important advantages of research conducted under controlled conditions are: 1) the repeatability and exact control of environmental factors, and 2) the capacity to work year-round. In addition the advantage of working with large populations at low cost, makes this method feasible for screening studies. Control of the environment under field conditions is impossible and great fluctuations occur from year to year, making it difficult to select for drought resistance in the field, and reducing the chance of obtaining a similar performance every year.

The objectives of this study were to develop a practical screening test for dessication tolerance in Grain Sorghum under water stress, and to demonstrate the effectiveness of the screening technique in improving water use efficiency of selected lines.

CHAPTER II

REVIEW OF LITERATURE

Plant Growth Stages and Effects of Water Stress

Plant growth has been divided into three stages with similar duration in time. GS-1 goes from planting to panicle initiation, GS-2 from panicle initiation to mid-bloom, and GS-3 from mid-bloom to physiological maturity, measured by black layer formation (13). Traditionally, GS-3 has been studied more thoroughly in respect to drought tolerance due perhaps to its economic importance and also because it is the most vulnerable stage. In recent years GS-1 and GS-2 are being studied in more detail. Their importance lies in the fact that these stages are the basis for the final product, and very little is known about their contribution to yield.

Maturity has been measured as total number of days from planting to flowering (63), but partitioning this period into stages facilitates evaluation of the particular contribution of each stage. It has been determined that as GS-1 increases more leaves are formed and time to reach maturity is increased (13). Number of seeds per head is a component of yield related directly to GS-1, where the potential of the plant to form the primary branches is determined (19, 54). Floret initiation is most influenced by conditions prevailing at the end of GS-1, especially high temperature. Under excessively high temperatures

the number of florets is reduced initially, and embryo abortion is observed as a delayed effect, causing a reduction in yield (19).

The formation of more leaves during GS-1 influences directly the production of photosynthates; these substances will help the plant to sustain larger heads with more seeds (13). Growth and yield are impaired if proper moisture conditions are not present during the stage of rapid growth and differentiation (70). Initiation and differentiation of reproductive primordia are two processes extremely sensitive to water stress, where rate of cell division is reduced but not suspended until very severe conditions occur (27). Stunted growth is usually related to an impairment of cell elongation under slight stress where even small diurnal variations in water content inhibit cell enlargement (37, 63).

Any adverse condition that the plant experiences under GS-1 or GS-2 will be reflected at later stages, either in morphological or physiological changes (37). Sullivan et al (77) found that short intervals of high temperatures at 15 days of age increased yield of treated plants. Hardening is a common phenomenon at this stage.

As the plant matures, all basic fuctions in the plant change, as well as requirements. Water consumption has a slow increase from emergence to the end of GS-1 and a steep increase during GS-2 until the end of the vegetative stage, decreasing progressively from then on (71). Respiration increases drastically during GS-1 and decreases steadily after 20 days of age (45). Water stress tolerance and heat tolerance decreases from the vegetative stage to the grain filling stage (6, 42) reaching the cycle's low in late boot to bloom stage (53), with head blasting at later stages (86). If water deficit occurs before the

period of floral initiation, the effect is minimized, and normal growth is observed. As the stress period approaches the stage of floral initiation an imbalance in hormone production develops (37) and flowering is hastened with a great reduction in vegetative growth, of as many as three leaves less than normal (83). Sorghum plants in GS-3 or grain-filling period are susceptible to water stress. A reduction in photo= synthate production at this stage leads to a large decrease in yield, as determined by lower test weight, especially after all photosynthate reserves have been depleted (24).

Effects of Water Stress on Stomatal Response

Plant behavior is not dependent upon atmospheric or edaphic conditions alone, rather it is a complex integration of factors that affect internal water balance in the plant (29). Kramer (48) indicates that any study of plant behavior under water stress should include all three components of the soil-plant-atmosphere continuum (SPAC).

The SPAC system defines the soil as a reservoir of available water and the atmosphere as a sink of unlimited capacity. The plant acts as a bridge linking both entities (35). Some controversy about the function of the plant in this system exists. Some authors support the characterization of the plant as a passive body, acting like wicks in the field (81). Others maintain that limited control of transpiration is observed, especially under certain environmental conditions. It has been determined that stomata close at certain intervals when evaporation demand is excessive and/or temperature is high (56, 80). Stomata remain closed during mid-day until water absorption recovers internal water potential and turgidity (70). Stomata also close with high

light intensities, high temperature and low external vapor pressure (50).

Closing of stomata benefits the plant by reducing transpiration, but may result in an increase in leaf temperature which may cause permanent injury. Carbon dioxide uptake is reduced if the stomata are closed, thus reducing synthesis of sugars (35).

The mechanism of stomatal response remains somewhat obscure. It is known that light and temperature trigger stomatal opening with K^+ having a predominant role (78). Potassium ions enter the guard cells raising osmotic pressure, and making water potential more negative. This causes water to flow into the guard cells raising their internal hydrostatic pressure and inducing an expansion of the cell. As guard cells increase in size, cell walls exert pressure in opposite directions forming an elliptical pore between them. This pore serves as an exit for water vapor out of the leaf, and as an entrance for CO_2 and O_2 (25).

Loss of water through stomata encounters the same group of resistances as that of CO_2 flux, with the exception of chloroplast resistance (60). Both fluxes are dependent upon stomatal opening and this makes it possible to measure both simultaneously. The validity of the use of Porometers to estimate leaf diffusive resistance has been questioned lately because it does not represent water status inside the plant (5), and it requires the sampling of several leaves on a single individual for a proper characterization (9).

Two types of water dificiency can be found in a plant at any moment. One is a day-time deficiency and the other is a residual

deficiency. The later results from the fact that plants are not able to compensate during the night all the water lost during the daytime (32). Water deficits caused by excessive transpiration are usually of short duration if water absorption remains high. If a lag between transpiration and absorption exists, a deficit is likely to occur. If this deficit is prolongued it may require all night for a complete recovery (81).

If the plant has been subjected to successive periods of water stress, it develops a conditioning effect by which the plants are hardened (76). Stomata of pre-conditioned cotton plants remained open at lower leaf water potential (-14 bars) than non-stressed plants (8). McCree (55) found a similar response on grain sorghum grown in a growth chamber. This alteration between leaf water potential and turgor potential results in a higher water content in the leaf of stressed plants at the same water potentials. Two main reasons are given for this modification: 1) an increase in tissue elasticity (Volumetric-Elastic Modulus) and 2) and increase in osmotic potential caused by a net increase in solute concentration (36, 82). Jones and Turner (41) have demonstrated that an increase in leaf water potential in grain sorghum is associated with a change in osmotic potential from -1.1 to -1.6 MegaPascals (-11 to -16 bars) after a period of stress. This preconditioning effect of dehydration has less effect in cells that contain a larger proportion of protoplasm and a smaller vacuole (40). Henckel (32) indicated that true xerophytes differed from mesophytes by having a higher protoplasmic elasticity which conferred to the cell a higher degree of resistance to water stress. Hydrophyllic viscosity of protoplasm increases in the leaf between emergence and tillering, then falls

sharply at flowering time. It seems that water reserve in the protoplasm is more stable than that of the vacuole, and among cell components, the chloroplast is the least affected (32).

Breeding for Drought Resistance

Breeding for drought resistance is not an easy task due to the complexity of the problem. It seems that the more it is known the more complex it becomes. Genetic improvement of mechanisms of avoidance should be distinguished from tolerance to water stress. Avoidance means that the plant is in reality never subjected to water stress during any phase of its life cycle. Tolerance means the ability to survive after water content inside the plant has been reduced drastically (52).

Plants with high tolerance are usually associated with decreased growth, small leaves, small cells and lower metabolic rates (71). Plants that avoid stress have little capacity to survive if such conditions occur. If the mechanism of avoidance cannot maintain an adequate level of water in the plant, the plant will be severely affected.

Breeding for drought resistance should produce cultivars that give economic yields under severe conditions of stress (38). It seems appropriate to include both avoidance and tolerance mechanisms in the breeding program (57). Selection must be performed on characters that are easy to identify and measure, otherwise it will be impractical (38). Some characters are not easy to measure, but sufficiently important to be included in any program. They are the characters associated with root growth, stomatal response, dessication tolerance, plant architecture, growth rate, etc. (57).

Because vegetative growth and yield are not always positively correlated, growth habit, as expressed by narrow leaves and low shoot/root ratio, can be misleading in breeding wheat for production under water stress (44). On the other hand, excessive vegetative growth can have a depressing effect on yield by using excessive amounts of water under limited moisture conditions (25).

It is known that stomata exert some degree of control upon transpiration during periods of darkness and low light (81), and that intra-varietal differences do occur either in stomatal sensitivity (33) or in survival capacity after succesive cycles of stress (61). Thus it is necessary to develop proper techniques that permit a fast and effective screening of large populations, in order to identify these characteristics.

C_4 Carbon Pathway

Higher plants have been recently divided into three main groups according to their mode of carbon fixation. C_3 plants have the normal Calvin cycle producing phosphorylated compounds. C_4 and CAM plants produce dicarboxylic acids as the initial products of photosynthesis, constituting two separate groups with a similar process (20).

C_4 plants originated in tropical regions under extremes of light, temperature, and dryness conferring on special characteristics needed for survival. Normal leaves of C_3 plants have spongy mesophyll layers and palisade cells differing from C_4 leaves. C_4 leaves have a Kranz anatomy, a specialized arrangement of vascular bundles surrounded by an inner parenchyma layer and an outer mesophyll layer (57). This type of arrangement increases leaf efficiency in trapping and distributing CO_2

due to the closeness of the mesophyll layer and the bundle sheath. Dicarboxylic acid is synthesized in the mesophyll and transported to the bundle sheath where it is decarboxylated to provide CO_2 to the Calvin cycle (20).

Grain sorghum is a C_4 plant and should be investigated more thoroughly in order to measure respiration and transpiration rates during photoperiod and during dark periods. Downes (18) points out that high temperature during the night cause a reduction in yield in grain sorghum, perhaps due to an impairement of the amount of substrates present in the plant for dark respiration. Carlson et al (10) found evidence that confirms this assumption, with dark CO_2 evolution increasing under moderate stress, and decreasing when water potential reached a level of -20 atmospheres. C_4 plants apparently lack photo-respiration, as measured by a low compensation point. Less CO_2 is released into free air, being accumulated as C_4-dicarboxylic acid and trapped in the mesophyll layer instead (20).

Slatyer (69) found evidence to support the theory that C_4 species are more efficient in water-use. He compared C_3 versus C_4 species of Atriplex and found that C_4 had higher net assimilation rates, more water consumption, higher leaf diffusive resistance, and as a consequence of these factors, more leaf area was developed during the first 15 days of growth. All this contributed to a higher efficiency in water-use as measured by the ratio growth/water-use, for C_4 species (78).

Under ideal conditions C_4 plants have an accelerated growth rate due to high photosynthetic rates (23). Leaves become light saturated at higher light intensities and can tolerate higher temperatures, with an optimum of 30 to 40 C (20). C_4 plants are more efficient in

water-use, producing more dry matter per unit of water consumed (51), either by decreasing transpiration or by increasing photosynthesis (23). Photorespiration depends on substrate produced during photosynthesis. Where C_4 plants have the capacity to maintain higher rates of photosynthesis under high moisture stress, a higher respiratory rate can be sustained (17). Moisture stress interacting with low light intensity affects photosynthesis by lowering the rate of synthesis of sugars (54).

Sources of CO_2 are the turbulent air layer outside the leaf, and the CO_2 that evolved from respiration and photorespiration. Flux of CO_2 finds several resistances in its path towards the site of synthesis in the chloroplast. The first obstacle is the air layer surrounding the leaf (r_a), and the next is leaf resistance (r_l) which can be partitioned into stomatal and intercellular-air space components. Inside the leaf a mesophyll resistance (r_m) composed of cell wall, plasmalemma and cytoplasm resistances act against the entrance of CO_2 into the cell. Finally inside the cell a chloroplast resistance (r_{chl}) composed of membrane and stroma resistances, is the last obstacle for the flux of CO_2 to reach the enzymes involved in CO_2 fixation inside the bundle sheath (60).

A Kranz leaf contains chloroplasts in the bundle sheath cells, and this permits these cells to photosynthesize as any normal cell (20). The proximity of the site of synthesis of sugars to the vascular bundle insures a rapid translocation of photosynthates.

Seed Germination in Osmotic Solutions

Although it is widely accepted that growth and yield are directly dependent on plant water potential and indirectly on soil water

potential (47), great consideration should be given to the inherent genotypic capacity of a plant to withstand water stress. For water to flow from soil to plant to atmosphere, a gradient in water potential is essential, and as long as the plant maintains an osmotic potential at a level higher than that of the soil, water will enter the root (35), until an equilibrium is reached.

A seedling exhibits a structural polarity starting on germination, where the radicle constitutes the absorbent organ and the epicotyl is the transpiration part (80). Water absorption is a characteristic of living cells, where metabolic inhibitors that cause death of root cells increase water absorption by overcoming the resistance created by the cell (46).

An increase in internal cell sap concentration, as a result of different solute concentrations between cells or organs within a plant determines the build-up of internal gradients of osmotic pressure (62).

Several chemicals have been used to simulate drought conditions for seed germination. These chemicals increase the osmotic potential in the solution, reducing the availability of water molecules (2). Any substance that modifies water availability could theoretically be used to select seeds with greater capacity of imbibition and absorption of water. NaCl, poly-vinyl phenol (PVP), sucrose, glucose, Mannitol, and others have been used (84), with different results. Some problems are encountered with certain chemicals when the concentration is high, developing a toxicity that impedes germination and normal growth.

Mannitol appears to be non-toxic (79), while PVP and NaCl can inhibit germination completely (84). Sodium ions affect plant development by reducing absorption of Ca, K, S, Mn, Cu, Zn, B, and Cl, and by

increasing Na, N, and Mo (4). Carbowax (polyethylene glycol) is used in germination tests, as well as a conductor in the evaluation of dessication tolerance with the leaf-disk method (75).

Seeds differ in total amount of water absorption and in the rate of absorption (72). In order to attain germination each seed must reach a specific level of hydration that varies from 30 to 50% according to species (38). Germination percentage is not influenced as long as the soil remains above the wilting coefficient at -15 atmospheres (14).

The difference between a poor and a good stand can be determined by genotypic differences. Variation among species is a proven fact (4) while variation within species remains obscure, yet it is evident in several crops (3, 12, 15, 28).

Germination in Mannitol and field emergence was positively correlated in Yogo wheat. Yogo wheat was significantly better than other cultivars in germination and seedling development in artificially-induced drought and under limited moisture conditions in the field (31). Dotzenko and Haus (15) demonstrated that selection effectively improved the ability to germinate under high concentrations of Mannitol in alfalfa. According to Younis et al (86) this ability is not related to the capacity of the plant to harden under moisture stress. Heat tolerance is more related to survival capacity under stress. Rodger et al (79) determined that hardy varieties of alfalfa manifested a greater decrease in germination at high concentrations of Mannitol, as compared to non-hardy varieties.

Schwen et al (67) working with legumes found that selections surviving at high concentrations of Mannitol produced progeny with

better germination in Mannitol solutions, but that heat, cold, and drought tolerance were not modified.

Crested wheatgrass tolerance to salinity was improved by selecting at high salinity levels; the best improvement was obtained from selections derived from seedlings that survived at 18,000 ppm producing progeny that had better germination at high osmotic concentrations (12).

CHAPTER III

MATERIAL AND METHODS

Screening and Selection Techniques

One hundred and sixteen grain sorghum cultivars were screened in the preliminary studies. These cultivars are listed in Appendix Table XXII. A wide range of responses were observed due to differences in origin and genetic background, making it possible to differentiate for resistance to stress among cultivars.

Partial germination

Twenty-five randomly selected seeds were uniformily spaced in 7.3 x 7.3 x 2.8 cm plastic boxes with lids, over two layers of germination substrate moistened with 6 cc of distilled water. Standard germination procedures were given as recommended by the Association of Official Seed Analysts (1). Alternate temperatures of 30 and 20 C for day and night, respectively, and a photoperiod of 9 hours were used. Germination counts were made at 24-hour intervals with a final count after 7 days. Seeds were pretreated with Captan-50.

Seeds that germinated during the second day (48-hour count) were isolated from the remaining seeds and constituted the group of seedlings used in the screening tests. The remaining seedlings were transplanted to the greenhouse for seed production and were considered as a representative sample from the original cultivar.

Transplant

Immediately after isolation, five seedlings from each cultivar were transplanted to 240 cc (8 oz) styrofoam cups filled with 250 grams of dry soil. The soil was obtained from the OSU Agricultural Experimental Station at Perkins, OK., and was sieved and autoclaved prior its utilization. One seedling was transplaned to each cup, and care was taken to avoid damaging the radicle by protecting it with a pair of tweezers in the process of insertion into the soil. This method limited variability between and within cultivars by selecting seedlings with uniform emergence and radicle length. Immediately after transplanting each cup was placed on a plate containing 50 cc of tap water. The water was admitted to the soil by capillarity through a 6 mm hole in the bottom of the cup. This method brought the soil to "field capacity" without disturbing the structure. Compaction was reduced to a minimum and the contact between roots and soil particles improved. It was observed that soil moisture was maintained longer using this procedure than when surface irrigation was used.

Growth Chamber Conditions

In order to reduce variability between cultivars with same origin, each group of cultivars was screened separately and in order to increase accuracy of estimates each group was screened twice. The screening tests were conducted in a Sherer-Gillete growth chamber, Model CEL 255-6, set to provide the following environmental conditions:

Temperature: 30 $\pm$ 0.5 C, constant for day and night.

P.A.R. : 120 uE $m^{-2}sec^{-1}$, at 75 cm from source (plant level)

Photoperiod of 11 hours, from 7 am to 6 pm.

No other factor was controlled in this study. Relative humidity was normally low with a minimum of 10% during light period and 20% at night. Air exchange with outside atmosphere was kept at a minimum by closing all vents, and air movement inside the chamber was maintained as uniform as possible.

A randomized block design was used, considering each plant as an experimental unit, with a total of five plants per cultivar. As mentioned above the experiment was run twice over time, thus the average of ten plants was used to characterize the cultivar. Cups were rotated inside the chamber to avoid a location effect for any particular seedling.

Classification for Stress Tolerance

The seedlings were allowed to grow for 7 days after which a second irrigation was applied by adding 50 cc of tap water to each cup by surface irrigation. This was considered as the start of the stress period and water was witheld from then on.

The basis of classification was the appearance of stress symptoms on any portion of the plant, such as leaf rolling, loss of turgidity, and discoloration of the leaf. All were identified visually. The day on which symptoms appeared was recorded for each seedling. Symptoms sometimes appeared as early as 24 hours after the second irrigation. The characterization of each cultivar was made by computing a weighted average with the product of the number of plants showing stress times

the number of the day on which symptoms appeared, all divided by the total number of seedlings that represented the cultivar in the test. This weighted average or score is presented along with the list of entries in the Appendix.

The mean score was computed for each group individually, in order to determine a reference point for the classification of cultivars. Those cultivars with a score above the mean were considered resistant and those below the mean susceptible.

Selection of Entries

Selection of entries was based, primarily, on the performance of each cultivar in the screening tests, and the availability of good quality seed of both the original cultivar and the selected line. Two cultivars were extracted from each group, representing a susceptible and a resistant entry, in accordance with their score in the screening tests. Selected as controls were Ryer Milo and M.35-1. Ryer had demonstrated a great capacity to recover after a period of severe stress in some preliminary studies, while M.35-1 was characterized as having heat and dessication tolerance (53, 84).

Results of Preliminary Test

Cultivars responded differently under stress conditions, but three symptoms were clearly visible. Leaf rolling caused by loss of turgidity was evident after 24 to 48 hours under stress. As stress became severe plants began to lodge, probably also caused by loss of turgidity of the sheath. A third symptom was a grayish color of the leaves, associated with the photosynthetic mechanism. These plants did not recover after

rewatering, indicating that the stress had caused irreversible damage. Since this test was intended for practical use, no attempt was made to measure soil or plant water potential. It was assumed that uniform conditions were present at all times, from the start of the experiment, through the dessication period. It was the plant itself that manifested its potential to survive under adverse conditions.

Susceptible seedlings usually died in the first 2 days under stress, thus a negative selection was applied by removing these plants. Only those individuals that remained erect and turgid were selected. Some plants lost part of their aerial parts but remained alive and recovered after rewatering. These plants were considered intermediate in stress tolerance. Plants that remained erect and turgid after 4 days under stress were classified as resistant. These plants were rewatered and allowed to recover for two days inside the growth chamber until recovery was evident. They were transferred to larger pots in the greenhouse for seed production, side by side with the representative sample from the original cultivar.

Variability was evident between and within groups, as reflected by their scores. The overall mean for each group indicated that the group from India had the highest tolerance to stress, followed by the groups from Sudan, improved lines from Oklahoma, Rio Bravo and Oklahoma B-lines. Apparently the inverse order holds for degree of homozygosity based on the distribution of individuals along the period of stress under consideration, where cultivars from Rio Bravo and Oklahoma had the least variation among individuals, while cultivars from India and Sudan were extremely variable.

Based on the score obtained on the screening tests, it was possible to select the following cultivars:

Susceptible: SU-6 (Gadam El-Hamam 33-2-1)
IN-15 (PI-288874)
OK-8

Resistant: SU-23 (L. R. Red B-23-27-1)
IN-2 (PI-288644)
OK-111

The code name will be used to refer to these cultivars from now on.

Determination of Effectiveness of Selection

It was assumed that effect of selection could be demonstrated if the transpiration rate, the amount of water consumed, dry matter accumulation, and seedling growth proved to be different for original and selected lines. Therefore a test was designed and conducted in a growth chamber to test this assumption.

Preparation of Cups

Styrofoam cups of 240 cc (8oz) of capacity were filled with 300 grams of sieved and sterilized soil. After filling, the cups were placed on a plate containing 65 cc of tap water. The water was absorbed by capillarity as explained elsewhere. After all the water had been taken up, the cup was covered with a plastic lid. This lid acted as the bottom when the cup was in an inverted position, but its primary function was to reduce soil water loss by evaporation. The cups were allowed to settle overnight after they were inverted.

Partially germinated seeds after 24 hours of incubation were transplanted to the cups by introducing them through the orifice in the cup into the moist soil by exserting a little pressure. The use of pre-imbibed seeds with a radicle of about 1 mm increased the probability of success in transplanting and reduced variability between and within cultivars. These seedlings emerged through the orifice after one or two days.

Growth Chamber Conditions

After transplanting, the cups were transferred to a walk-in growth chamber and arranged in a split-plot design with cultivar as main plot and selection as subplot. Each seedling was considered a replication, with a total of five per entry. This experiment was repeated two times and averaged to characterize each cultivar.

The growth chamber was set to provide for the following conditions:

Temperatures: Alternate 30 and 20 C for day and night, respectively.

P.A.R.: 180 uE $m^{-2}sec^{-1}$, with a photoperiod of 14 hours, from 6 am to 8 pm.

Air Velocity: No control applied on speed or flow. It was measured with mean speed of 11.7 m min^{-1}.

Rel. Humidity: Also non-controlled. Means were 10% during photoperiod and 20% during the night.

No other factor was controlled or measured in this experiment.

Techniques Used to Measure Selection Effectiveness

Water Consumption

Water consumption was estimated by weight difference. Weighing cups twice daily at 8 am and 2 pm permitted an estimate of water loss on an hourly basis. Readings taken on days 7 and 11 permitted the calculation of water consumption during a 4 day period as a measure of water-use efficiency. Water loss from cups with unexposed areas served to estimate water losses not related to evapotranspiration, while cups with an orifice but without plants estimated evaporation alone.

Leaf Diffusive Resistance

Leaf or stomatal diffusive resistance was measured with an Autoporometer LI-65 (Lambda Instruments; Lincoln, Neb.) using an LI-20 sensor with a narrow aperture of 3.5 x 20 mm. Care was taken to follow each seedling throughout the experiment in order to have valid comparisons. Readings were taken twice daily at 8 am and 2 pm on day 7 and day 11. The adaxial surface of the third, well developed leaf characterized 7-day-old seedlings, and the fourth leaf was used to characterize 11-day old plants. Both readings were obtained from the middle portion of the leaf and no attempt was made to correlate soil and leaf water potential.

Five seedlings from original and selected lines were measured pairwise in order to minimize variation within sub-plots. All values were standardized to 25 C for uniformity in response to leaf temperature, as recommended in the instructions booklet with the instrument.

The assumption that a plant had the highest and lowest values of leaf resistance at 8 A.M. and 2 P.M., respectively, in response to environmental demand, needed corroboration in this study. It was necessary to evaluate daily resistance rates during photoperiod and during the dark period. Leaf diffusive resistance was measured every hour from 9:30 A.M. to 4:30 P.M. on 7-day-old seedlings, and from 7:30 A.M. to 5:30 P.M. in 11-day-old seedlings. Night leaf resistance was measured from 8:30 P.M. to 6:30 A.M. in an inverted cycle scheme, where the dark period was given during working hours. Two separate experiments were conducted on different sets of plants in order to characterize day and night leaf resistance rates, using two plants per entry in each case. These values were also standardized to 25 C for uniformity purposes.

Leaf Area

Total leaf area was estimated for each seedling as the sum of the product of (Length) (Maximum Width) (0.75) on each leaf (26). Leaf area was measured on days 7 and 11 for the same seedling and the difference was considered as growth rate in cm^2 of leaf area per day. It was used to compute growth efficiency per unit of water consumed.

Stomatal Density

A matrix of the adaxial surface of the fourth leaf on 13-day-old seedlings was obtained by applying a film of clear nail polish. The sample was obtained on the same seedling used to evaluate water loss and leaf resistance during photoperiod. Care was taken to use the same position where the sensor was attached.

Two readings were made under the miscroscope on each sample, and averaged to characterize the seedling. Three seedlings served to characterize each entry. Stomatal density was calculated by converting the number of stomata present in the field of the microscope to stomata per cm^2. Total number of stomata on the adaxial surface was estimated by the product of total leaf area and stomatal density. No attempt was made to count stomata on the abaxial surface.

Dry Matter Accumulation

Seedlings were removed from the cup after 15 days of growth and dried in an oven set at 105 C for a period of 48 hours. Dry weight was determined and reported in grams. This value was used to calculate water-use efficiency also.

Water-Use Efficiency

Two methods were used to estimate water-use efficiency:

1) Efficiency in growth = $\frac{\text{Increase in Leaf Area}}{\text{Total Water Consumed}}$, over a 4-day period.

2) Conversion Efficiency = $\frac{\text{Total Dry Matter Produced}}{\text{Total Water Consumed}}$

The first method is a measure of growth per unit of water consumed for a period under consideration. It was assumed that more efficiency in water utilization could determine better development of leaf area. The second method measures the efficiency of conversion of water to dry matter, that is, the capacity of the plant to use available water for cell growth and multiplication, rather than for transpiration.

Germination in Osmotic Solutions

A stock solution with a formula-estimated osmotic pressure of 15 atmospheres was prepared by dissolving 108 grams of Mannitol, ($C_6H_{14}O_6$), in distilled water to 1 liter, according to Vant Hoff's formula for the pressure of gases (84). Succesive dilutions rendered osmotic pressures of 3, 6, 9, and 12 atmospheres with proportional parts of solution and distilled water in a ratio of 1:4, 2:3, 3:2, and 4:1, respectively.

Ten randomly selected seeds from each entry were germinated in 7.3 x 7.3 x 2.8 plastic boxes with lids, over two layers of germination substrate moistened with 5 cc of solution. The box was sealed with masking tape to prevent air and moisture exchange with the exterior environment.

The experimental design was a randomized block in a split-plot arrangement with two replications over time. Main plot was group of cultivars with similar origin, contained in one tray inside the germinator. Sub-plot was concentration of solution, with original and selected lines from both cultivars contained in a large plastic box of 17.5 x 12.5 x 6.5 cm, used to reduce variation between selections within each cultivar. Cultivar was arranged vertically while selection was horizontal inside the box. Selection was considered as sub-sub-plot only for practical reasons, ignoring this effect by assuming uniform environments inside the larger box. Randomization was applied at all levels, to avoid any bias in the arrangement of the treatments.

Standard germination conditions were followed as explained elsewhere, except for germination counts and seedling measurement. Counts were obtained at 24 hour intervals for four consecutive days, and a

final count was made at the end of seven days. Seeds were not treated with chemicals. Thus it was necessary to remove diseased seedlings as needed.

A seed was considered germinated when the radicle had a length of 1 mm, and non-germinated if no structure was visible at the end of the seven-day period. Germination speed was estimated by the number of seeds that germinated after 24 hours (first count), on a percentage basis of the total germination. Total germination was considered as the total number of seeds that germinated in the control treatment with distilled water at the end of the 7th day. Emergence Rate Index (E.R.I.) represents the uniformity on germination of any cultivar along the period of 4 days under consideration; it was computed as follows:

E.R.I. = $Y_1 + 1/2(Y_2) + 1/3(Y_3) + 1/4(Y_4)$, where Y represents the number of seeds germinated during the nth. day, multiplied by the reciprocal of the number of days from the start.

Seedling Development in Osmotic Solutions

Root and shoot lengths were measured in millimeters and removed for fresh weight determination on the 4th day of the experiment. Dry weights were obtained by placing roots and shoots in an oven, set at a constant 105 C for a period of 48 hours. Five seedlings randomly selected were measured from each treatment. Germinated as well as non-germinated seeds were included in the sample. Dry matter percentage was estimated as the ratio of Dry Weight/Fresh Weight x 100. A weighted average was also calculated to characterize overall performance of each line at all concentrations of Mannitol. It was computed as follows:

Average = $Y_0 + 1.2(Y_3) + 1.4(Y_6) + 1.6(Y_9) + 1.8(Y_{12}) + 2.0(Y_{15})/$ $\Sigma(1 + 1.2 + \ldots 2.0)$ where Y represents the factor quantity at each level of solution, and the denominator is the sum of weighing factors for each day.

CHAPTER IV

RESULTS AND DISCUSSION

Water Consumption

Water loss per unit of time can be considered from two different points of view: 1) plants that lose less water are more efficient in conserving water than those that have high water consumption, thus increasing their resistance to water stress, and 2) less water loss means lower growth rate and lower yields, both grain and stover, thus on a productivity basis, higher resistance to water stress is detrimental to yield.

The second approach has been considered in this study, where higher water loss indicated higher yield potential, based on the assumption of a direct relationship between yield and water loss.

Statistical analysis for water consumption of 7-day-old seedlings indicated significance at $\alpha = 0.127$ for entries, $\alpha = 0.267$ for selections, and $\alpha = 0.137$ for the interaction entry x selection (Appendix Table XXVIII). Table I contains mean water consumption in grams for 7-day-old seedlings.

Individual comparisons can be made in each cultivar, even though no statistical significance was detected. Among original cultivars, SU-23 and OK-111 classified previously as resistant had more water loss than SU-6 and OK-8, their susceptible counterparts. The original cultivars from India showed this response inverted, that is, the

TABLE I

MEAN WATER CONSUMPTION IN GRAMS PER DAY
FOR 7-DAY-OLD SEEDLINGS

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	1.01	1.06	0.05
	IN-15	1.17	1.19	0.02
	OK-8	1.03	1.09	0.07
RESISTANT	SU-23	1.05	1.17	0.12
	IN-2	1.01	0.99	-0.02
	OK-111	1.13	1.03	-0.10
CONTROLS	RYER	0.92		
	M.35-1	1.04		

F-tests were not statistically significant for Entries (P=0.1270), Selections (P=0.2673) nor their interaction (P=0.1369).

susceptible line IN-15 lost more water, than the resistant line. Within the selected goup, this difference was maintained only by SU-23 and IN-15. Selected OK-111 was replaced by OK-8 which had higher water consumption. The classification as susceptible and resistant was no longer valid for all selected cultivars because they were derived from superior individuals which supposedly were resistant to water stress.

The effect of selection was non-significant, but mean water loss was higher for all selected lines except IN-2 and OK-111. If higher rates of water transpired are related to more yield (7), those cultivars with greater water consumption should increase also their productivity, both in fodder and grain. This difference was maintained only by IN-15 among the selected lines. As seen in Table I, OK-111 reduced water loss by 0.10g, while the best improvement was obtained in SU-23 with 0.12g over its original line. All susceptible lines were improved over the original cultivars, while among the resistant lines, SU-23 had a large increase and OK-111 had a reduction in the level of water consumption.

From Figure 1 it is apparent that water consumption per day was much higher on day 11 than on day 7. There was some increase in water consumption from original to selected lines for SU-6, SU-23, IN-65, and OK-8.

The amount of water consumed by the plant during a period of 4 days, from day 7 to 11, was used to estimate a mean daily consumption. This average represented a better estimate than one reading in a single day. The data are presented in Table II.

Among original lines a small advantage was observed for SU-23 and OK-111 among the resistant lines, and for IN-15 among the susceptible lines. Within the selected lines there was an advantage for IN-15

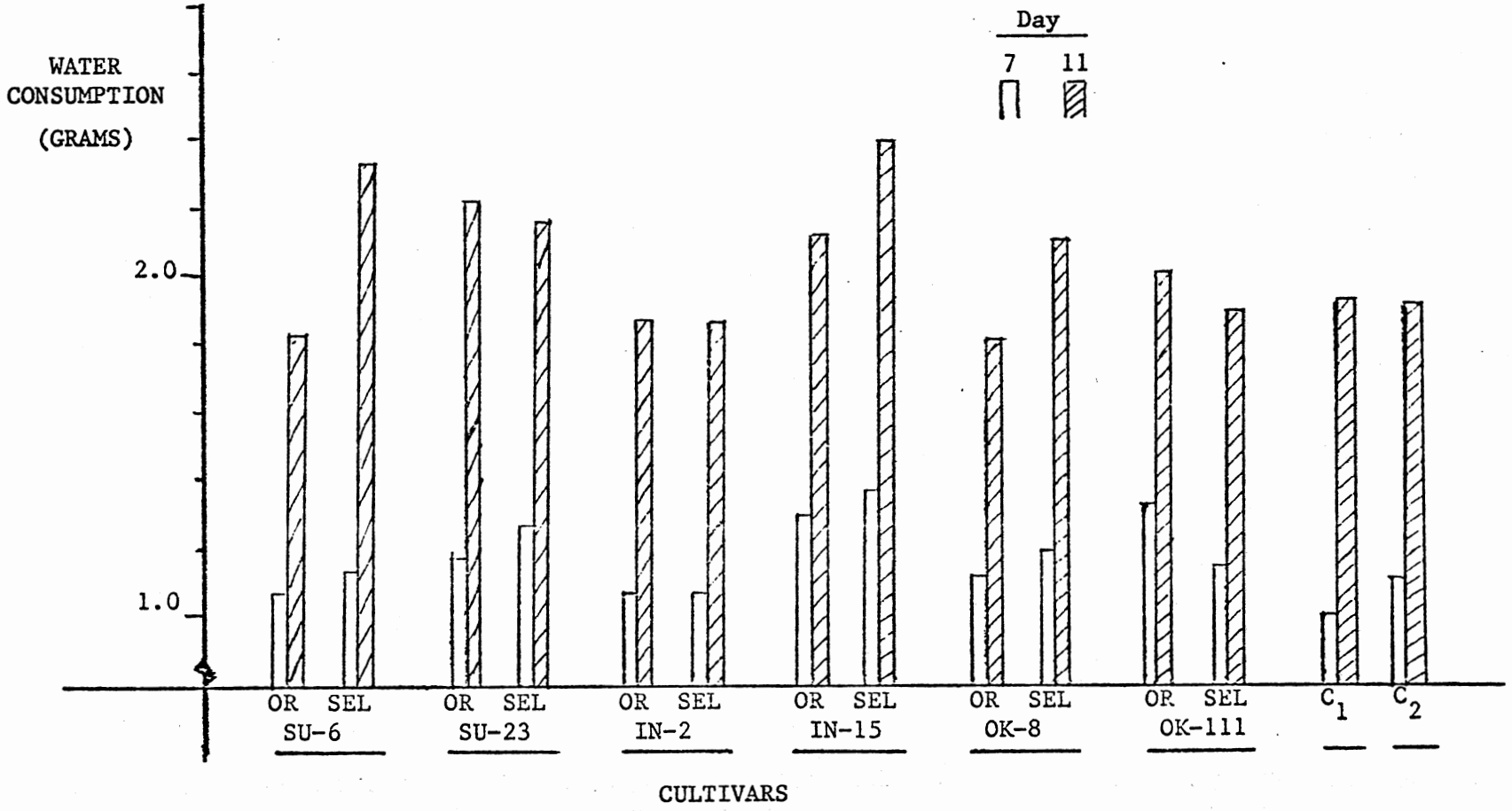


Figure 1. Mean Water Consumption in Grams for 7 and 11-Day-Old Seedlings from Original and Selected Lines.

TABLE II

MEAN WATER CONSUMPTION IN GRAMS, DURING A PERIOD OF FOUR DAYS, FROM DAY 7 TO DAY 11

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	2.44	2.78	0.34
	IN-15	2.97	3.21	0.24
	OK-8	2.68	2.87	0.19
RESISTANT	SU-23	2.65	2.75	0.10
	IN-2	2.54	2.67	0.13
	OK-111	2.80	2.65	-0.15
CONTROLS	RYER	2.55		
	M.35-1	2.65		

F-tests were statistically significant for Entries (P=0.0089), nonsignificant for Selections (P=0.1041) and Entry x Selection (P=0.6591).

among the susceptible group. This indicated a greater effect of selection, on these cultivars although statistically nonsignificant (Table XXIX).

There is evidence of a direct relationship between water loss at day 7 and total water consumed during a period of 4 days, from day 7 to day 11. Figures 2, 3, and 4 present individual regression lines for each cultivar. A ratio of 1:10 was evident between variables although this ratio was not consistent. Each cultivar had a specific ratio with a different slope to its regression line. Cultivars from India were the closest to 1:10 ratio, all others had scattered individual values with no correlation among readings. OK-8 and OK-111 tended to cluster at high values on both variables, indicating high degree of homozygosity among individuals. The usefulness of this relationship is evident in the prediction of water loss at later stages of growth by measuring water consumption of young seedlings.

Leaf Diffusive Resistance

No statistical difference was detected among entries on any day in particular, nor when all readings were pooled together. There was no difference between times of reading either (Appendix, Table XXX). A difference in leaf resistance within susceptible and resistant groups was detected. This can be seen in Table III, where mean resistance values for 7-day-old seedlings are pooled to characterize each cultivar. SU-23 and IN-2 had highest resistance values among original lines, and SU-23 was highest among selected lines. Selected IN-15 was higher than IN-2, a resistant line.

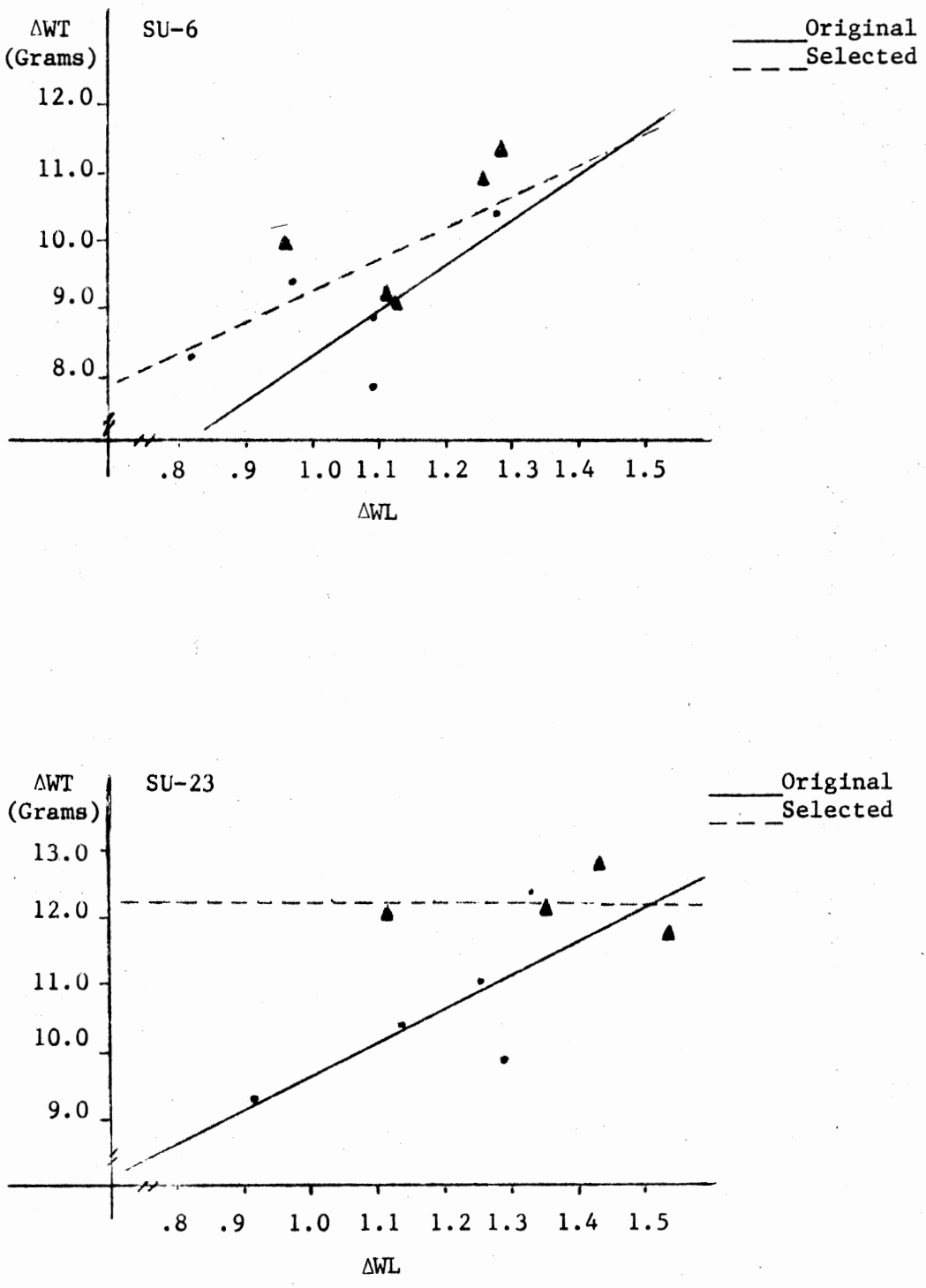
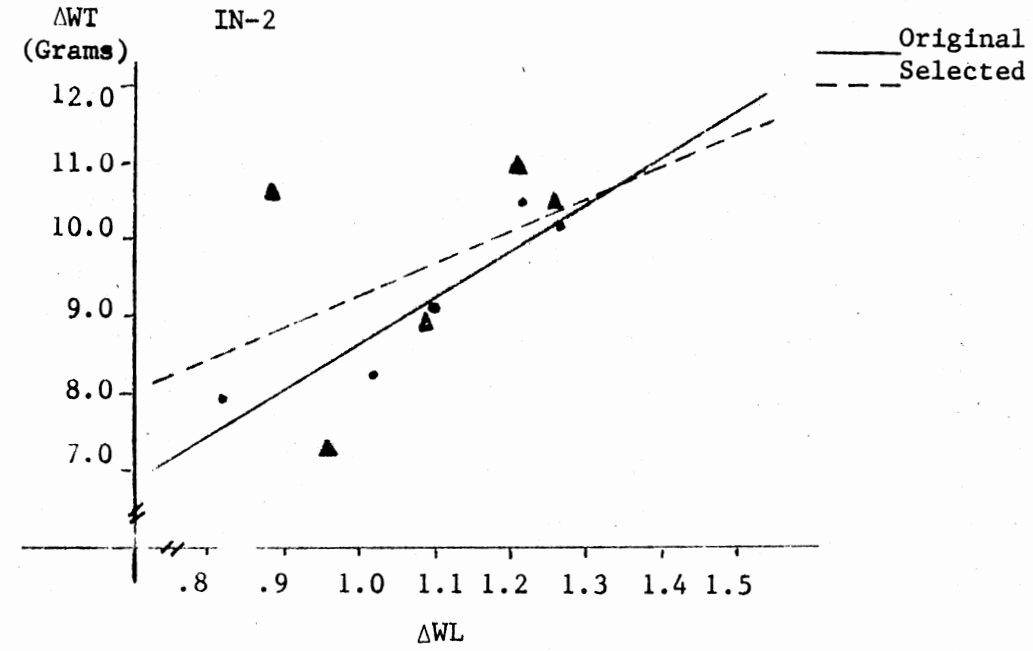


Figure 2. Ratio of Total Water Consumption (ΔWT) in a Period of 4 Days and Water Consumption (ΔWL) of 7-Day-Old Seedlings, for Cultivars from Sudan.



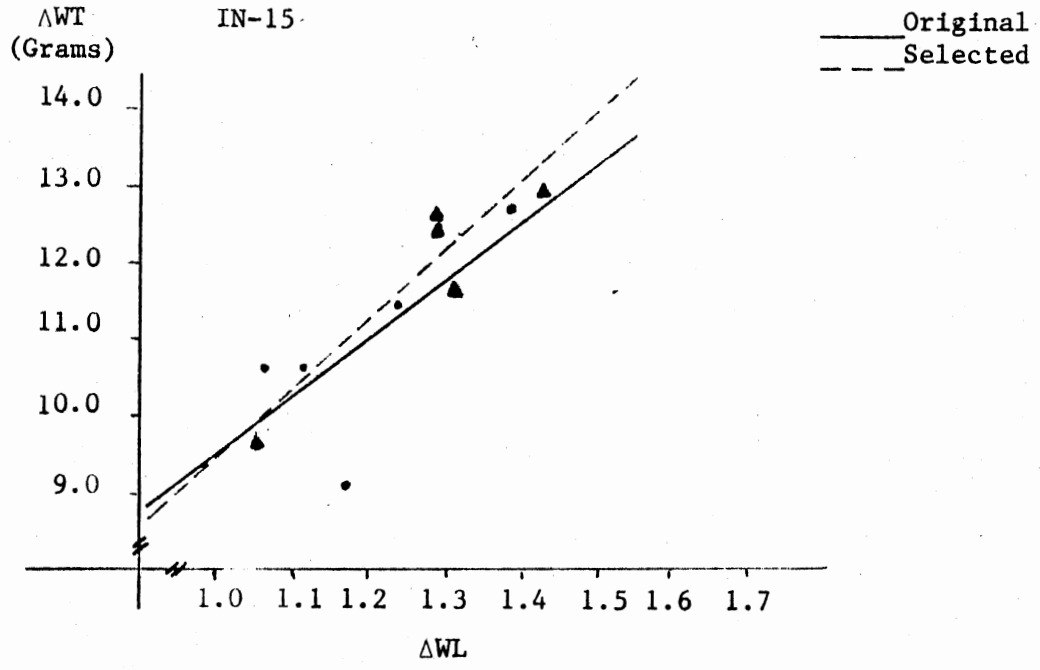


Figure 3. Ratio of Total Water Consumption (ΔWT) in a Period of 4 Days and Water Consumption (ΔWL) of 7-Day-Old Seedlings, for Cultivars from India.

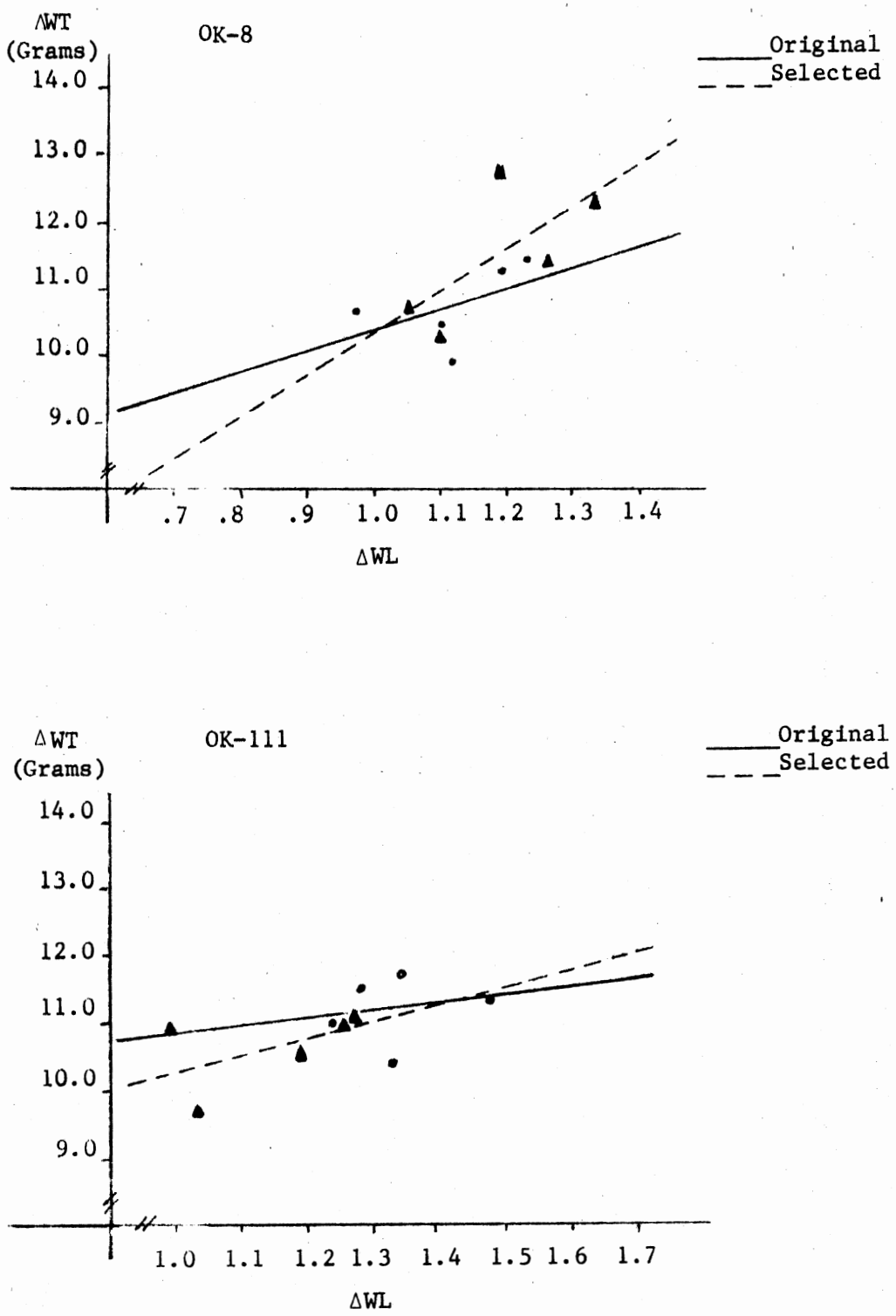


Figure 4. Ratio of Total Water Consumption (ΔWT) in a Period of 4 Days and Water Consumption (ΔWL) of 7-Day-Old Seedlings, for Cultivars from Oklahoma.

TABLE III

MEAN LEAF DIFFUSIVE RESISTANCE (SEC CM^{-1}) FOR 7-DAY-OLD SEEDLINGS. AVERAGE OF TWO READINGS TAKEN TWICE DAILY IN 10 PLANTS.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	40.9	42.8	1.9
	IN-15	40.8	48.4	7.6
	OK-8	45.3	44.2	-1.1
RESISTANT	SU-23	46.4	76.3	29.9
	IN-2	47.1	40.5	-6.6
	OK-111	37.7	38.8	1.1
CONTROLS	RYER	53.8		
	M.35-1	58.0		

F-tests were statistically nonsignificant for Entries (P=0.456), Selections (P=0.089) nor Entry x Selection (P=0.529).

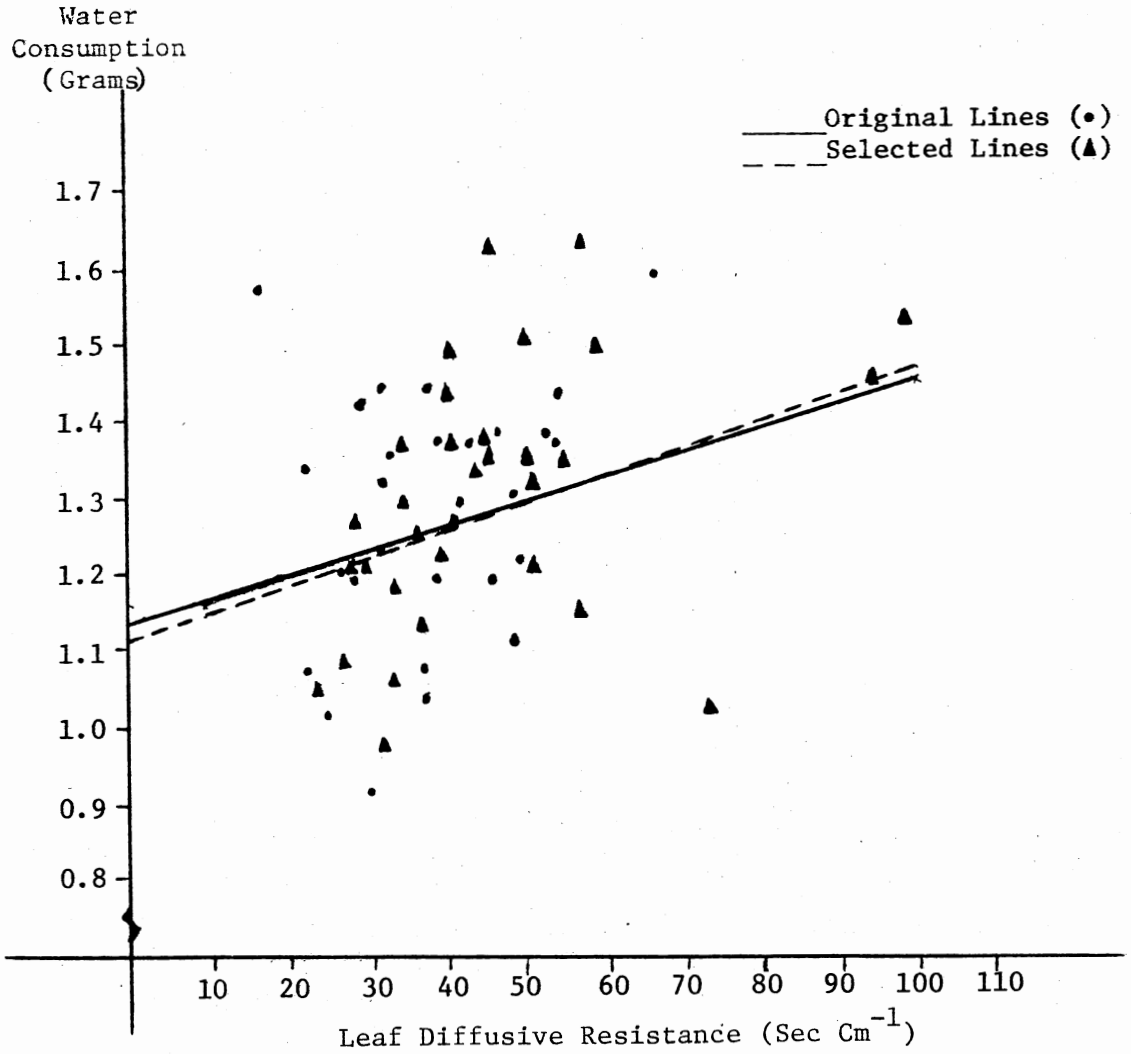
High leaf resistance indicates sensitivity to adverse conditions and capacity to reduce water loss by closing stomata. This will bring an increase in leaf water content which maintains the leaf turgidity.

Plants with a high rate of water consumption also had high mean leaf resistance, as can be observed in Figure 5, especially on selected lines. The overall correlation of these two factors on 7-day-old seedlings had a value of $r = 0.36$ with water loss as dependent variable, meanwhile, individual analysis indicated a different performance for original and selected lines with $r = 0.27$ and $r = 0.42$, respectively (Appendix, Table XXXI). Selected lines had more water loss and still developed more leaf resistance during photoperiod. Possible explanations are: 1) the plants had a partial closure of stomata, 2) an increase in leaf osmotic potential, which reduced water loss (78), or 3) higher loss of water through the cuticle (76).

When original and selected lines were compared, all selections except IN-2 and OK-8 had improved resistance over the original line. Selection was significant at $\alpha = 0.089$, the interaction entry by time was significant at $\alpha = 0.1287$ (Appendix, Table XXX). The latter supported the finding of a cycling pattern of leaf resistance. This cycling reduced the accuracy of characterization of the entries.

Frequency Distribution for Leaf Resistance

The mean itself is not a good indicator of superiority among cultivars; it should be complemented with an individual study of the frequency distribution. Frequency distributions of each entry are presented in Figures 6, 7, and 8, based on mean leaf resistance from 10 individuals per entry.



Firgure 5. Overall Ratio Between Leaf Diffusive Resistance and Water Consumption for 7-Day-Old Seedlings.

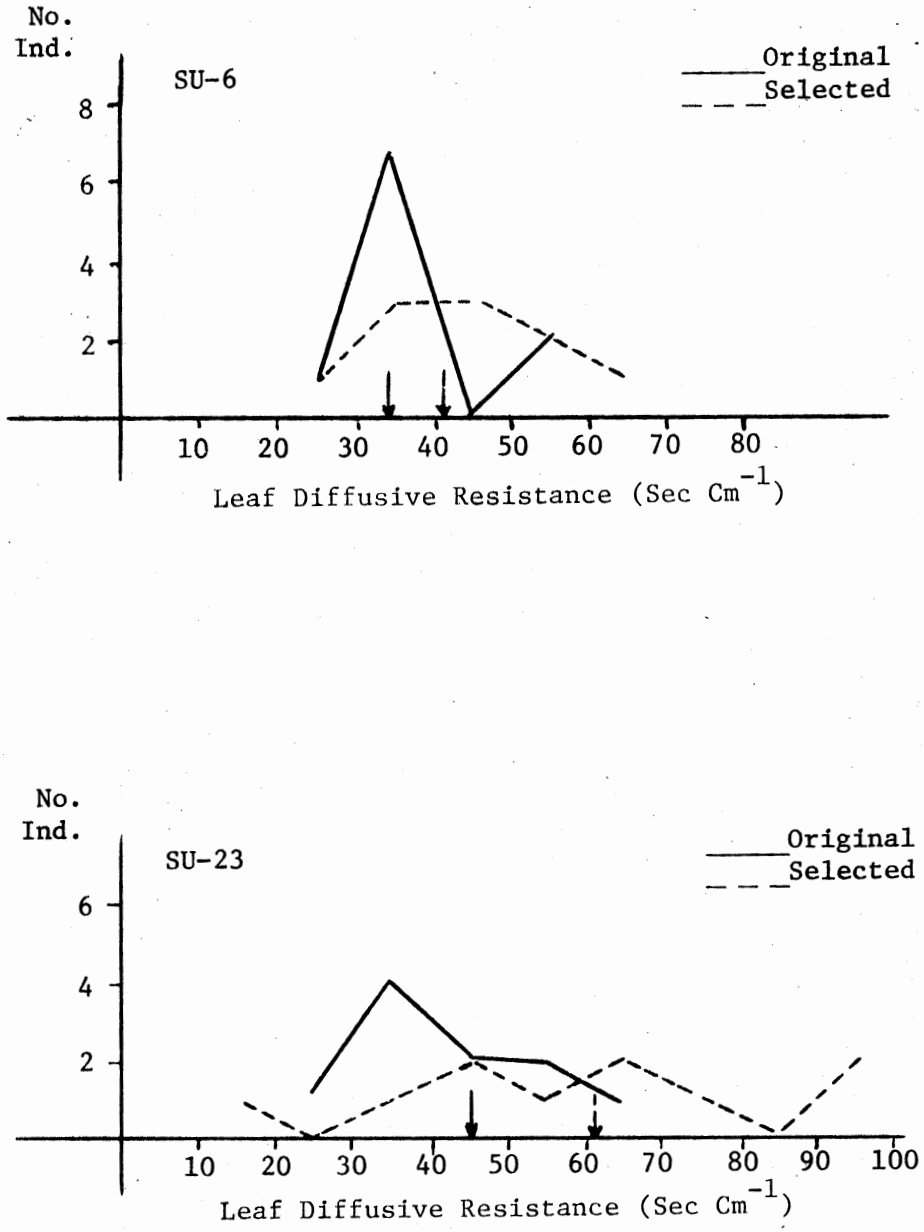
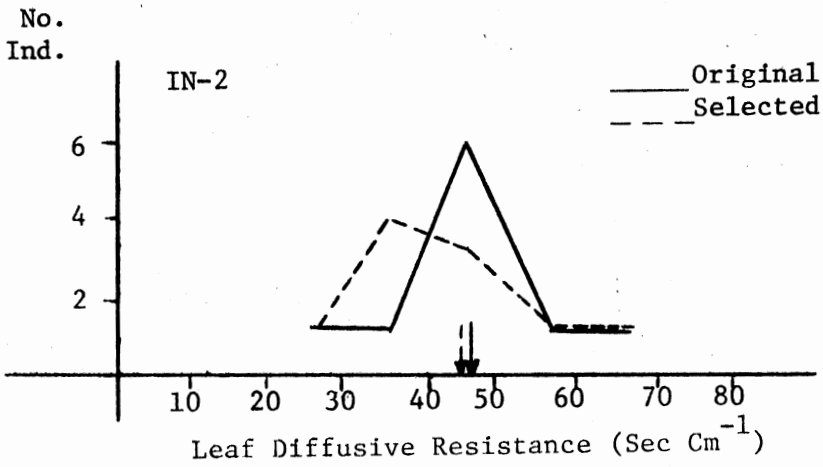


Figure 6. Frequency Distributions for Mean Leaf Diffusive Resistance for Cultivars from Sudan. Arrows Represent the Mean of Each Entry.



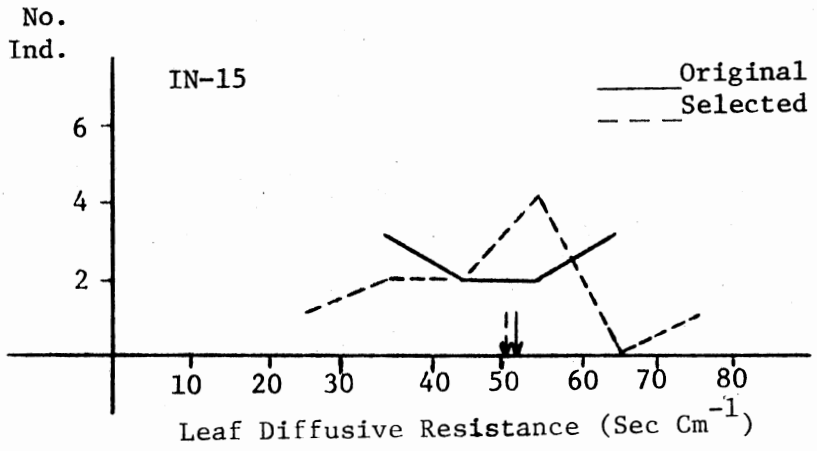
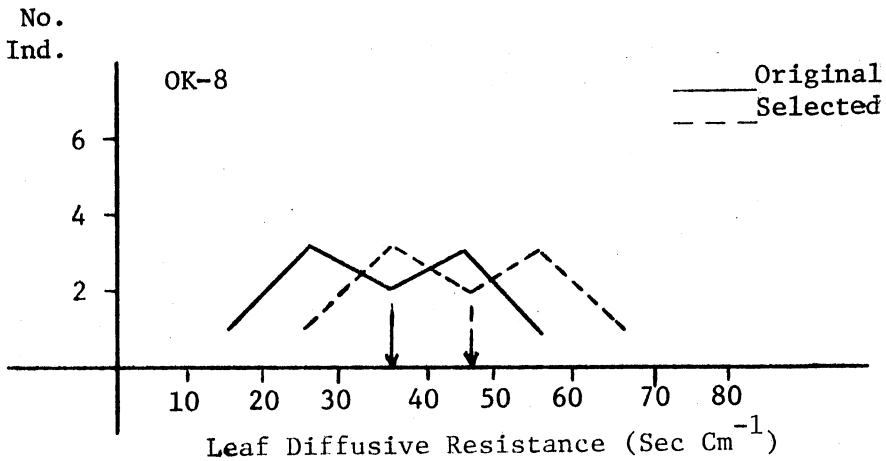


Figure 7. Frequency Distributions for Mean Leaf Diffusive Resistance for Cultivars from India. Arrows Represent the Mean of Each Entry.



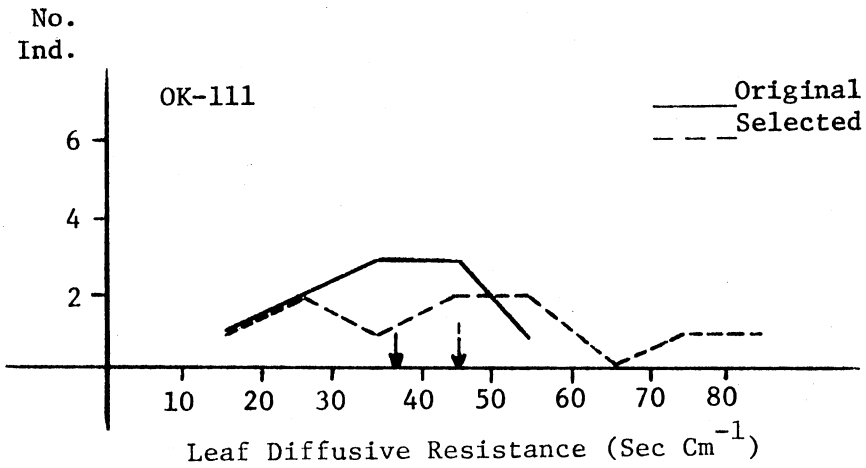


Figure 8. Frequency Distributions for Mean Leaf Diffusive Resistance for Cultivars from Oklahoma. Arrows Represent the Mean of Each Entry.

SU-6 had the mean displaced to the right as an effect of selection, with a more normal distribution of the selected line. New genotypes appeared in higher and lower classes for the selected lines of SU-23 as if recombination were taking place in a line which supposedly was homozygous. This cultivar had the widest range in mean leaf resistance and it may be a possible source-population of genetic material.

The mean for the selected lines of IN-2 was displaced very slightly to the left, but the range between values remained unchanged. New genotypes appeared in higher and lower classes in IN-15, but the mean of the selected lines was essentially the same as the mean of the original lines.

A positive effect of selection was observed for OK-8, with a total shift of the population to the right. New genotypes appeared in higher classes of OK-111, which was supposed to be highly homozygous, causing a displacement of the mean to the right.

In summary, a positive effect of selection in SU-6, SU-23, OK-8, and OK-111, and a negative effect on both lines from India was evident. It is important to note that new genotypes appeared in all lines except in IN-2 indicating that selection is still possible even if the inbred line is highly homozygous for other characters. These new genotypes could remain undetected unless a proper screening technique is applied.

Leaf Diffusive Resistance for Day and Night

The extremely complex effect of the environment upon plant transpiration prohibits an accurate comparison among a group of cultivars under field conditions. There is always at least one factor varying during the day, e.g., light, temperature, water potential of plant or

soil. Furthermore, the interaction among these factors makes it very difficult to separate single-factor effects, and almost impossible to duplicate the conditions present at any moment.

It is extremely difficult to detect endogenous cycles of transpiration when the environment is dynamic. Under controlled conditions the plant becomes more independent of the environment and cycles are easier to detect. It is then necessary to measure leaf resistance several times during the day to characterize a plant (33), especially when each individual has a particular rythm of transpiration, with differences in range and periods between similar points of resistance.

Eight readings were obtained during photoperiod at 1-hour intervals from 9:30 A.M. to 4:30 P.M. Two seedlings from each entry grown in individual styrofoam cups were used to characterize each entry; the means are presented in Table IV, and Table XXIII in the Appendix.

Among original ines, all those regarded as resistant proved to have higher leaf resistance than their susceptible counterparts. The difference between cultivars from Sudan and India was not as wide as the difference between entries from Oklahoma. The increase in leaf resistance due to selection was apparent for all cultivars except IN-15 and OK-111. Among selected lines, higher leaf resistance could be indicated for all lines, except IN-15 and OK-111.

To complement the information given by the mean, the distribution of this factor during the day is presented in Figures 9, 10, 11, and 12. A cycling pattern was evident in the original lines with periods of 3 and 4 hours between 1:30 to 2:30 pm. The distribution of selected lines differed from the distribution of the original lines in the length of periods, with the extremes falling outside the time period under

TABLE IV

MEAN LEAF DIFFUSIVE RESISTANCE (SEC CM^{-1}) FOR 7-DAY-OLD SEEDLINGS. AVERAGE OF 8 READINGS TAKEN HOURLY FROM 9:30 A.M. TO 4:30 P.M.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	49.2	53.6	4.4
	IN-15	51.7	49.2	-2.5
	OK-8	37.3	57.2	19.9
RESISTANT	SU-23	50.7	69.7	19.0
	IN-2	53.9	59.1	5.2
	OK-111	57.3	46.4	-10.9
CONTROLS	RYER	28.9		
	M.35-1	46.6		

F-tests were statistically nonsignificant for Entries (P=0.5136), Selections (P=0.2007), nor Entry x Selection (P=0.3620).

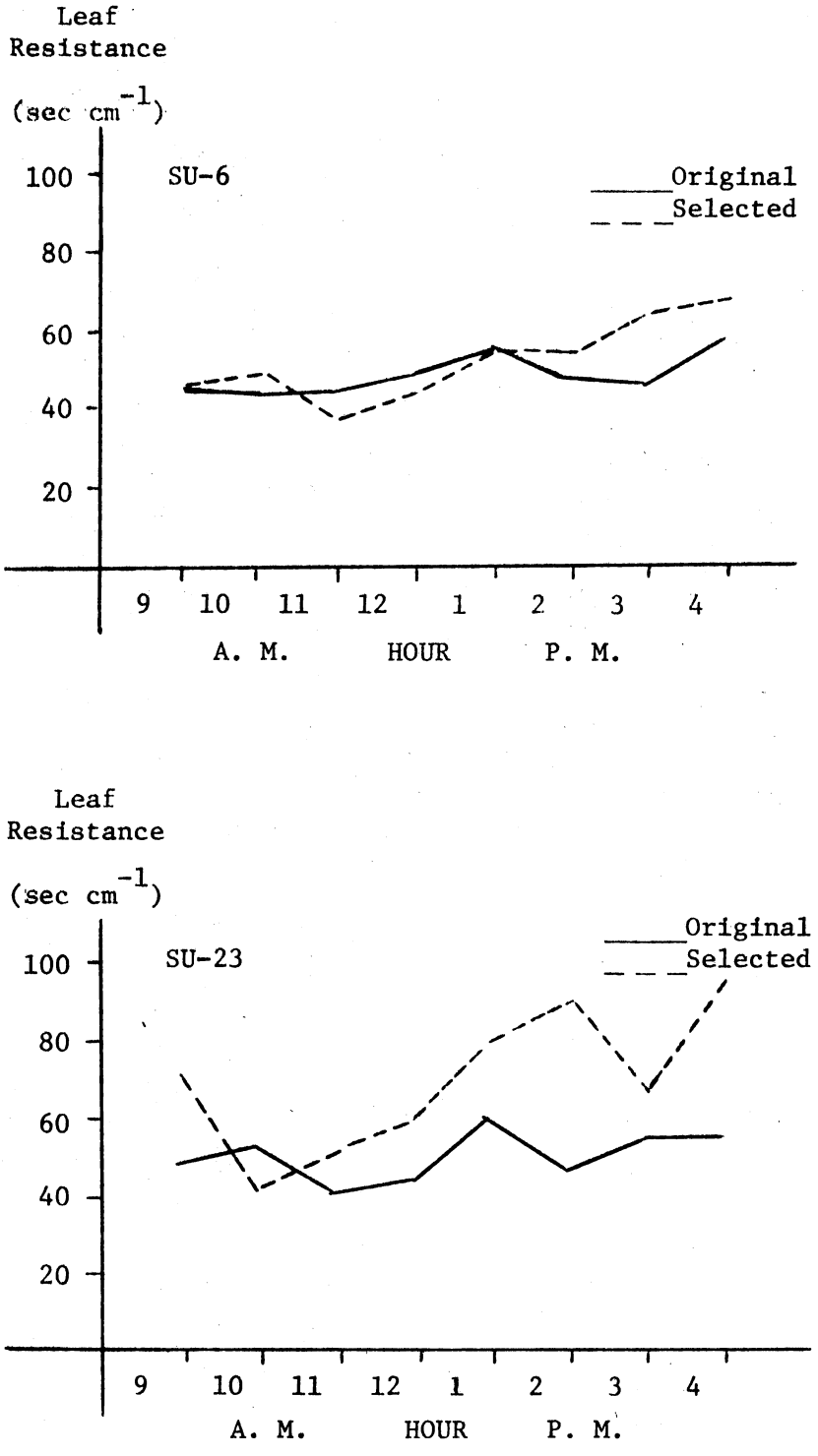
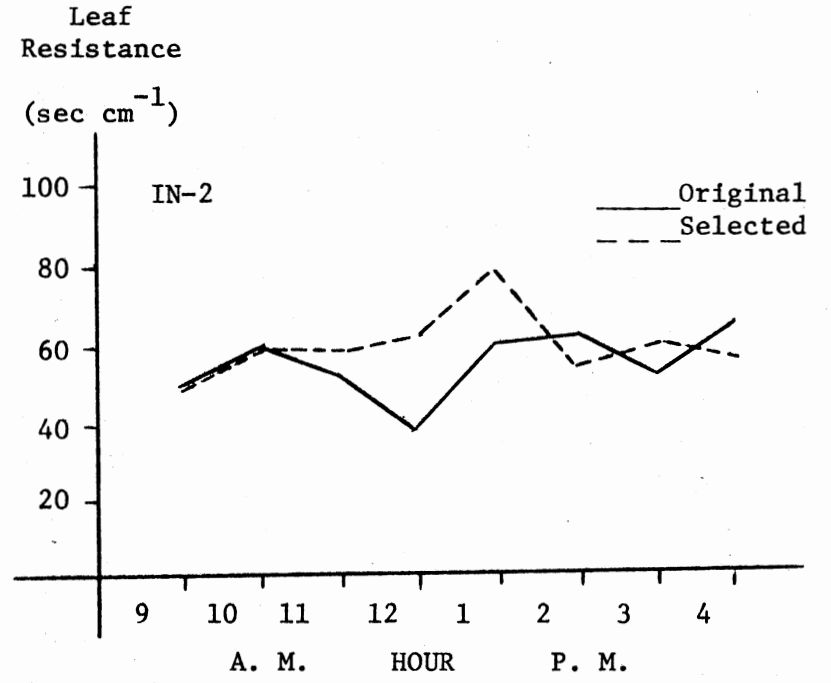


Figure 9. Leaf Diffusive Resistance Pattern During Photoperiod, for 7-Day-Old Seedlings for Cultivars from Sudan.



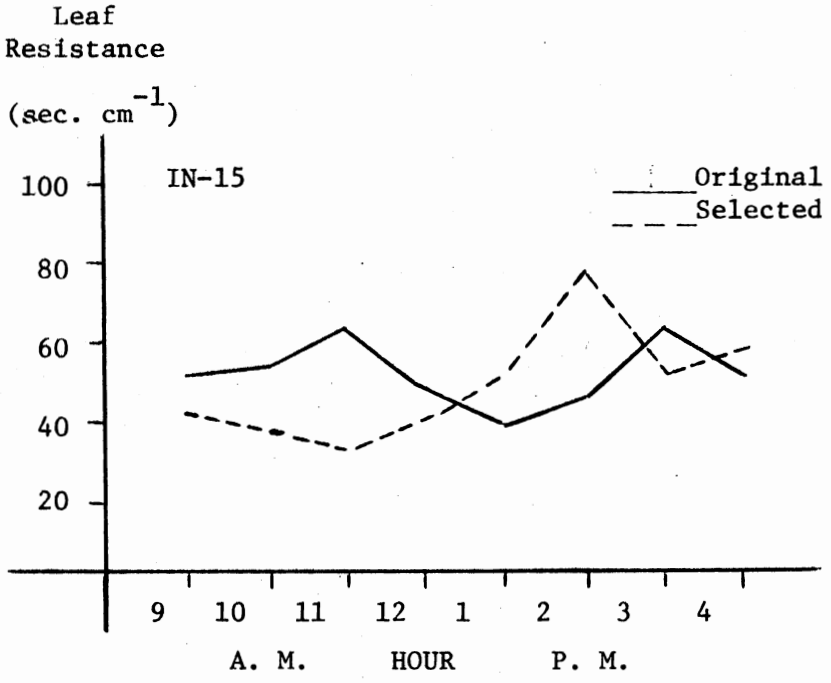
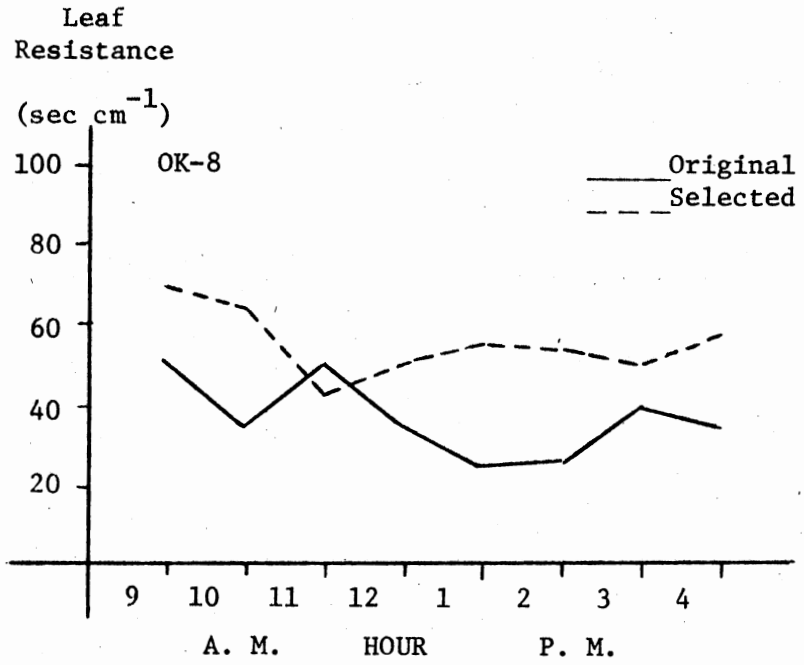


Figure 10. Leaf Diffusive Resistance Pattern During Photoperiod, for 7-Day-Old Seedlings for Cultivars from India.



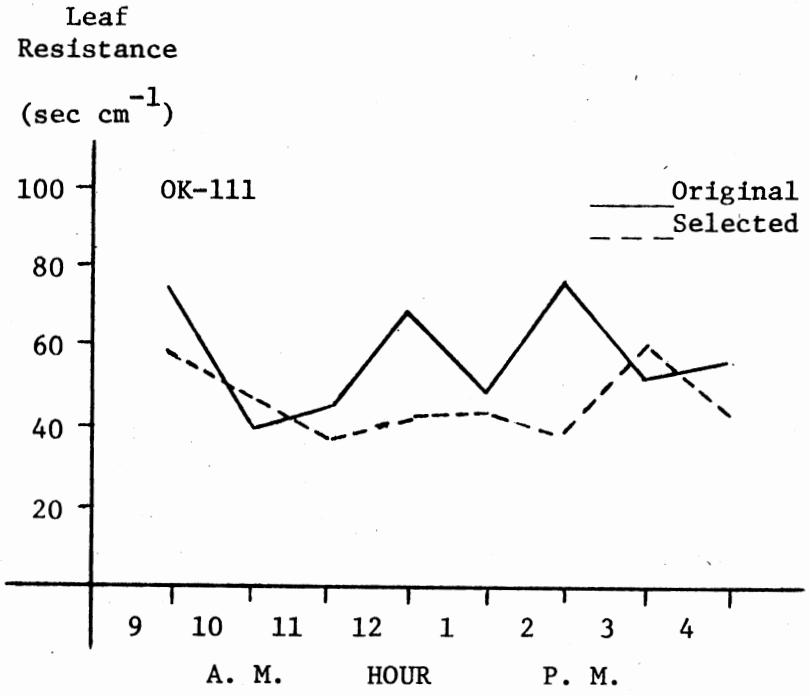


Figure 11. Leaf Diffusive Resistance Pattern During Photoperiod, for 7-Day-Old Seedlings for Cultivars from Oklahoma.

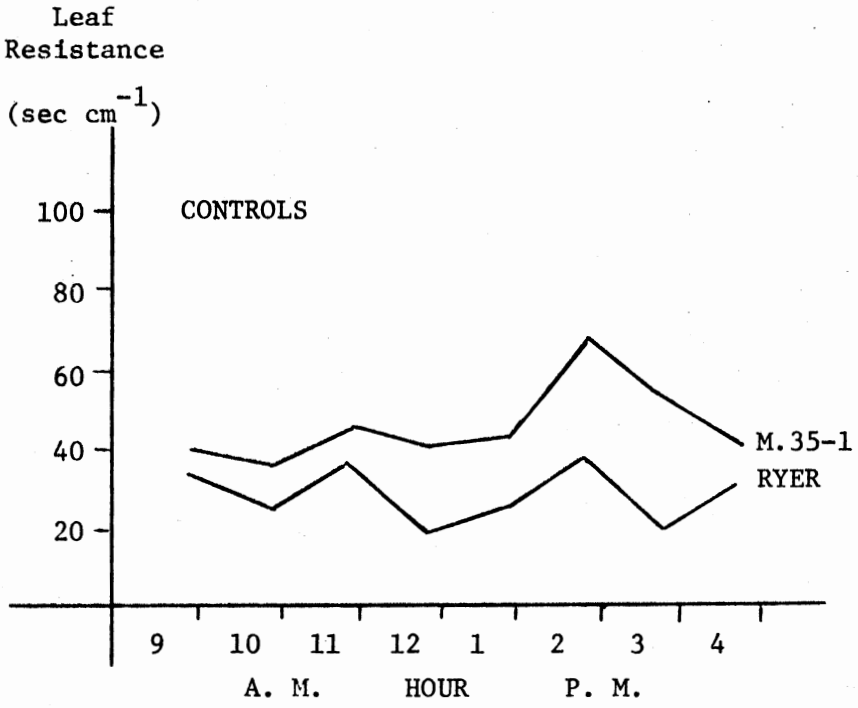


Figure 12. Leaf Diffusive Resistance Pattern During Photoperiod, for 7-Day-Old Seedlings for Controls Ryer and M.35-1.

consideration; sometimes just one peak was observed. The fact that period lengths were wider on selected lines, indicated the capacity of the seedlings to maintain stomata open or closed for longer periods. A steep increase in leaf resistance during the afternoon was observed on selected lines of SU-6, SU-23, IN-15, and control M.35-1, an indication of possible hardening of the plant during the day.

Some problems were encountered with the instrument used to measure leaf resistance, and very high readings were obtained on 11-day-old plants. At times it was necessary to repeat a reading on a seedling after an interval of one or two minutes, but time after time the same levels of resistance were encountered. These values should be viewed with caution. It was assumed that the bias affected all seedlings uniformily and in the same direction, thus emphasis should be placed upon differences between selections only. Mean leaf resistance for 11-day-old seedlings is presented in Table V.

SU-23, IN-15, and OK-8 among the selected lines remained superior to their original lines; all other entries showed reduced leaf resistance. Hardening of seedlings was evident as an increase in leaf resistance from day 7 to day 11.

If a C_4 plant, such as sorghum, has reduced photorespiration as compared to C_3 plants, its CO_2 uptake should be closely related to transpiration. Thus diffusive resistance could be a measure of both, because they depend directly upon stomata opening. High resistance during photoperiod and low resistance at night may indicate that the plant is restraining itself from excessive loss of water during the day by closing stomata, while the opposite occurs at night.

TABLE V

MEAN LEAF DIFFUSIVE RESISTANCE (SEC CM^{-1}) FOR 11-DAY-OLD SEEDLINGS. AVERAGE OF 11 READINGS TAKEN HOURLY FROM 9:30 A.M. TO 4:30 P.M.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	94.2	72.7	-21.5
	IN-15	73.5	95.3	21.8
	OK-8	84.8	90.6	5.8
RESISTANT	SU-23	62.4	107.3	44.9
	IN-2	82.0	76.2	-5.8
	OK-111	79.3	68.0	-11.3
CONTROLS	RYER	63.4		
	M.35-1	76.2		

F-tests were statistically nonsignificant for Entries (P=0.9324), Selections (P=0.5049) nor Entry x Selection (P=0.1861).

Night leaf diffusive resistance is presented in Table VI. When original and selected lines were compared, all lines except IN-2 and OK-111 showed a reduction in leaf resistance. The reduction in leaf resistance was favorable to these entries, apparently by promoting more growth, as will be discussed later.

The ratio of Night/Day leaf diffusive resistance measured the relative reduction for each entry. This ratio is presented in Table VII. Selected lines SU-6, IN-15, OK-8, and SU-23 had a reduction between 21.1% and 44.2% in Night/Day ratio, while IN-2 and OK-111 increased 15.7% and 30.9%, respectively. Night/Day ratio corresponded well with leaf area, thus it may be valid to indicate that more seedling growth is the consequence of a lower Night/Day ratio.

Lower Night/Day ratios mean that more water is available to the seedling, as tranpsiration is reduced during the day and increased at night. This could be beneficial for Auxin production during the dark period, as Quinby (63) indicates, where the hormone has more favorable conditions for its synthesis and translocation inside the plant.

Leaf diffusive resistance rates during the night are shown in Figures 13, 14, 15, and 16. Closing of stomata is expected after the lights are off and this was seen in some entries, with the exception of OK-8 among original lines, and SU-6, SU-23 and OK-111 among the selected lines. This could be attributed to a cycling pattern that is maintained day and night. A factor other than light may intervene in the opening and closing of stomata under uniform environmental conditions, perhaps temperature or plant water potential. The initiation of the dark period and lower temperatures at night had a joint effect upon leaf resistance. It was observed that leaf temperature and leaf diffusive resistance were

TABLE VI

MEAN LEAF DIFFUSIVE RESISTANCE (SEC CM^{-1}) FOR 7-DAY-OLD SEEDLINGS. AVERAGE OF 9 READINGS TAKEN HOURLY IN THE DARK PERIOD FROM 8:30 P.M. TO 6:30 A.M.

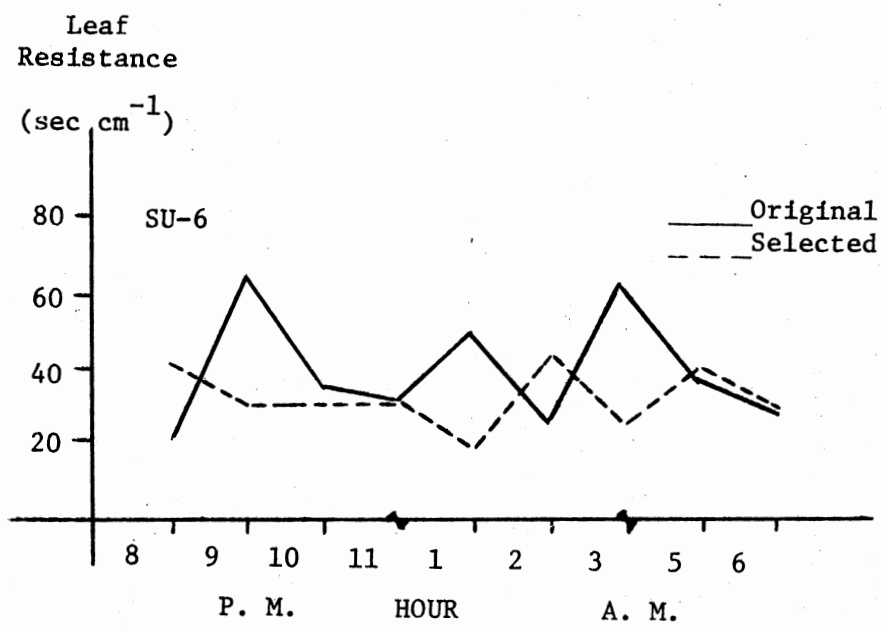
CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
	SU-6	39.94	32.21	-7.73
SUSCEPTIBLE	IN-15	51.01	26.78	-24.23
	OK-8	31.79	31.67	-0.12
	SU-23	41.16	39.45	-1.71
RESISTANT	IN-2	50.82	74.00	23.18
	OK-111	29.55	31.22	1.67
CONTROL	RYER	36.70		

F-tests were statistically significant for Entries (P=0.0053), and nonsignificant for Selections (P=0.7797) and Entry x Selections (P=0.2989).

TABLE VII

NIGHT/DAY LEAF DIFFUSIVE RESISTANCE RATIO
FOR 7-DAY-OLD SEEDLINGS.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	RELATIVE %
	SU-6	81.2	60.1	-21.1
SUSCEPTIBLE	IN-15	98.7	54.4	-44.2
	OK-8	85.2	55.4	-29.9
	SU-23	81.2	56.6	-24.6
RESISTANT	IN-2	94.3	125.2	30.9
	OK-111	51.6	67.3	15.7
CONTROL	RYER	127.1		



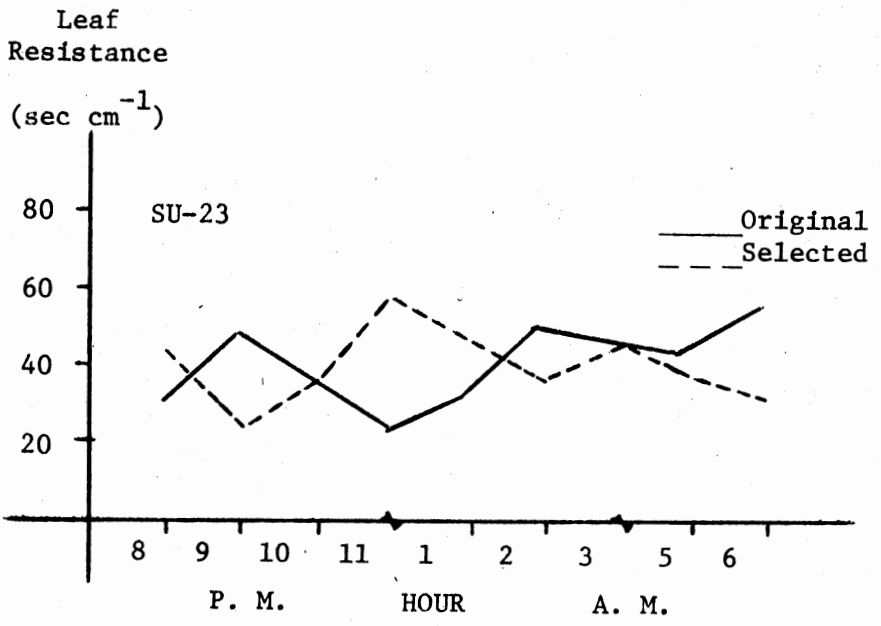


Figure 13. Leaf Diffusive Resistance Pattern During Dark Period, for 7-Day-Old Seedlings for Cultivars from Sudan.

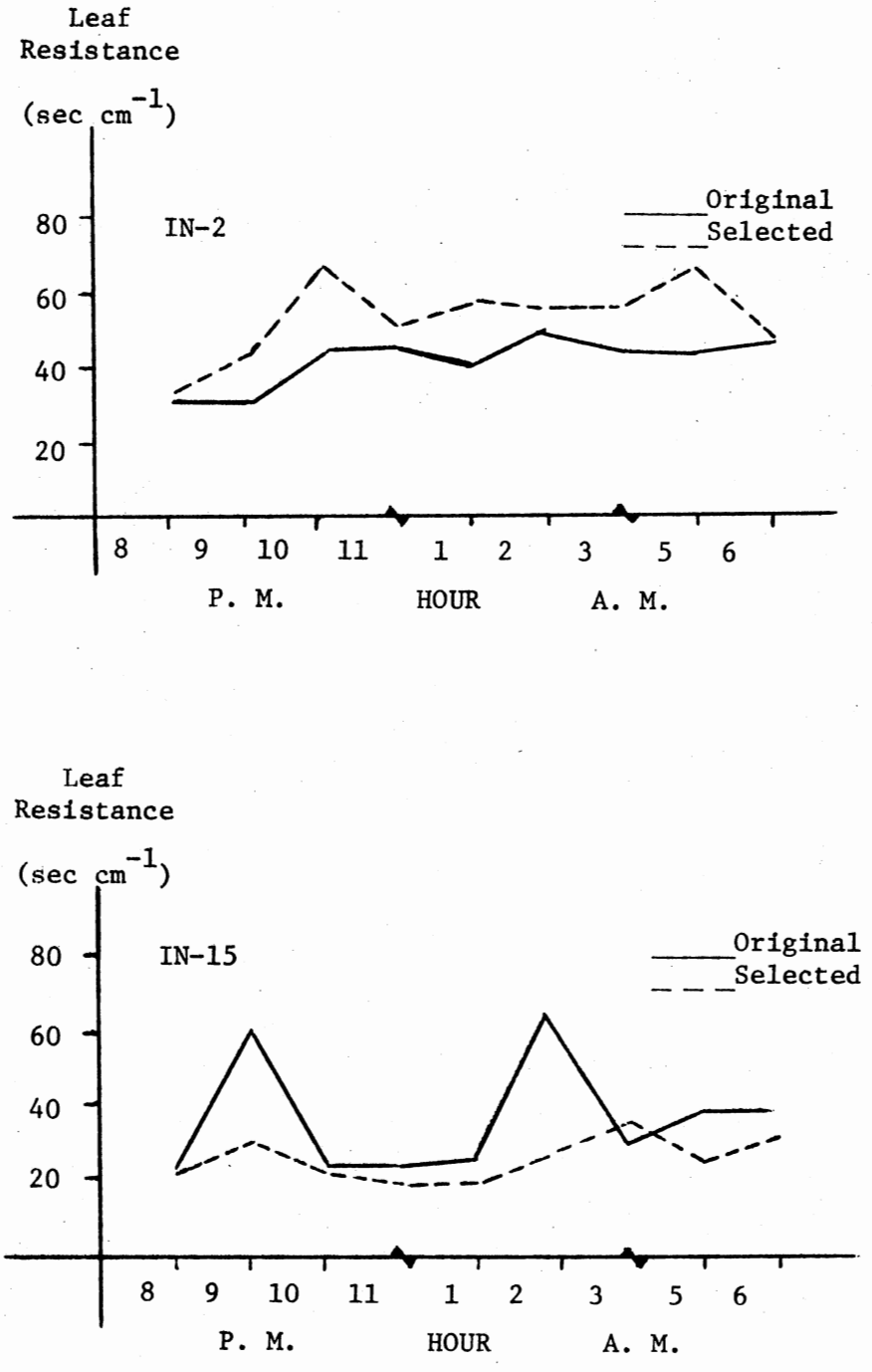


Figure 14. Leaf Diffusive Resistance Pattern During Dark Period, for 7-Day-Old Seedlings for Cultivars from India.

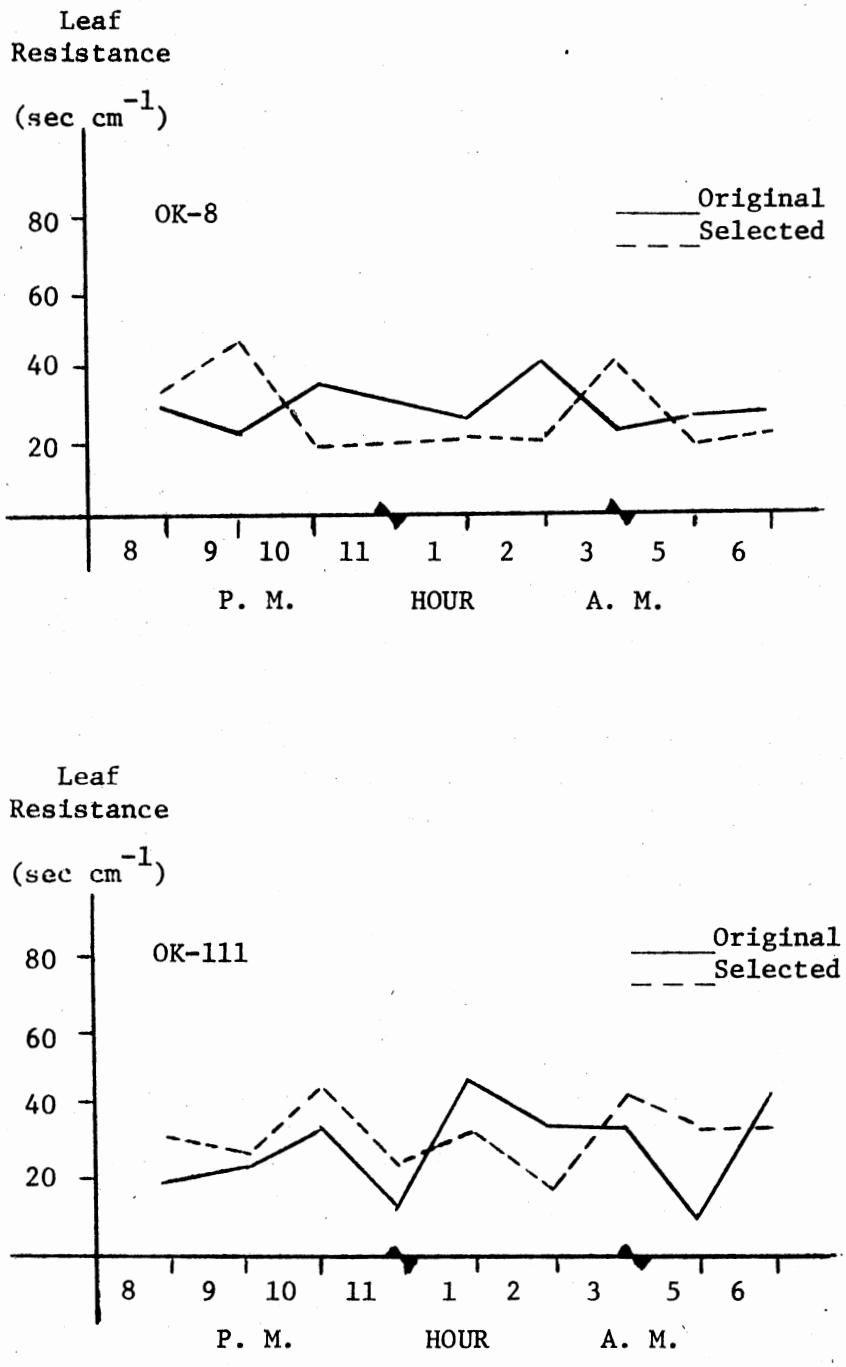


Figure 15. Leaf Diffusive Resistance Pattern During Dark Period, for 7-Day-Old Seedlings for Cultivars from Oklahoma.

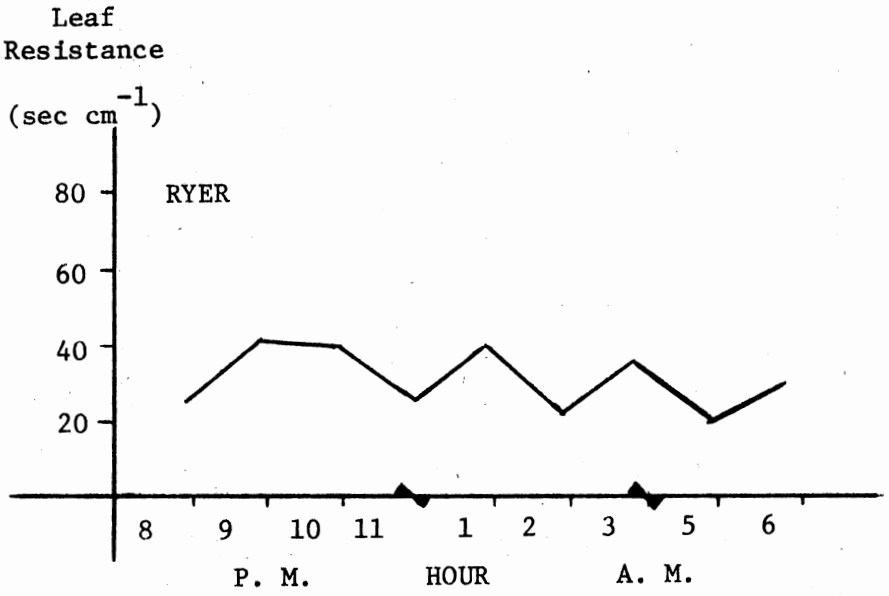


Figure 16. Leaf Diffusive Resistance Pattern During Dark Period, for 7-Day-Old Seedlings for Control Ryer.

reduced in some entries as a result of the change in environment from light to dark periods inside the chamber. This modification in plant response indicated difference in stomatal sensitivity; as soon as the lights were on again, some seedlings resumed transpiration, as indicated by a reduction in leaf resistance.

Night leaf resistance followed a pattern with periods somewhat shorter than during photoperiod. The range of values was of less magnitude, with less difference between minimum and maximum points, perhaps as an indication of less time needed to recover plant turgidity after a period of transpiration, (Table XXIV).

It was difficult to visualize why the plant kept losing water during the night, if all factors causing transpiration demand were absent, with exception of air stirring inside the chamber. No plausible explanation could be found, unless we accept that a biological clock is controlling transpiration, somehow independently from environmental factors, and that it is manifested only under uniform conditions.

Overall mean leaf diffusive resistance for original and selected lines, as seen in Figure 17, also showed difference due to selection. On day 7, during photoperiod original lines had a uniform level of resistance until noon, increasing in value as the day proceeded. A clear effect of cycling is observed in 11-day-old seedlings, with two periods of different range and amplitude. Minimum resistance is observed at 8 A.M. and 1 P.M., which completes the first cycle of transpiration, the peak on resistance is observed at 10:00 A.M. on both original and selected lines. The second cycle is shorter for original lines, with a period of 2 hours between points of low resistance, and the range of values is reduced by 50% as compared to the first cycle.

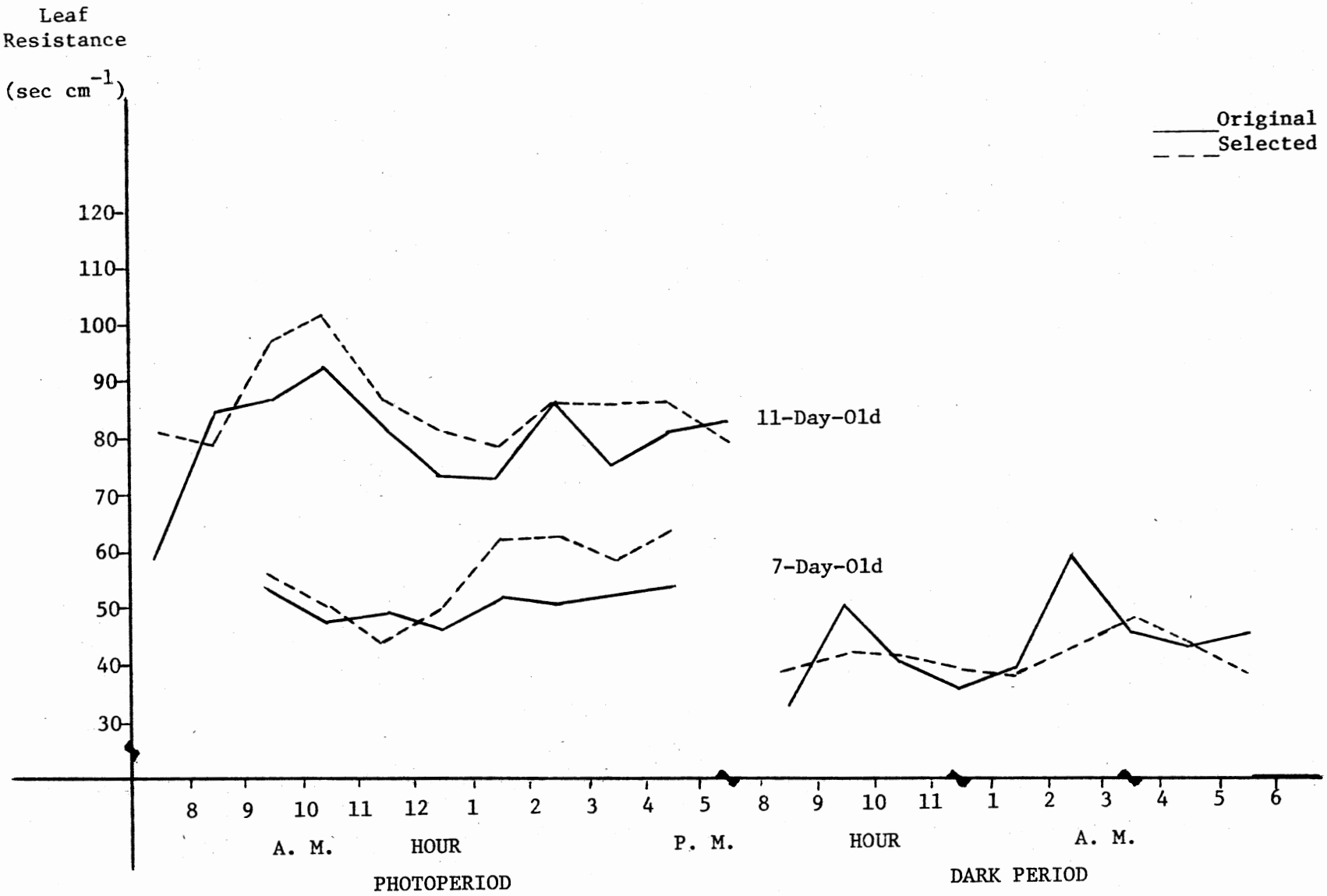


Figure 17. Overall Daily Pattern of Leaf Diffusive Resistance During Photoperiod and Dark Period, Measured on 7 and 11-Day-Old Seedlings for Original and Selected Lines. All Values Adjusted at 25 C.

While original lines maintained a cycling pattern, selected lines kept a constant rate of resistance, doubling the period between points of lower resistance on the second cycle. As can be seen in Figure 17, mean leaf resistance was higher for selected lines at virtually all hours during the time-period under consideration. During the dark period, original lines had two definite peaks of maximum resistance while selected lines had a more uniform response throughout the night. This may be related to water potential in the plant. If selected lines cut transpiration early in the day, more water is available and ready to be used, while original lines maintained a uniform rate of transpiration depleting available water from the soil. Also, and under the same assumption, more time is required to recover turgidity, as seen in the night pattern for original lines, while selected lines were not subjected to this delay.

Leaf Area

The analysis of this factor indicated significance at $\alpha = 0.064$ for entries, $\alpha = 0.099$ for selections, and $\alpha = 0.102$ for interaction entry x selection (Appendix, Table XXXII). Mean leaf area is presented in Table VIII. Among original cultivars, the resistant lines SU-23 and OK-111 had more leaf area than their susceptible counterparts, while IN-2 did not. Among selected lines, SU-23 and IN-15 remained superior while OK-111 had less leaf area than OK-8. Selected lines surpassed the original lines in leaf area except IN-2 and OK-111. All other entries had larger leaves and also faster rates of growth (Appendix, Tables XXV and XXVI).

TABLE VIII

MEAN LEAF AREA IN CM^2 FOR 7-DAY-OLD SEEDLINGS

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	6.7	8.4	1.7
	IN-15	8.4	8.8	0.4
	OK-8	6.3	7.5	1.2
RESISTANT	SU-23	7.2	10.2	3.0
	IN-2	7.6	6.4	-1.2
	OK-111	7.2	6.5	-0.7
CONTROLS	RYER	7.9		
	M.35-1	9.1		

F-tests were statistically significant for Entries (P=0.0644) and nonsignificant for Selections (P=0.099) and Entry x Selections (P=0.1021).

The increase in leaf area varied widely among entries, with all susceptible lines developing more leaf area than their respective original lines. Among the resistant lines SU-23 showed a large increase of 3.0 cm^2 over the original line, while IN-2 and OK-111 showed a decrease in leaf area of selected lines.

Growth rate, as measured by the increase in leaf area from day 7 to day 11 is presented in Table IX. This factor is difficult to characterize on a daily basis due to its logarithmic response during early stages, thus it is presented as total growth for a period of 4 days. Leaf area doubled for most of the entries during the period under consideration. Statistical analysis is presented in Table XXXIII.

If pregermination and transplanting of seedlings of equal size had some control on variability between and within entries, it may be, that this advantage in development was the result of a faster rate of cell multiplication, or cell elongation, or both. Hence, the selected lines had this advantage in growth due to this early boost in growth. Growth rate was improved in all selected entries except in IN-2 and OK-111. This advantage, if present only during the first 30 days of the plant's life, may be useful in avoiding competition from weeds, in the early shading of the soil which reduces water loss, and the establishment of a better stand.

Stomatal Density

Stomata counts were made under the microscope and converted to stomata per cm^2. Mean stomatal densities appear in Table X. Great differences were apparent among original lines. Cultivars from Sudan

TABLE IX

INCREASE IN LEAF AREA FROM DAY 7 TO DAY 11.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
	SU-6	9.8	14.2	4.4
SUSCEPTIBLE	IN-15	10.0	11.7	1.7
	OK-8	11.2	11.9	0.7
	SU-23	12.5	16.6	4.1
RESISTANT	IN-2	11.4	9.2	-2.2
	OK-111	12.1	8.7	-7.6
	RYER	14.7		
CONTROLS				
	M.35-1	10.9		

F-tests were statistically significant for Entries (P=0.020), nonsignificant for Selections (P=0.2143) and significant for Entry x Selections (P=0.0157).

TABLE X

MEAN STOMATAL DENSITY IN $10^3 CM^{-2}$, MEASURED ON THE ADAXIAL SURFACE, ON THE MID-PORTION OF THE THIRD LEAF OF 13-DAY-OLD SEEDLINGS.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	1.52	1.44	-0.08
	IN-15	1.01	1.26	0.25
	OK-8	0.77	0.79	0.02
RESISTANT	SU-23	1.45	1.31	-0.14
	IN-2	1.07	0.88	-0.19
	OK-111	0.91	0.92	0.01
CONTROLS	RYER	0.74		
	M.35-1	1.07		

had nearly twice as many stomata as cultivars from Oklahoma, and more variation was observed between groups than within groups.

The effect of selection was somewhat puzzling; SU-6 and SU-23 had decreased stomatal density and increased leaf area (previous Table). IN-2 had decreased stomatal density and decreased leaf area. Further, OK-111 had about the same stomatal density while it had a sizeable decrease in leaf area. Total number of stomata on the adaxial surface was calculated as the product of mean leaf area times mean stomatal density. These values are presented in Table XI. No consideration was given to stomatal density on the abaxial surface. Among original lines, all resistant lines had a higher number of stomata than their susceptible counterparts. Still the difference between groups was evident, with Oklahoma, India, and Sudan cultivars in order from lowest to highest density. The fact that total number of stomata was dependent upon total leaf area was clearly seen in the correlated improvement of both factors in selected lines (Tables IX and XI). Also these results corresponded fairly well with dry matter accumulation, indicating that fewer stomata per unit area was the result of cell enlargement, which increased the distance between stomata. IN-2, OK-8 and OK-111 had the lowest total number of stomata and also the lowest efficiency in growth, an indication that a relationship existed between growth and transpiration capacity.

Efficiency in Growth

The ratio of leaf area increase (ΔLA) over total water consumed (ΔWT) from day 7 to day 11 was considered as a measure of the amount of growth per unit of water. Mean values are presented in Table XII.

TABLE XI

TOTAL NUMBER OF STOMATA IN 10^3, ESTIMATED BY THE PRODUCT OF TOTAL LEAF AREA AND MEAN STOMATAL DENSITY IN ADAXIAL SURFACE, FOR 13-DAY-OLD SEEDLINGS.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	38.08	49.59	11.51
	IN-15	27.22	40.08	12.86
	OK-8	20.51	22.36	1.85
RESISTANT	SU-23	43.14	52.23	9.09
	IN-2	29.75	21.12	-8.63
	OK-111	25.95	20.63	-5.32
CONTROLS	RYER	25.11		
	M.35-1	33.01		

TABLE XII

GROWTH EFFICIENCY AS MEASURED BY THE INCREASE IN LEAF AREA PER UNIT-WATER USED, DURING A PERIOD OF 4 DAYS, FROM DAY 7 TO DAY 11.

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	1.00	1.28	0.28
	IN-15	0.83	0.90	0.07
	OK-8	1.05	1.02	-0.03
RESISTANT	SU-23	1.19	1.63	0.44
	IN-2	1.11	0.85	-0.26
	OK-111	1.08	0.81	-0.27
CONTROLS	RYER	0.69		
	M.35-1	0.97		

F-tests were statistically significant for Entries (P=0.0199), nonsignificant for Selections (P=0.5452), and Significant for Entry x Selection (P=0.0041).

SU-6 and IN-15 were improved in efficiency from original to selected lines. SU-23 and IN-2 were not changed, and both entries from Oklahoma suffered a reduction in efficiency. Ryer might be an example of this; it had the highest rate of growth with 1.96 cm^2 of leaf area developed per gram of water, confirming field observations (D. E. Weibel, personal comunication).

Conversion Efficiency of Water into Dry Matter

The measurement of dry matter produced per unit water consumed was a second approach to estimate water-use efficiency. This ratio measured the capacity of the plant to convert water into dry matter. Mean daily water consumption and dry matter accumulated per day are presented in Table XIII.

SU-6 and IN-15 among susceptible lines, and SU-23 among resistant lines increased dry matter production due to selection, corresponding well to the increase in leaf area already discussed. These entries had the largest increase in leaf area among all cultivars. OK-8 reduced its dry matter production, as well as IN-2. This resulted in a reduction in the efficiency of conversion of water to dry matter in both cultivars.

There was poor correlation of leaf area and production of dry matter, which could mean that some entries increased cell number and size, while others increased cell size alone. By observing these ratios, the improvement due to selection was evident in SU-6, IN-15, and OK-111, while SU-23 remained unchanged. Selected lines of OK-8 and IN-2 had less effciency than their original lines, due primarily to a reduction in dry matter production. IN-2 had an increase in leaf area with less dry matter produced, while OK-111 had less leaf area but still

TABLE XIII

WATER-USE EFFICIENCY, AS MEASURED BY THE RATIO OF DRY MATTER PRODUCED PER GRAM OF WATER CONSUMED PER DAY

CLASSIFICATION	ENTRY	SELECTION	DRY MATTER (g/day)	WATER LOSS (g/day)	RATIO (DM/WL)	IMPROVEMENT
SUSCEPTIBLE	SU-6	Or.	0.034	2.44	1.4	0.4
		Sel.	0.051	2.78	1.8	
	IN-15	Or.	0.033	2.97	1.1	0.2
		Sel.	0.041	3.21	1.3	
	OK-8	Or.	0.044	2.68	1.6	-0.2
		Sel.	0.039	2.87	1.4	
RESISTANT	SU-23	Or.	0.044	2.65	1.7	0.0
		Sel.	0.046	2.75	1.7	
	IN-2	Or.	0.040	2.54	1.6	-0.4
		Sel.	0.031	2.67	1.2	
	OK-111	Or.	0.039	2.81	1.4	0.1
		Sel.	0.039	2.65	1.5	
CONTROLS	RYER	Or.	0.054	2.52	2.1	
	M.35-1	Or.	0.040	2.65	1.5	

maintained the same dry weight, an indication of more cell enlargement without in increase in cell number in the first case, and the opposite in the latter.

Germination in Osmotic Solutions

No statistical difference was detected in germination percentage for concentration of Mannitol, as can be seen in Table XIV. However, entries were significantly different at $\alpha = 0.0001$, and the interaction entry X concentration at $\alpha = 0.0071$ (Appendix, Table XXXIV).

Germination speed was affected, as expressed by the percentage of germinated seeds at the first count. Data for this factor are presented in Table XV. Entries were different at $\alpha = 0.0113$ and concentration of Mannitol indicated differences at $\alpha = 0.0001$, and the interaction entry x concentration at $\alpha = 0.0165$ (Appendix, Table XXXV).

Germination speed was different among cultivars and among selections at 0 atmospheres. Selected lines of SU-6, SU-23, IN-15, and OK-111 had higher percentages at first count than their original lines. Most selected lines showed consistently higher germination speed at all levels of osmotic pressure, except OK-8. A marked reduction was observed in original lines of SU-23 and IN-2 as the concentration of Mannitol increased. It seemed like the interval from 9 to 12 atmospheres was a critical threshold that acted as a selective barrier among genotypes. Among the original cultivars IN-15, OK-8 and OK-111 had some germination above the level of 9 atmospheres, while among selected lines all but SU-23 and IN-2 had some germination at these concentrations, with SU-6 and OK-111 being improved in germination speed over their original lines. The average indicated improvement for all selected lines except OK-8,

TABLE XIV

GERMINATION PERCENTAGE IN OSMOTIC SOLUTIONS.

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15	AVERAGE
SU-6	Or.	75	80	80	70	75	95	79
	Sel.	100	100	100	95	100	100	99
SU-23	Or.	20	25	65	45	30	20	34
	Sel.	95	95	90	95	90	95	93
IN-2	Or.	100	95	100	100	95	95	98
	Sel.	100	95	90	95	85	85	92
IN-15	Or.	95	100	95	90	95	80	93
	Sel.	90	100	95	100	100	95	97
OK-8	Or.	75	85	85	85	85	85	83
	Sel.	85	75	90	85	95	90	87
OK-111	Or.	95	95	95	95	90	90	93
	Sel.	100	90	95	100	100	100	98
Ryer	Or.	90	85	95	85	95	95	91
M.35-1	Or.	90	75	80	80	60	80	76

F-tests were statistically significant for Entry x Osmotic level (P=0.0071) and Entry x Selection (P=0.0037), and nonsignificant for Entry x Osmotic level x Selection (P=0.4697).

TABLE XV

MEAN GERMINATION SPEED, MEASURED AS PERCENT GERMINATION AT FIRST COUNT

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15	AVERAGE
SU-6	Or.	40	35	25	15	0	0	19.2
	Sel.	80	65	20	45	20	5	39.2
SU-23	Or.	10	0	0	5	0	0	2.5
	Sel.	35	10	10	5	0	0	10.0
IN-2	Or.	80	50	5	5	0	0	23.3
	Sel.	60	35	40	15	0	0	25.0
IN-15	Or.	50	10	10	5	0	5	13.3
	Sel.	80	80	50	35	5	5	42.5
OK-8	Or.	65	70	60	20	20	5	40.0
	Sel.	50	25	20	10	0	5	18.3
OK-111	Or.	60	50	10	20	5	0	24.2
	Sel.	80	60	45	25	5	5	36.7
Ryer	Or.	65	70	50	35	5	0	45.0
M.35-1	Or.	20	25	10	25	5	0	17.0

F-tests were statistically nonsignificant for Entry x Osmotic level (P=0.1446) and significant for Entry x Selection (P=0.0093) and Entry x Osmotic level x Selection (P=0.0408).

with the largest increase in IN-15, and the smallest on SU-23 and IN-2. If germination speed is a measure of the capacity of the seed to imbibe water, then all selected lines, except OK-8 improved their absorption capacity, perhaps as an indication that selected lines had higher seed osmotic potentials.

Emergence rate index is a measure of germination on a time basis. The weighted average could be considered as a measure of the response of a cultivar to the increase in osmotic pressure in the media. This index is presented in Table XVI. Statistical analysis indicated high significance at $\alpha = 0.0003$ for entries $\alpha = 0.0004$ for concentrations of Mannitol, and $\alpha = 0.1199$ for the interaction entry x concentration (Appendix, Table XXXVI).

Selected lines of SU-6, SU-23, IN-15, and OK-111 were improved in rate of emergence at all concentrations of Mannitol. The original line of IN-2 was best at low concentrations (0 and 3 atm) while the selected line had better response at intermediate levels (6 to 12 atm). The original line of OK-8 remained superior to the selected line at all levels. Entries that expressed a better rate of emergence at the 0 and 3 atmospheres also showed superiority at the highest concentration of 15 atmoshperes, although a reduction of near 50% in emergence was observed between the levels of 0 and 15 atmospheres.

Seedling Development in Osmotic Solutions

The average dry weight of roots from 10 seedlings was used to characterize each entry. Figures 18, 19, and 20 represent the response of each cultivar to variation in osmotic pressure where SU-6, SU-23 and IN-15 were the only selected lines that proved to be superior to the

TABLE XVI

EMERGENCE-RATE INDEX FROM DAY 1 TO DAY 4
IN OSMOTIC SOLUTIONS.

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15	WEIGHTED AVERAGE
SU-6	Or.	5.7	5.8	5.2	4.0	3.6	3.6	4.6
	Sel.	9.0	8.3	5.8	6.9	5.8	4.5	6.7
SU-23	Or.	1.4	1.3	3.1	2.3	1.2	0.9	1.7
	Sel.	6.4	5.3	4.8	4.5	3.1	3.4	4.6
IN-2	Or.	9.0	7.3	5.3	5.2	3.9	3.4	5.7
	Sel.	8.0	6.4	6.5	5.5	4.0	3.2	5.6
IN-15	Or.	7.3	5.3	5.3	4.3	3.8	3.2	4.8
	Sel.	8.5	9.0	7.3	6.6	5.0	4.0	6.7
OK-8	Or.	6.9	7.8	7.3	5.3	5.3	4.2	6.1
	Sel.	6.5	4.9	5.3	4.8	4.2	4.0	4.9
OK-111	Or.	7.8	7.3	5.0	5.8	4.6	4.1	5.7
	Sel.	9.0	7.5	7.0	6.2	5.1	4.5	6.5
Ryer	Or.	7.5	7.3	7.3	6.6	4.8	4.3	6.3
M.35-1	Or.	5.2	4.5	4.3	4.5	3.1	2.5	4.0

F-tests were statistically nonsignificant for Entry x Osmotic level (P=0.1199), significant for Entry x Selection (P=0.0125) and nonsignificant for Entry x Osmotic level x Selection (P=0.3113).

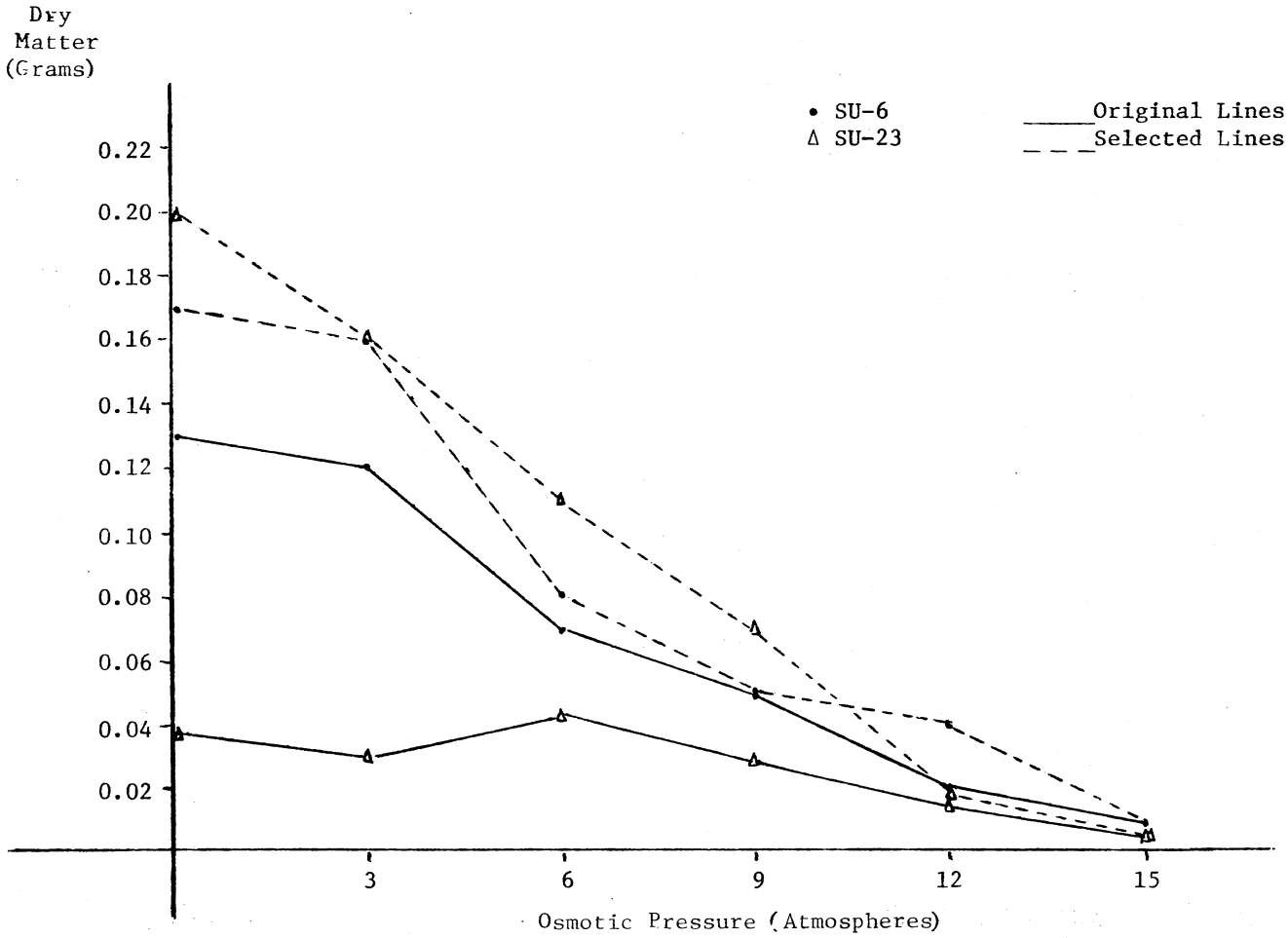


Figure 18. Root Dry Weight for Original and Selected Lines from Sudan. Average of 10 Seedlings after 4 Days of Development in Mannitol Solutions.

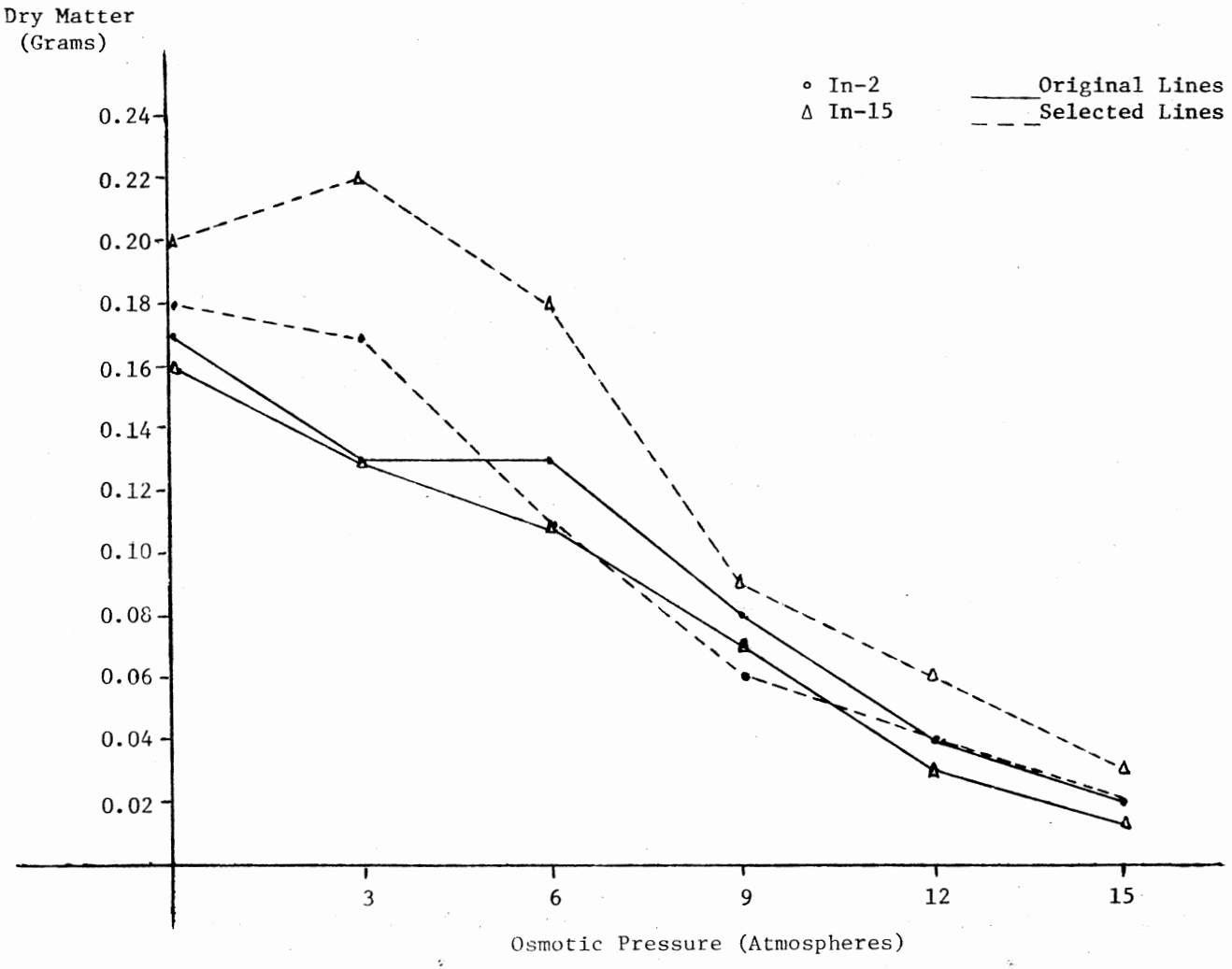


Figure 19. Root Dry Weight for Original and Selected Lines from India. Average of 10 Seedlings after 4 Days of Development in Mannitol.

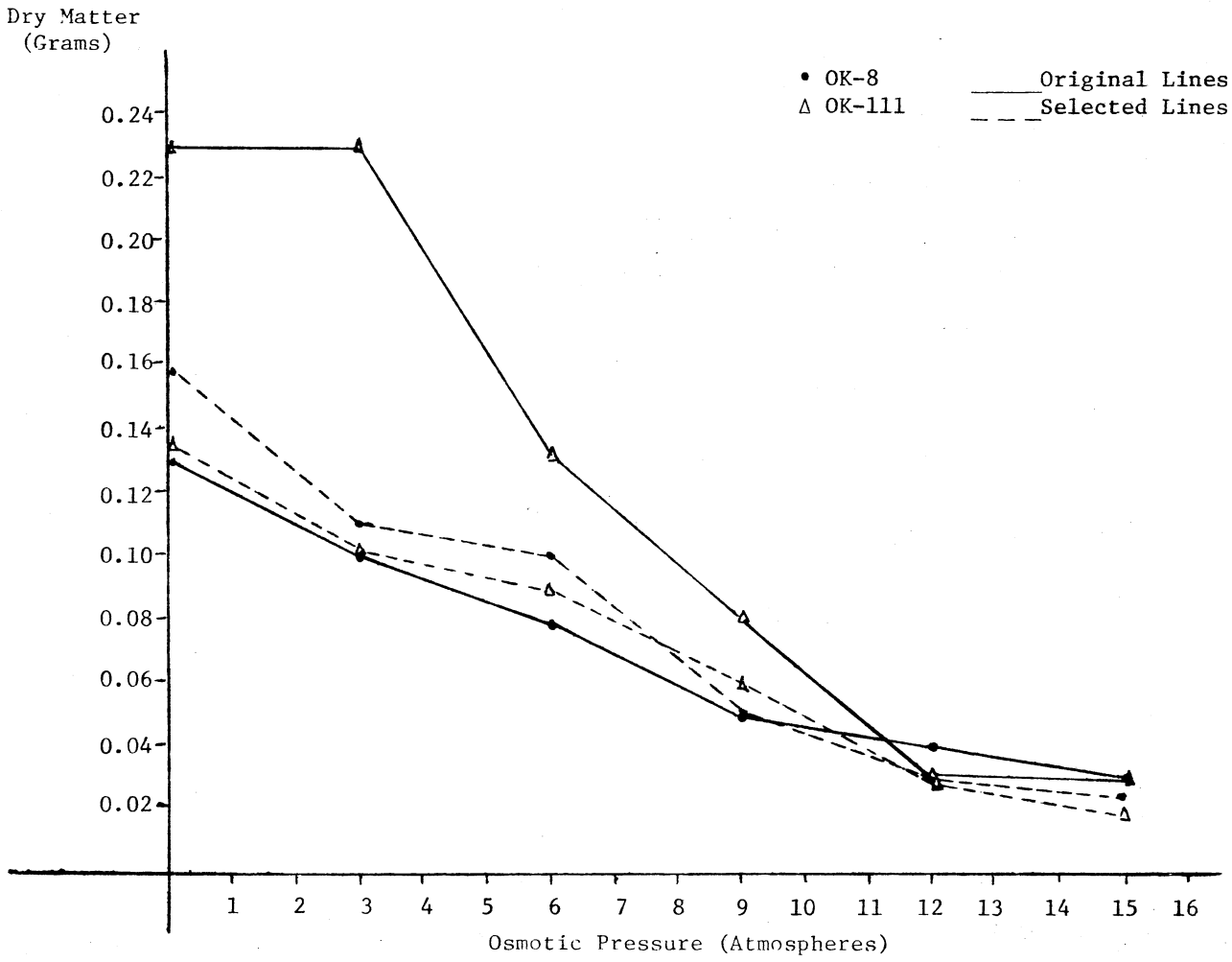


Figure 20. Root Dry Weight for Original and Selected Lines from Oklahoma. Average of 10 Seedlings after 4 Days of Development in Mannitol.

original line at all concentrations of Mannitol. IN-2 and OK-8 were superior only at osmotic pressures of 6 atmospheres or less. Above the level of 9 atmospheres, little or no difference was observed between original and selected lines except in SU-6 and IN-15. The limit of 9 atmospheres again appeared to be a tough barrier for all entries, and could be considered as the natural limit for future tests.

In Table XVII, it can be appreciated that cultivars from Oklahoma had the largest accumulation of dry matter at high osmotic potentials followed by cultivars from India and Sudan.

There seemed to be an increase in dry matter accumulation in the rotts at intermediate levels of osmotic pressure. Apparently this increase is a consequence of lower absorption of water by the roots. Lower percentage of root dry matter indicated higher root water percentage. Selections of SU-6, IN-2 and IN-15 were superior in relative water content to their original lines at concentrations below 9 atm. If it is assumed that higher water percent means greater capacity to absorve water, then these selected lines were improved in their capacity to extract water from the media. Selected lines of SU-23 and OK-8 were superior to their original line at concentration of 9 and 12 atm, while that of OK-111 had more water percentage at 3 and 6 atm.

Attention should be given to the fact that all selected lines of each cultivar, reached the maximum of dry matter accumulation one concentration level above the level of maximum accumulation for the original lines. If this was a measure of performance during early stages of growth, then the selected lines would have greater potential to survive due to the advantage in water absorption and root development at higher osmotic concentrations, or under higher water potentials. The

TABLE XVII

ROOT DRY MATTER PERCENTAGE ON 4-DAY-OLD SEEDLINGS GERMINATED IN MANNITOL

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15	AVERAGE
SU-6	Or.	14	13	16	16	13	13	14.4
	Sel.	13	11	13	14	19	13	13.7
SU-23	Or.	6	6	11	18	15	10	11.0
	Sel.	10	12	13	13	10	14	12.0
IN-2	Or.	11	14	15	22	18	20	16.7
	Sel.	7	13	14	16	22	17	14.8
IN-15	Or.	11	11	18	23	23	16	16.9
	Sel.	10	1	15	18	23	20	14.5
OK-8	Or.	14	12	14	17	25	23	17.6
	Sel.	15	13	14	16	18	23	16.2
OK-111	Or.	11	15	16	18	15	27	23.6
	Sel.	14	13	13	18	17	25	16.7
Ryer	Or.	15	16	15	20	17	17	16.7
M.35-1	Or.	10	13	15	16	13	9	12.7

F-tests were statistically nonsignificant for Entry x Osmotic level (P=0.6054), Entry x Selection (P=0.4504) and Entry x Osmotic level x Selection (P=0.4846).

cultivar M.35-1, used as control, had a particular response with dry matter accumulation following a bell-shaped curve, with the maximum accumulation between 6 and 9 atmospheres.

Root length was measured on 5 seedlings per entry, and the average used to characterize the cultivar. Table XVIII contains these values. Selected lines of SU-6 and IN-2 had the longest roots at all levels selected. In-15 was superior at all levels, except at 0 atm. SU-23 was superior to its original line from 0 to 9 atomospheres and OK-111 from 0 to 6 atmospheres only. SU-23 appeared to have shorter roots with less dry matter production than other entries. The response in OK-8 was variable and no tendency was detected between selections, except a slight superiority of the original line at osmotic pressures above 9 atmospheres. Again the largest differences appeared at concentrations of 9 atmospheres or less. All entries had similar lengths at 12 and 15 atmospheres, thus the highest level for screening among cultivars appears to be 9 atmospheres.

Root development on these lines had some relation to germination speed and rate of emergence. Roots that emerged earlier had more time to grow and to develop as compared to the later roots. The relation to dry matter accumulation was not consistent though, and it should be studied further. Those entries that developed longer roots also had more water percentage and dry matter accumulation, as a result of earlier germination and of a faster rate of growth. Probably an increase in internal water potential had something to do with this advantage, by permitting the seed to have more imbibition of water during germination.

TABLE XVIII

ROOT LENGTH IN CM OF 4-DAY-OLD SEEDLINGS GERMINATED IN MANNITOL.

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15	AVERAGE
SU-6	Or.	5.5	4.7	3.4	1.9	1.2	0.2	2.8
	Sel.	6.1	6.0	3.5	2.8	1.5	1.0	3.5
SU-23	Or.	0.9	0.7	1.7	0.8	0.5	0.4	0.8
	Sel.	5.5	3.6	2.9	1.9	0.5	0.3	2.5
IN-2	Or.	6.2	5.0	4.2	2.2	1.2	0.5	3.2
	Sel.	7.3	6.3	4.6	2.7	1.7	0.8	3.9
IN-15	Or.	5.7	3.9	2.9	1.9	1.1	0.7	2.7
	Sel.	4.7	5.6	4.6	2.3	1.4	0.9	3.2
OK-8	Or.	5.2	4.4	2.7	2.0	1.0	0.8	2.7
	Sel.	6.3	3.7	2.9	1.6	0.9	0.7	2.7
OK-111	Or.	5.8	4.9	4.0	2.2	0.9	0.7	3.1
	Sel.	6.1	5.2	4.5	1.7	0.9	0.3	3.1
Ryer	Or.	4.8	4.6	3.5	2.5	1.8	1.3	2.8
M.35-1	Or.	7.5	4.0	3.4	1.5	1.2	0.6	2.6

F-tests were statistically significant for Entry x Osmotic level ($P=0.0001$), Entry x Selection ($P=0.0001$) and Entry x Osmotic level x Selection ($P=0.0001$).

Shoot dry weight was obtained simultaneously to root dry weight. The means of five seedlings of each cultivar are presented in Table XIX. Shoot dry weight had no consistent response to the increase in osmotic pressure. Higher weights were observed on all original lines at 0 atm except on SU-23. SU-6, SU-23 and OK-111 had higher dry weight on selected lines at 3 and 6 atm. Selected IN-15 was consistently better at levels above 6 atm. IN-15 and OK-8 among selected lines, were the only entries that could develop a shoot at 15 atmospheres the highest pressure used in this study.

Little difference was observed in dry matter accumulation between original and selected lines, especially at low osmotic levels (0 to 6 atm). The means for shoot dry matter are presented in Table XX. Original and selected lines had a striking similarity in dry matter from 0 to 9 atmoshperes, differing only at higher concentrations of Mannitol. It was, in most cases, the selected line that had a higher percentage of dry matter while the original line had no growth at all.

Mean shoot lengths are presented in Table XXI. With the exception of OK-8 all selected lines developed larger shoots than original lines, at all concentrations of Mannitol. The effect of selection was positive by increasing growth rate; this effect could be observed throughout the time under consideration in this study, and was reflected in larger leaf area at the seedling stage (see Table IX). IN-2 and OK-111 suffered a reduction in growth rate, and no reason was evident for this behavior.

A genetic difference could be appreciated in shoot development at the 0 level, where the order among groups of cultivars, from highest to lowest was India, Oklahoma, and Sudan. This difference could be related to the genotype of these cultivars, where both entries from India were

TABLE XIX

SHOOT DRY WEIGHT IN GRAMS, OF 4-DAY-OLD SEEDLINGS GERMINATED IN MANNITOL

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15	AVERAGE
SU-6	Or.	0.8	0.8	0.5	0.3	0.1	0	0.40
	Sel.	0.6	1.2	0.6	0.3	0.1	0	0.44
SU-23	Or.	0.1	0.1	0.2	0	0	0	0.07
	Sel.	1.2	0.7	0.4	0.3	0	0	0.42
IN-2	Or.	1.7	1.1	0.7	0.2	0	0	0.59
	Sel.	1.5	1.3	0.5	0.2	0.1	0	0.60
IN-15	Or.	1.8	1.3	0.6	0.3	0.1	0	0.67
	Sel.	1.4	1.0	1.1	0.6	0.3	0.1	0.72
OK-8	Or.	0.9	0.5	0.4	0.2	0.1	0	0.31
	Sel.	0.6	0.7	0.3	0.1	0.1	0.1	0.30
OK-111	Or.	1.6	1.2	0.4	0.1	0	0	0.53
	Sel.	1.1	1.4	0.6	0.2	0.1	0	0.53
Ryer	Or.	1.7	0.9	0.6	0.2	0.2	0	0.60
M.35-1	Or.	2.1	1.0	0.4	0.1	0.2	0	0.63

F-tests were statistically nonsignificant for Entry x Osmotic level (P=0.1776), Entry x Selection (P=0.3711) and Entry x Osmotic level x Selection (P=0.4361).

TABLE XX

SHOOT DRY MATTER PERCENTAGE ON 4-DAY-OLD SEEDLINGS GERMINATED IN MANNITOL.

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15
SU-6	Or.	11	12	13	16	13	0
	Sel.	12	11	13	16	17	0
SU-23	Or.	7	5	12	2	8	0
	Sel.	10	10	13	19	0	0
IN-2	Or.	11	12	15	21	6	0
	Sel.	11	13	14	20	18	45
IN-15	Or.	12	13	14	20	19	11
	Sel.	12	13	15	22	30	17
OK-8	Or.	13	13	16	16	57	0
	Sel.	13	13	16	15	57	83
OK-111	Or.	11	15	18	21	0	0
	Sel.	13	12	30	15	33	0
Ryer	Or.	10	13	15	11	50	0
M.35-1	Or.	12	13	16	15	25	0

F-tests were statistically nonsignificant for Entry x Osmotic level (P=0.9780), Entry x Selection (P=0.6128), and Entry x Osmotic level x Selection (P=0.9415).

TABLE XXI

SHOOT LENGTH IN CM OF 4-DAY-OLD SEEDLINGS GERMINATED IN MANNITOL.

ENTRY	SEL.	ATMOSPHERES OF OSMOTIC PRESSURE 0	3	6	9	12	15	AVERAGE
SU-6	Or.	2.5	1.1	1.4	0.4	0.2	0	1.0
	Sel.	3.0	2.8	1.3	0.7	0.3	0.1	1.4
SU-23	Or.	0.3	0.5	0.8	0.2	0	0	0.3
	Sel.	2.9	1.4	1.1	0.5	0	0	1.0
IN-2	Or.	5.1	2.8	1.5	0.4	0.1	0	1.7
	Sel.	5.2	3.6	2.2	0.6	0.3	0.1	2.0
IN-15	Or.	5.2	3.8	1.2	0.5	0.2	0.1	1.9
	Sel.	5.3	4.9	2.5	0.8	0.3	0.2	2.3
OK-8	Or.	3.2	1.5	1.1	0.4	0.1	0	1.1
	Sel.	1.6	2.2	0.6	0.1	0.1	0.1	0.8
OK-111	Or.	4.1	3.1	0.8	0.2	0	0	1.4
	Sel.	2.7	3.6	1.3	0.6	0.1	0	1.4
Ryer	Or.	2.8	2.1	1.0	0.4	0.1	0	1.1
M.35-1	Or.	5.7	3.3	2.2	0	0	0.1	1.9

F-tests were statistically significant for Entry x Osmotic level (P=0.0001), Entry x Selection (P=0.0001) and Entry x Osmotic level x Selection (P=0.0001).

2-dwarf, and cultivars from Oklahoma and Sudan were 3-dwarf. The difference between cultivars from Oklahoma and Sudan might be related to differences in maturity, where the cultivars from Oklahoma were earlier than those from Sudan.

CHAPTER V

SUMMARY AND CONCLUSIONS

A complex environment demands high specialization among individuals where only those that are capable of withstanding severe variations of soil and atmosphere can survive. This is natural selection working for the improvement of the species by selecting the best individuals within a population. Actually the plant breeder applies selection procedures that identify and separate individuals with a certain trait. Sometimes a specific trait can not be measured or evaluated without the proper technique.

Drought or dessication tolerance is a complex factor, difficult to understand and to evaluate. A reliable technique that measures and selects for water stress tolerance is still unavailable. This study is a contribution to the development of a practical and effective method of selection for tolerance to water stress. The objectives were: 1) to identify possible sources of resistance among several grain sorghum cultivars, selecting individuals that survived a period of edaphic stress, and 2) to demonstrate the effectiveness of this technique in improving water-use efficiency of the progeny of those individuals selected within each cultivar.

Screening tests were performed on 7-day-old seedlings of cultivars from Sudan, India, Oklahoma, and Mexico, selecting those plants that survived one period of 5 days of water stress under conditions of high

temperature and reduced light. These seedlings were transplanted to the greenhouse, side by side with a representative sample of the original unselected line for seed production under similar conditions.

Pregerminated seeds from the greenhouse material were transplanted to individual styrofoam cups using soil as rooting media. The use of partially germinated seeds reduced variability between and within cultivars. Simultaneous tests were performed on original and selected lines on seedlings of 7 and 11 days of age, for water consumption, leaf diffusive resistance, leaf resistance patterns for day and night, leaf area, and stomatal density. These factors were used to calculate water-use efficiency as growth in cm^2 of leaf area per unit of water consumed, and grams of dry matter produced per unit of water.

Average water consumption, as measured by weight difference was 1.08g per day on 7-day-old seedlings, and 2.06g per day for 11-day-old plants. If it is assumed that higher rates of water consumption are associated with higher yields of either grain or forage, then those entries with higher water consumption should also have higher yield potential. Water consumption per day increased in SU-6, SU-23, IN-15 and OK-8 at 7 days of age; only IN-15 was statistically significant at $\alpha = .05$. Total water consumption measured in a period of 4-days also increased in all cultivars except OK-111; no statistical significance was detected among selections. Average water consumption per day during this period was estimated at 2.75 g, with the selected line of IN-15 showing the highest level with 3.2 g day^{-1} and the original line of SU-6 the lowest with 2.4 g day^{-1}. The highest improvement was observed in SU-6, with 0.34 g day^{-1} above its original line.

Leaf diffusive resistance was measured twice daily at 8 A.M. and 2 P.M. with an Autoporometer, using a sensor with a narrow aperture of 7 mm^2 placed on the middle portion of the third leaf of 7-day-old seedlings, and on the fourth leaf of 11-day-old plants. Stomatal resistance was measured on the adaxial surface only. The highest level of resistance among 7-day-old seedlings in the original lines was observed in IN-15 with 50.7 sec cm^{-1}, and the lowest in OK-8 with 36.5 sec cm^{-1}. Among selected lines, SU-23 had the highest resistance with 60.4 sec cm^{-1} while IN-2 had the lowest value of 43.5 sec cm^{-1}. SU-6, SU-23, OK-8, and OK-111 had increased resistance in selected lines, while IN-2 and IN-15 had reduced resistance.

The daily pattern for leaf diffusive resistance was determined by taking measurements at 1-hour intervals on 7-day-old and 11-day-old seedlings during the light period. Similar measurements were taken during the dark period on a separate group of 7-day-old seedlings. The means from eight readings during the day did not coincide with the means of the previous estimate using two readings per day. It was observed that two readings per day tended to underestimate leaf diffusive resistance, at least in half of the entries. However, selected line SU-23 had the highest resistance in both methods, with readings above 60 sec cm^{-1}, and the original line of OK-8 had the lowest readings in both methods. SU-6, SU-23, IN-2 and OK-8 had increased leaf resistance in selected lines, while IN-15 and OK-111 suffered a reduction in selected lines.

The difference in means obtained with the two methods could be attributed to a cycling pattern in leaf resistance which becomes evident under uniform conditions inside a growth chamber. A mean obscures the cycling, if present, and makes comparisons of means less meaningful.

Leaf resistance during the dark period proved to be an effective way to differentiate between cultivars and among selections. OK-8 and OK-111 had the lowest values among original cultivars with 31.8 and 29.6 sec cm^{-1} respectively. IN-2 and IN-15 had the highest resistance with 50.8 and 51.0 sec cm^{-1}. Among selected lines, IN-2 had the highest value of 74.0 sec cm^{-1}, while IN-15 had the lowest of 26.8 sec cm^{-1}.

A reduction in leaf diffusive resistance was observed in selected lines of SU-6, SU-23, and IN-15, while IN-2 and OK-111 had increased resistance during the dark period and OK-8 remained unchanged. The reduction in resistance could indicate that stomata remained open during the night, thus water vapor and CO_2 were being exchanged, or that water was being lost through the cuticle, regardless of stomatal opening.

The ratio night/day leaf resistance measured the difference in stomatal behavior regardless of the level of resistance. Four out of six selected lines had lower ratios, with a minimum reduction of 21.0 and a maximum of 31.8. Only IN-2 and OK-111 had lower resistance during the day and higher during the night. IN-2 had the highest ratio among all entries, either original or selected with 125.3% of N/D ratio.

As observed in the pattern of day leaf resistance, an increase in leaf resistance late in the evening in selected lines, indicated a reduction in transpiration and consequently less depletion of water from the soil surrounding the roots. This suggested that more water remained available in the soil and less time was required for recovery of turgidity of the plant. On the other hand, those original lines that maintained a uniform rate of transpiration throughout the day probably had a higher water deficit both in the soil and in the plant, thus more time was needed for a complete recovery of turgidity.

It was interesting to note that leaf area for 7-day-old seedlings was superior for selected lines of SU-6, SU-23, IN-15, and OK-8, and also these lines developed more leaf area in a 4-day period from day 7 to day 11. The largest increment was observed in SU-23 with 3.0 cm^2 of leaf area, while selected lines of IN-2 and OK-111 suffered a reduction with respect to their original lines. The rate of growth was related to other factors; selected lines of SU-6, SU-23 IN-15 and OK-8 consumed more water on day 7 and during a 4-day period from day 7 to 11, all but IN-15 had higher leaf resistance during the day, and lower resistance during the night. The ratio of night/day leaf resistance also suggested the superiority of these selected lines over their original lines. In all instances, higher water loss was positively related to more leaf area developed either in selected or in original lines.

Stomatal density was also modified by selection. SU-6, SU-23, and IN-2 showed a reduction of stomatal density of 0.08, 0.14, and 0.19 stomata cm^{-2} respectively. IN-15 had an increase of 0.25 stomata cm^{-2} over its original line while OK-8 and OK-111 remained unchanged. It was not determined if these modifications were induced by cell enlargement or an increase in cell number. Apparently both cultivars from Sudan had an increase in leaf area and a reduction in stomatal density as a consequence of cell enlargement, while IN-15 had a small increase in leaf area but a large increase in stomatal density, an indication of more cells per unit area or consequently a smaller cell size.

The efficient use of water, as measured by growth obtained per unit of water, reflects the capacity of the plant to grow and develop before moisture becomes a limiting factor, permitting the airial portions of the plant to shade the ground so evaporation is reduced. Selection was

effective for SU-6, SU-23, and IN-15, which increased efficiency by 0.28, 0.44 and 0.07 $cm^2\ g^{-1}$ respectively. IN-2, OK-8 and OK-111 suffered a reduction of 0.26, 0.07 and 0.27 $cm^2\ g^{-1}$ respectively. The reduction in efficiency could be related to less leaf area developed, lower growth rate, higher night/day leaf resistance ratios, and lower water consumption in 7-day-old seedlings of selected lines of IN-2, OK-8 and OK-111.

Water-use efficiency, as measured by dry matter accumulation per unit of water, was higher for all selected lines except of IN-2 and OK-8. This was related almost exclusively to leaf area development and plant height. Selected lines of IN-2 and OK-111 had less leaf area developed, while SU-23 had a reduction in height even though its leaf area increased. The lower amount of dry matter accumulated in the leaves and in the stalk was responsible for this reduction in efficiency, and only SU-6 and IN-15 were improved altogether in dry matter leaf area, plant height, and water-use efficiency.

Germination percentage was not affected by the osmotic levels of Mannitol. Similar percentages were observed within each entry at the 4-day count, and on the last count after 7 days. Germination speed, measured as germination percentage after 24 hours, was modified by selection. All selected lines were improved in germination speed, except OK-8. SU-23 had the lowest germination values at all levels of osmotic pressure.

Lower dry matter percentage in the seedling indicated higher water percentage. By observing root dry matter percentage, it could be determined that all selected lines had higher water percentage in the root as compared to their original lines, except SU-23. This could be an

indirect measure of the capacity of the root to absorb water. When root length was considered, all cultivars from Sudan and India were improved over the original line, while cultivars from Oklahoma remained unchanged. The largest increment in root length was obtained in SU-23, but still this cultivar had the smallest root among all entries with 0.8 cm, as well as the lowest percent of dry matter accumulation. The selected line of IN-2 had the longest root with 3.9 cm at the 4-day count. Genetic variability for root length was observed in cultivars from Sudan and India, while the entries from Oklahoma were very uniform.

Shoot dry weight was improved on all selected lines except OK-8 and OK-111. Selected lines of SU-23 and IN-15 had the largest increment over the original line, however, it was considered that original line of SU-23 had an abnormal root development hence no clear effect could be adscribed to selection.

Shoot dry matter percentage became meaningless at high concentrations of Mannitol, where little shoot development was observed with the emergence of the epicotyl alone. The structure formed had very low dry matter accumulated after 4 days of growth, and its quantification was difficult, obtaining zeros in some cases. The level of nine atmospheres was considered the highest level for detection of differences among entries and between selections.

Root and shoot lengths were positively correlated with germination speed and emergence rate index. It should be expected that early emergence determined more time for root and shoot development, however, dry matter accumulation in the root and in the shoot was not related in all cases to early germination.

Based on this information, the following conclusions could be obtained:

1. The screening technique developed was effective in the identification of resistance to water stress among cultivars.
2. Selection was effective in increasing leaf diffusive resistance during the day in selected lines of SU-6, SU-23, IN-2 and OK-8. Selected lines of IN-15 and OK-111 had reduced resistance as compared to their original lines.
3. Leaf diffusive assistance was lower during the night in selected lines of SU-6, SU-23 and IN-15, and higher for IN-2 and OK-111.
4. The ratio of night/day leaf resistance was lower in selected lines of SU-6, SU-23, IN-15 and OK-8, and higher for IN-2 and OK-111.
5. Better utilization of water by reducing transpiration late in the evening and a higher transpiration rate at night resulted in an advantage for selected lines, as reflected by an increase in leaf area on 7-day-old seedlings, and a better growth rate from day 7 to day 11.
6. Water loss during the night was high, even though stomatal resistance was also high, indicating a possible loss of water through the cuticle during the night.
7. Higher consumption of water was positively associated to lower night/day leaf resistance ratio, and more leaf area developed, regardless of type of selection.
8. From the studies with Mannitol solutions, SU-6, SU-23, IN-15 and OK-111 were improved by selection in germination percentage, germination speed and emergence rate.

9. Root and shoot lengths were improved by selection in SU-6, SU-23, IN-2 and IN-15.
10. Higher germination speed and higher emergence rate produced more root and shoot development in selected lines, with longer roots associated with longer shoots.
11. Higher water percentage in the root was negatively associated with water percentage in the shoot when seeds were germinated in Mannitol solutions.
12. Seed germination and seedling development were severely reduced above 9 atmospheres of osmotic pressure.
13. These techniques of screening and evaluation should be studied further, in order to compare these findings with those of other methods.

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APPENDIXES

TABLE XXII

LIST OF CULTIVARS FOR SCREENING TESTS

Code	Common Name	Days under Stress 1	2	3	4	5	6	Score
	Group from Sudan							
SU-1	Cross 3:17-7	1	1	6	2	-	-	2.90
SU-2	L. R. White 20-27-1	-	1	4	7	-	-	4.20
SU-3	Zirizira II B-23-1-1	-	1	5	4	-	1	3.55
SU-4	Zirizira I 3-5-1	3	4	5	1	-	1	2.57
SU-5	Cross 1:36-14	4	3	4	7	1	-	2.89
SU-6	Gadam El-Hamam 33-2-1	1	3	4	2	-	-	2.70*
SU-7	Bargawi A-56-1	1	1	-	1	-	-	2.33
SU-8	Gassabi II A-3-1-2	-	-	4	1	-	-	3.20
SU-9	Gorib 10-3-1-1	2	2	2	7	-	-	3.08
SU-10	Cross 12:9-6-1	-	1	-	8	1	-	3.90
SU-11	Mayo A-239:7-1-11	1	1	3	2	-	-	3.43
SU-12	Tozi Wad Akar 51-3	-	-	6	4	-	-	3.40
SU-13	Karkatib 4-1-1	1	1	3	-	-	-	2.40
SU-14	Tozi Wad Yabis	-	-	-	-	-	-	----**
SU-15	Zanab El-Shah 1-3-1	2	2	1	-	-	-	1.80
SU-16	Croxx 4:43-32	2	-	2	-	-	-	2.00
SU-17	Tozi Unbinein 22	-	1	7	1	1	-	3.20
SU-18	Tozi Unbinein 7	-	1	4	-	-	-	2.80
SU-19	Tozi Fet. Maatug 7	-	-	-	-	-	-	----**
SU-20	Zinnari	-	-	-	-	-	-	----**
SU-21	Gadam El-Hamam A5-1-3-1	-	-	-	-	-	-	----**
SU-22	Dabar 1-1-1-1	1	-	3	1	-	-	2.80
SU-23	L. R. Red B-23-27-1	1	3	3	4	4	-	3.47*
SU-24	Faki Mustahi A-121	-	2	6	11	4	-	3.74
SU-25	Cross II 46-11-8	2	1	4	6	-	3	3.63
SU-26	Mugud Akiad A-251	-	-	-	-	-	-	----**
SU-27	Nyan Doil A-263	-	-	-	-	-	-	----**
SU-28	Lwel-2 A-216	2	3	2	2	-	-	2.44
SU-29	Query I A-269	1	-	2	2	-	-	3.00
SU-30	Wad Fahal	3	1	1	1	-	-	2.40
							Mean	2.99
	Group from India							
IN-1	PI-288643	-	2	2	1	-	-	2.80
IN-2	PI-288644	-	-	-	1	3	1	5.00*
IN-3	PI-288645	-	-	-	-	4	1	5.20
IN-4	PI-288865	-	-	1	-	2	2	5.00
IN-5	PI-288866	-	-	1	2	-	-	3.67
IN-6	PI-288867	-	-	2	1	1	-	3.75
IN-7	PI-288868	-	-	4	1	-	-	3.20
IN-8	PI-288868-2	-	-	4	1	-	-	3.20

TABLE XXII (CONTINUED)

Code	Common Name	Days under Stress 1	2	3	4	5	6	Score
	Group from India (Continued)							
IN-9	PI-288869-1	-	1	3	-	-	-	2.75
IN-10	PI-288869-2	1	2	-	1	-	-	2.25
IN-11	PI-288871	-	2	3	-	-	-	2.60
IN-12	PI-288872-1	-	5	-	-	-	-	2.00
IN-13	PI-282	1	-	-	1	2	1	4.20
IN-14	PI-288873	-	1	2	1	1	-	3.40
IN-15	PI-288874	-	2	-	1	2	-	3.60*
IN-16	PI-288875	1	1	2	3	2	-	3.44
IN-17	PI-288876	3	2	-	-	-	-	1.40
IN-18	PI-288877	-	-	-	1	1	1	5.00
IN-19	PI-288878	-	-	-	-	-	-	----**
IN-20	PI-288879	2	1	1	1	-	-	2.20
IN-21	PI-288880	1	-	4	1	-	-	2.83
IN-22	PI-288881	3	-	-	1	-	-	1.75
IN-23	PI-288882	-	2	-	1	-	-	2.67
IN-24	PI-289724	-	-	-	-	-	-	----**
IN-25	IS-9181	-	-	-	-	-	-	----**
IN-26	I 899 2-9-21	1	-	2	1	-	-	2.75
IN-27	I 899 1-9-19	-	4	4	2	-	-	2.80
							Mean	3.23
	Group from Oklahoma: A-B Isolines and B Lines							
1	A Martin	-	9	1	-	-	-	2.10
2	B Martin	-	5	4	1	-	-	2.60
3	A Redlan	1	9	-	-	-	-	1.90
4	B Redlan	-	10	-	-	-	-	2.00
5	A Wheatland	2	7	1	-	-	-	1.90
6	B Wheatland	-	8	2	-	-	-	2.20
7	A Dwarf Redlan	-	8	2	-	-	-	2.20
8	B Dwarf Redlan	-	5	2	1	-	-	2.50
9	A OK-8	1	5	2	-	-	-	2.13
10	B OK-8	-	3	4	-	-	-	2.57*
11	B OK-11	-	4	2	-	-	-	2.33
12	B OK-12	-	1	2	2	-	-	3.20
13	B OK-24	-	1	2	2	-	-	3.20
14	B OK-93	2	2	-	1	-	-	2.00
15	B OK-94	-	5	-	-	-	-	2.00
16	B OK-98	-	4	1	-	-	-	2.20
17	B OK-99	-	4	1	-	-	-	2.20
18	B OK-111	-	-	2	2	-	-	3.50*
19	B OKY-54	-	1	1	-	-	-	3.00
20	B OKY-55	-	2	1	-	-	-	2.33
21	B OKY-99	-	3	2	-	-	-	2.40

TABLE XXII (CONTINUED)

Code	Common Name	Days under Stress 1	2	3	4	5	6	Score
	Group from Oklahoma: A-B Isolines and B Lines (Continued)							
22	B WD-4	-	2	2	-	-	-	2.50
23	B WD-5	2	3	-	-	-	-	1.60
24	B WD-18	5	-	-	-	-	-	1.00
25	B Combine Kafir-60	1	3	1	-	-	-	2.00
							Mean	2.30
	Group From Oklahoma: Improved Lines							
DI-1	Tx.63 x Sol Kafir 1-3-1-2	-	2	-	-	-	-	2.00
DI-2	Bonar Day x #1-7-1-2-2	-	-	2	-	-	-	3.00
DI-3	57 x 2E 1-1-1-1-2	-	1	1	-	-	-	2.50
DI-4	57 x 2E 3-1-1-1-2 WD	-	1	2	-	-	-	2.67
DI-5	57 x 16 3-1-1-2-2	-	-	3	-	-	-	3.00
DI-6	58 x 16 3-2-1-2-1-2	-	2	3	-	-	-	2.60
DI-7	58 x 38E 2-2-2-2	-	-	4	-	-	-	3.00
DI-8	58 x 38E 7-1-1-2	1	1	1	-	-	-	2.00
DI-9	DR Cross 4-5-2	2	1	1	-	-	-	1.75
DI-10	(Redlan x Kaura) x DR 2-1-1-2	-	1	2	-	-	-	2.67
DI-11	Stand. White Milo CI-352	1	2	1	-	-	-	2.00
DI-12	Sooner Milo 241	-	-	4	-	-	-	3.00
DI-13	Ryer Milo	-	1	3	-	-	-	2.75*
DI-14	Stand. Yellow Milo CI-234	1	1	3	-	-	-	2.40
DI-15	Shantung Kaoliang CI-293	-	-	3	-	-	-	3.00
DI-16	Early Kaoliang CI-791	-	3	1	-	-	-	2.25
DI-17	Hegari CI-750	-	-	5	-	-	-	3.00
DI-18	Sooner Milo GC-241	-	-	5	-	-	-	3.00
DI-19	Def. Endo X Ryer 1-5-1-1-1-2	-	-	5	-	-	-	3.00
DI-20	61 x 15 1-2-1-1-2-2-2	-	-	5	-	-	-	3.00
DI-21	68 x 29E 2-1-1-1-1	-	-	5	-	-	-	3.00
DI-22	68 x 29E 2-11-1-1	-	-	5	-	-	-	3.00
DI-23	68 x 30E 3-3-1-1-2-1	-	-	5	-	-	-	3.00
DI-24	Ryer 73 F_2 3367-1	-	-	5	-	-	-	3.00
DI-25	(Redlan x Kaura) x Ryer	-	-	5	-	-	-	3.00
DI-26	TP-11	-	-	5	-	-	-	3.00
							Mean	2.72
	Group from Rio Bravo, Tamaulipas, Mexico							
RB-1	SHE-610	-	1	2	-	-	-	2.67
RB-2	SHE-808	-	1	3	-	-	-	2.75
RB-3	SHE-1008 (Maratin)	-	1	4	-	-	-	2.80
RB-4	SHE-1148 (Malinche)	-	3	1	-	-	-	2.25

TABLE XXII (CONTINUED)

Code	Common Name	Days under Stress 1	2	3	4	5	6	Score
	Group from Rio Bravo, Tamaulipas, Mexico (Continued)							
RB-5	SHE-2042	-	-	2	-	-	-	3.00
RB-6	SHE-2264 (Zacapil)	-	-	2	-	-	-	3.00
RB-7	SHE-2300 (Tejon)	-	-	3	-	-	-	3.00
RB-8	SHE-356 x 415	-	-	1	-	-	-	3.00
							Mean	2.5

*Selected

**Discarded

TABLE XXIII

MEAN LEAF DIFFUSIVE RESISTANCE IN SEC. CM^{-1} FOR 7-DAY-OLD SEEDLINGS DURING LIGHT PERIOD FROM 9:30 A.M. TO 4:30 P.M.

ENTRY	SEL.	A.M. 9:30	10:30	11:30	HOUR 12:30	1:30	P.M. 2:30	3:30	4:30	ENTRY MEAN
SU-6	Or.	44.87	44.64	44.68	48.58	56.02	48.15	47.61	58.76	49.164
	Sel.	45.76	50.88	37.61	45.49	56.68	57.72	64.67	69.63	53.555
SU-23	Or.	48.41	52.68	40.43	44.40	60.07	47.71	55.14	56.95	50.724
	Sel.	72.55	41.39	52.27	59.73	80.10	91.18	65.24	95.02	69.685
IN-2	Or.	49.15	58.07	51.83	37.57	58.79	60.65	51.83	63.57	53.933
	Sel.	48.25	58.52	57.32	61.85	78.35	53.68	59.00	55.57	59.068
IN-15	Or.	50.90	53.37	62.59	47.95	39.17	45.07	63.63	50.54	51.653
	Sel.	41.98	37.81	33.96	40.90	52.04	77.96	52.01	57.25	49.239
OK-8	Or.	51.54	35.67	51.21	30.55	25.82	26.91	40.67	36.09	37.308
	Sel.	70.68	67.99	43.47	52.35	57.76	55.17	50.89	59.56	57.284
OK-111	Or.	74.12	39.23	44.09	68.00	47.57	77.22	52.68	55.56	57.309
	Sel.	58.54	46.58	37.85	42.21	43.93	38.08	59.48	45.76	46.554
Ryer	Or.	33.32	25.83	37.47	19.63	25.75	38.12	20.13	30.87	29.015
M.35-1	Or.	40.39	36.68	45.86	42.40	43.32	68.25	53.95	41.96	46.601

F-tests were statistically nonsignificant for Entry x Selection (P=0.3620), Entry x Hour (P=0.8874), Selection x Hour (P=0.4330) and Entry x Selection x Hour (P=0.5374).

TABLE XXIV

MEAN LEAF DIFFUSIVE RESISTANCE IN SEC. CM^{-1} FOR 7-DAY-OLD SEEDLINGS DURING DARK PERIOD FROM 8:30 P.M. TO 6:30 A.M.

ENTRY	SEL.	HOUR P.M. 8:30	9:30	10:30	11:30	1:30	2:30	A.M. 3:30	5:30	6:30	MEAN
SU-6	Or.	28.34	63.08	36.81	31.64	50.09	37.52	61.57	36.13	26.89	41.341
	Sel.	41.28	30.71	30.97	33.15	19.35	42.07	23.24	40.28	28.91	32.218
SU-23	Or.	30.49	48.83	37.57	24.14	31.61	50.96	47.62	44.74	55.44	41.267
	Sel.	43.89	23.60	34.52	57.24	46.29	35.24	45.51	36.81	31.93	39.448
IN-2	Or.	35.75	35.77	53.99	57.22	49.88	62.91	54.02	51.25	56.63	50.824
	Sel.	39.57	55.78	91.51	65.52	76.13	72.40	72.40	89.07	61.83	69.357
IN-15	Or.	26.95	76.72	23.32	24.27	26.34	84.51	28.73	44.08	42.65	41.952
	Sel.	21.72	33.21	22.23	19.29	18.31	26.83	41.65	25.29	32.52	26.783
OK-8	Or.	32.61	21.99	40.62	33.15	29.11	49.34	23.72	26.34	29.26	31.793
	Sel.	40.53	59.50	15.97	17.62	22.29	20.48	51.65	18.59	24.56	30.132
OK-111	Or.	21.12	24.51	34.22	11.32	46.91	34.85	33.75	9.51	43.07	28.807
	Sel.	33.91	27.61	45.66	25.70	34.40	18.38	33.27	42.30	35.17	32.933
Ryer	Or.	28.03	53.20	49.71	27.67	49.76	21.69	45.40	20.70	33.70	36.651

F-tests were statistically nonsignificant for Entry x Selection (P=0.2989), Entry x Hour (P=0.3115), Selection x Hour (P=0.6352) and Entry x Selection x Hour (P=0.1528).

TABLE XXV

MEAN LEAF LENGTH AND WIDTH IN CM. FOR 7-DAY-OLD SEEDLINGS.

ENTRY	SEL.	LEAF 1		LEAF 2		LEAF 3	
		L	W	L	W	L	W
SU-6	Or.	1.98	0.56	6.16	0.52	12.46	0.60
	Sel.	2.22	0.66	7.04	0.56	16.02	0.64
SU-23	Or.	2.38	0.58	8.74	0.46	13.66	0.54
	Sel.	2.58	0.62	6.56	0.58	18.46	0.66
IN-2	Or.	3.18	0.56	9.04	0.42	15.86	0.44
	Sel.	3.20	0.56	7.46	0.40	14.90	0.42
IN-15	Or.	3.08	0.60	7.82	0.48	15.42	0.52
	Sel.	3.20	0.58	8.54	0.54	17.22	0.56
OK-8	Or.	1.94	0.62	6.12	0.56	13.02	0.54
	Sel.	1.64	0.62	6.32	0.56	13.86	0.56
OK-111	Or.	2.04	0.60	6.64	0.58	14.80	0.50
	Sel.	2.06	0.66	6.26	0.54	11.80	0.46
Ryer	Or.	1.82	0.68	6.70	0.50	18.50	0.52
M.35-1	Or.	3.46	0.68	8.38	0.54	14.92	0.60

TABLE XXVI

MEAN LEAF LENGTH AND WIDTH IN CM. FOR 11-DAY-OLD SEEDLINGS.

ENTRY	SEL.	LEAF 1		LEAF 2		LEAF 3		LEAF 4	
		L	W	L	W	L	W	L	W
SU-6	Or.	1.98	0.56	6.94	0.54	14.80	0.56	16.10	0.72
	Sel.	2.22	0.66	7.00	0.56	17.84	0.60	22.40	0.82
SU-23	Or.	2.38	0.58	9.34	0.48	21.72	0.54	17.74	0.72
	Sel.	2.58	0.62	8.06	0.60	19.42	0.62	24.36	0.88
IN-2	Or.	3.18	0.56	8.48	0.44	21.68	0.46	22.30	0.54
	Sel.	3.20	0.56	8.06	0.44	19.94	0.42	19.06	0.54
IN-15	Or.	3.08	0.60	7.72	0.50	17.20	0.50	17.38	0.68
	Sel.	3.20	0.58	8.40	0.56	19.16	0.52	19.04	0.78
OK-8	Or.	1.94	0.62	6.14	0.56	14.12	0.58	19.16	0.72
	Sel.	1.64	0.62	6.24	0.58	15.00	0.56	20.06	0.74
OK-111	Or.	2.04	0.60	6.48	0.62	16.10	0.50	21.76	0.70
	Sel.	2.06	0.66	6.92	0.56	15.26	0.48	15.26	0.56
Ryer	Or.	1.82	0.68	6.58	0.56	17.88	0.50	26.72	0.54
M.35-1	Or.	3.46	0.68	9.04	0.58	19.14	0.58	15.54	0.74

TABLE XXVII

PLANT LENGTH IN CM. FROM SOIL SURFACE TO TIP OF THIRD LEAF OF 7-DAY-OLD SEEDLINGS

CLASSIFICATION	ENTRY	ORIGINAL	SELECTED	IMPROVEMENT
SUSCEPTIBLE	SU-6	9.90	11.62	1.72
	IN-15	12.46	13.54	1.08
	OK-8	9.88	10.04	0.16
RESISTANT	SU-23	13.42	11.50	-1.92
	IN-2	14.84	12.60	-2.24
	OK-111	10.64	9.82	-0.82
CONTROLS	RYER	13.72		
	M.35-1	13.44		

TABLE XXVIII

ANALYSIS OF VARIANCE FOR WATER CONSUMPTION
FOR 7-DAY-OLD SEEDLINGS.

SOURCE	df	MS	F	OSL
Replication	1	1.476		
Entry	5	0.081	2.998	0.127
Error a	5	0.027		
Selection	1	0.017	1.493	0.267
Entry x Sel.	5	0.029	2.615	0.137
Error b	5	0.011		

TABLE XXIX

ANALYSIS OF VARIANCE FOR WATER CONSUMPTION (ΔWL)
DURING A 4-DAY PERIOD FROM DAY 7 TO DAY 11.

SOURCE	df	MS	F	OSL
Entry	5	5.19	4.22	0.0089
Error a	20	1.22		
Selection	1	4.69	2.79	0.1041
Entry x Sel.	5	1.10	0.66	0.6591
Error b	24	1.68		

TABLE XXX

ANALYSIS OF VARIANCE FOR LEAF DIFFUSIVE RESISTANCE FOR 7-DAY-OLD SEEDLINGS

SOURCE	df	MS	F	OSL
Replication	1	1843.81	2.41	
Entry	5	851.70	1.11	0.4555
Error a	5	766.61		
Selection	2	2015.66	0.26	
Entry x Sel.	5	521.81	0.07	0.5292
Error b	1	7739.20		
Time	1	115.83	0.53	0.9211
Entry x Time	5	654.41	2.97	0.1287
Error c	5	219.99		
Time x Sel.	1	74.24	0.12	0.7300
Entry x Time x Sel.	5	756.29	1.26	0.3411
Error d	12	599.22		

TABLE XXXI

REGRESSION AND CORRELATION FOR LEAF DIFFUSIVE RESISTANCE (X) AND WATER LOSS (Y), FOR 7-DAY-OLD SEEDLINGS.

STATISTIC	ORIGINAL	SELECTED	POOLED
$\bar{x}$	39.02	46.13	42.58
ΣX	1170.56	1384.00	2554.57
ΣX^2	49466.99	73282.09	122749.08
σX	11.24	17.73	15.27
$\bar{y}$	1.18	1.20	1.19
ΣY	35.37	35.95	71.32
ΣY^2	42.46	43.97	86.43
σY	0.16	0.17	0.17
b_o	1.031	1.009	1.020
b_1	0.004	0.004	0.004
r	0.27	0.42	0.36

TABLE XXXII

ANALYSIS OF VARIANCE FOR LEAF AREA FOR 7-DAY-OLD SEEDLINGS.

SOURCE	df	MS	F	OSL
Replication	1	695.88		
Entry	5	14.59	4.45	0.0644
Error a	5	3.23		
Selection	1	15.09	3.73	0.0999
Entry x Sel.	5	12.45	3.07	0.1021
Error b	6	4.04		

TABLE XXXIII

ANALYSIS OF VARIANCE FOR INCREASE IN LEAF AREA FROM DAY 7 TO DAY 11.

SOURCE	df	MS	F	OSL
Entry	5	25.62	3.47	0.0200
Error a	20	7.37		
Selection	1	11.55	1.61	0.2143
Entry x Sel.	5	25.21	3.51	0.0157
Error b	24	7.16		

TABLE XXXIV

ANALYSIS OF VARIANCE FOR PERCENT GERMINATION
IN OSMOTIC SOLUTIONS OF MANNITOL

SOURCE	df	MS	F	OSL
Reps	1	277.77		
Entry	5	3528.33	79.39	0.0001
Error a	5	44.44		
Osmotic level	5	63.33	2.15	0.2102
Error b	5	29.44		
Entry x O. level	25	104.66	2.75	0.0071
Error c	25	38.11		
Selection	1	7225.00	72.25	0.0746
Error d	1	100.00		
Entry x Sel.	5	3331.66	16.94	0.0037
Error e	5	196.66		
O. level x Sel.	5	106.66	2.56	0.1627
Error f	5	41.66		
Entry x O. level x Sel.	25	99.33	1.03	0.4697
Error g	25	96.33		

TABLE XXXV

ANALYSIS OF VARIANCE FOR GERMINATION SPEED
IN OSMOTIC SOLUTIONS OF MANNITOL

SOURCE	df	MS	F	OSL
Reps	1	1534.02		
Entry	5	2032.36	10.39	0.0113
Error a	5	195.69		
Osmotic level	5	10999.02	66.38	0.0001
Error b	5	165.69		
Entry x O. level	25	303.36	1.54	0.1446
Error c	25	197.36		
Selection	1	2417.36	139.24	0.0538
Error d	1	17.36		
Entry x Sel.	5	1839.02	11.33	0.0093
Error e	5	162.36		
O. level x Sel.	5	182.36	1.41	0.3567
Error f	5	129.02		
Entry x O. level x Sel.	25	342.02	2.04	0.0408
Error g	25	168.02		

TABLE XXXVI

ANALYSIS OF VARIANCE FOR EMERGENCE-RATE INDEX
IN OSMOTIC SOLUTIONS OF MANNITOL

SOURCE	df	MS	F	OSL
Reps	1	0.0077		
Entry	5	0.2843	47.86	0.0003
Error a	5	0.0059		
Osmotic level	5	0.4247	45.70	0.0004
Error b	5	0.0092		
Entry x O. level	25	0.0113	1.61	0.1199
Error c	25	0.0070		
Selection	1	0.4162	78.28	0.0716
Error d	1	0.0053		
Entry x Sel.	5	0.1355	9.91	0.0125
Error e	5	0.0136		
O. level x Sel.	5	0.0060	0.73	0.6284
Error f	5	0.0082		
Entry x O. level x Sel.	25	0.0114	1.22	0.3113
Error g	25	0.0093		

TABLE XXXVII

ANALYSIS OF VARIANCE FOR ROOT DRY WEIGHT OF 4-DAY-OLD SEEDLINGS GERMINATED IN OSMOTIC SOLUTIONS

SOURCE	df	MS	F	OSL
Reps	1	0.1362		
Entry	5	0.2084	2.99	0.1272
Error a	5	0.0696		
Osmotic Level	5	1.6335	622.92	0.0001
Error b	5	0.0026		
Entry x O. level	25	0.0571	2.92	0.0047
Error c	25	0.0195		
Selection	1	0.1230	1.25	0.4649
Error d	1	0.0987		
Entry x Sel.	5	0.1879	11.61	0.0088
Error e	5	0.0161		
O. level x Sel.	5	0.0146	0.18	0.9597
Error f	5	0.0825		
Entry x O. level x Sel.	25	0.0602	3.68	0.0009
Error g	25	0.0818		

TABLE XXXVIII

ANALYSIS OF VARIANCE FOR ROOT DRY MATTER PERCENTAGE OF 4-DAY-OLD SEEDLINGS GERMINATED IN OSMOTIC SOLUTIONS OF MANNITOL.

SOURCE	df*	MS	F	OSL
Reps	1	24575.09		
Entry	5	10717.89	0.75	0.6193
Error a	5	14265.95		
Osmotic level	5	28744.73	0.99	0.5061
Error b	5	29160.76		
Entry x O. level	25	11440.93	0.94	0.5643
Error c	25	12214.90		
Selection	1	25315.81	0.80	0.5358
Error d	1	31711.09		
Entry x Sel.	5	12223.60	1.07	0.4722
Error e	5	11447.05		
O. level x Sel.	5	28341.87	0.86	0.5627
Error f	5	32879.87		
Entry x O. level x Sel.	25	12576.11	0.97	0.5295
Error g	23	12935.78		

*Note: All entries with fresh weight = 0 as divisor, were made equal to zero, with a loss of 1 degree of freedom.

TABLE XXXIX

ANALYSIS OF VARIANCE FOR SHOOT DRY WEIGHT OF 4-DAY-OLD SEEDLINGS GERMINATED IN OSMOTIC SOLUTIONS OF MANNITOL

SOURCE	df	MS	F	OSL
Reps	1	0.0014		
Entry	5	0.7303	3.41	0.1022
Error a	5	0.2141		
Osmotic level	5	4.9117	205.59	0.0001
Error b	5	0.0238		
Entry x O. level	25	0.1610	1.97	0.0480
Error c	25	0.0816		
Selection	1	0.1950	0.66	0.5664
Error d	1	0.2970		
Entry x Sel.	5	0.1144	0.77	0.6084
Error e	5	0.1482		
O. level x Sel.	5	0.0716	0.40	0.8322
Error f	5	0.1797		
Entry x O. level x Sel.	25	0.0775	0.84	0.6690
Error g	25	0.0925		

TABLE XL

ANALYSIS OF VARIANCE FOR SHOOT DRY MATTER PERCENTAGE OF 4-DAY-OLD SEEDLINGS GERMINATED IN OSMOTIC SOLUTIONS OF MANNITOL

SOURCE	df*	MS	F	OSL
Rep	1	20283.41		
Entry	5	3699.55	0.71	0.6431
Error a	5	5226.63		
Osmotic level	5	12614.29	1.32	0.3846
Error b	5	9570.46		
Entry x O. level	22	5774.24	147	0.2034
Error c	18	3917.19		
Selection	1	2508.14	0.71	0.5540
Error d	1	3527.65		
Entry x Sel.	5	2474.25	1.19	0.4253
Error e	5	2072.42		
O. level x Sel.	5	6003.23	0.86	0.5751
Error f	4	6998.60		
Entry x O. level x Sel.	19	1197.02	0.20	0.9993
Error g	15	5928.16		

*Note: All entries with fresh weight = 0 as divisor, were made equal to zero, with a loss of 1 degree of freedom.

TABLE XLI

ANALYSIS OF VARIANCE FOR ROOT LENGTH OF 4-DAY-OLD SEEDLINGS GERMINATED IN OSMOTIC SOLUTIONS OF MANNITOL.

SOURCE	df	MS	F	OSL
Entry	5	25.90	45.68	0.0001
Osmotic level	5	224.68	396.21	0.0001
Entry x O. level	25	2.97	5.25	0.0001
Selection	1	32.04	56.50	0.0001
Entry x Sel.	5	5.09	8.99	0.0001
O. level x Sel.	5	3.13	5.53	0.0001
Entry x O. level x Sel.	25	1.96	3.47	0.0001
Error	288	0.56		

TABLE XLII

ANALYSIS OF VARIANCE FOR SHOOT LENGTH OF 4-DAY-OLD SEEDLINGS GERMINATED IN OSMOTIC SOLUTIONS OF MANNITOL

SOURCE	df	MS	F	OSL
Entry	5	17.69	56.04	0.0001
Osmotic level	5	118.44	375.18	0.0001
Entry x O. level	25	4.40	13.96	0.0001
Selection	1	6.69	21.21	0.0001
Entry x Sel.	5	1.64	5.21	0.0002
O. level x Sel.	5	1.79	5.69	0.0001
Entry x O. level x Sel.	25	1.07	3.40	0.0001
Error	288	0.31		

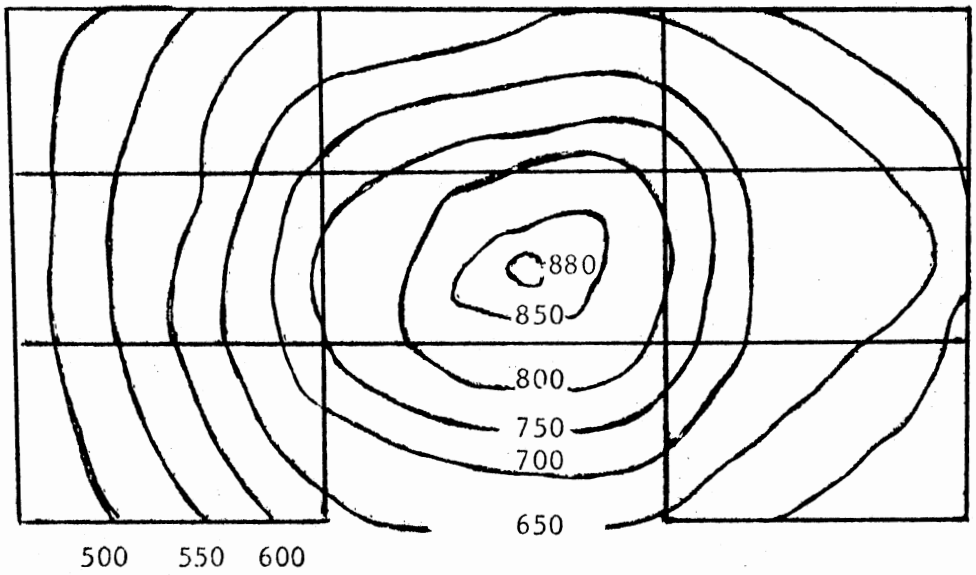

Figure 21. Light Characterization of Growth Chamber 5 (CERL-OSU): Photometric Scale (Footcandles) at 75 cm from Light Source. Mean = 660 ftc.

175	200	180
150	210	190
150	200	175

Figure 22. Light Characterization of Growth Chamber 5 (CERL-OSU): Radiometric (P.A.R.) Scale (uE/cm/sec) at 75 cm from Light Source. Mean = 180 uE/cm/sec.

VITA

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