SCREENING FOR DROUGHT TOLERANCE IN COTTON

(*GOSSYPIUM HIRSUTUM* L.)

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1972

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1979

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ACKNOWLEDGMENTS

The author wishes to express sincere gratitude to his major adviser, Dr. L. M. Verhalen, for his assistance, guidance, friendship, constructive criticisms, and encouragement throughout the author's research and academic studies. His appreciation is also extended to Dr. R. W. McNew for his meaningful help in the statistical analyses and interpretation of the data. He also thanks the other members of his advisory committee, Dr. M. B. Kirkham and Dr. D. E. Weibel, for their interest and advice during this research work. All of the above made numerous valuable suggestions when reviewing this manuscript.

Appreciation is expressed to Dr. J. E. Quisenberry and Dr. Bruce Roark, Lubbock, Texas, for informative discussions on the evaluation of drought tolerance. Dr. Quisenberry also contributed seed of the race stocks tested herein.

Deep thanks are also due to Mr. G. H. Hartman, in charge of the Controlled Environment Research Laboratory, for his valuable help in dealing with problems which occurred with the growth chamber during the period of its use.

Gratitude is likewise acknowledged to EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais) in the person of its President, Dr. Helvécio Mattana Saturnino, and to EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) in the person of its former Chief of the Department of Human Resources, Dr. Matheus Bressan, for making this training

possible and for financial support, respectively. Thanks are also extended to Mr. E. A. Menezes, Mrs. M. N. Penna, and Mr. A. A. C. Purcino for their help in setting up and maintaining these experiments. Mrs. Penna also typed the preliminary draft of this manuscript.

The author wishes to thank Mrs. Janey Logan for typing the final copy of this thesis.

Deep gratitude is extended to the author's wife, Maria Nilce P. Penna, and to his daughter, Juliana, for their love, patience, and friendship which were continually demonstrated throughout his graduate work.

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Screening for Drought Tolerance in Cotton (*Gossypium hirsutum* L.)[1]

ABSTRACT

The objectives of this study were to adapt a screening procedure previously used for seedling drought tolerance work in cereals for use in cotton (*Gossypium hirsutum* L.), to identify genotypes of cotton tolerant to drought from among a wide range of cultivars and race stocks, and to evaluate selected root and shoot characters for their possible use in predicting drought tolerance.

Ninety genotypes were screened in seven growth chamber experiments. Fifteen days after germination, seedlings in each experiment were subjected to four successive four-day drought cycles, each followed by irrigation, and a count of plant survival two days later. Analyses over all four cycles in an experiment generally revealed significant entry X cycle interactions. When the last two cycles were analyzed together, interactions were generally minimal; and significant differences among entries were obtained in four of the experiments. Differences among entries were sometimes evident after the first cycle, but generally were more pronounced after the onset of the third cycle. Three cycles are probably the minimum required in cotton work. One of the later experiments, a "confirmation" test, was composed of entries evaluated in

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[1] To be submitted for publication in *Crop Science*.

previous experiments and included both "tolerant" (those with higher percent survival) and "susceptible" genotypes (those with lower survival). A number of entries duplicated their performance (whether resistant or susceptible) of previous experiments; others did not. This experience emphasizes the importance of reevaluating selections in duplicate experiments to increase one's confidence in previous classifications. In general, the technique appears to have practical value for screening a large number of genotypes for drought tolerance, especially if combined with confirmation experiments.

The root-shoot experiment consisted of 15 entries (both tolerant and susceptible from previous experiments) grown in pots, filled with sand, for 35 days with no stress for moisture or nutrients. Eight root and shoot measurements were taken at harvest; six displayed significant differences among entries at the 0.05 or higher level. However, these results could not be directly related to entry performance in the drought-screening experiments. Rapid growth and development may be of importance for seedling survival under drought conditions. A similar experiment, but submitting plants to several drought cycles, should supply further information on this issue.

Overall, the results from the drought screening technique appear to be positively correlated with subjective field information. It is a relatively simple, though time consuming, method. In working with growth chambers, we would emphasize that it is essential to precisely characterize their lack of internal uniformity to permit more adequate designs of the experiments to be conducted therein.

Additional index words: Upland cotton, Cotton breeding, Seedling evaluation.

INTRODUCTION

The general functions of water in plants are described as being (37):

(a) the major component of physiologically active tissue;

(b) a common reagent to photosynthesis and hydrolytic processes;

(c) the solvent in which salts, sugars, and other solutes move throughout the plant; and

(d) essential to maintain the turgidity required for cell enlargement and growth.

It is probable that water deficits affect almost every process taking place in the plant.

Plant water balance is influenced by complex interactions among the components which constitute its environment, i.e., the soil, the plant itself, and atmospheric conditions (37). Most land plants are subjected at one time or another to a degree of water deficit. Internal balance within the plant and degree of water stress depend on the relative rates of water absorption and loss. For this reason, plants growing in soils near field capacity (or even in solution culture) may develop water deficits on hot, sunny days. Heatherly et al. (20) believe that many species have their development and yield severely repressed even by moderate water stress.

Fischer and Turner (16) state that the majority of crop production in the world's semi-arid regions derives from wheat (Triticum spp.),

barley (Hordeum spp.), sorghum (Sorghum spp.), and the millets with the remainder produced by cotton (Gossypium spp.), oilseed, and leguminous crops. Cotton (Gossypium hirsutum L.), however, is not classified as a drought-tolerant crop, and it is not very efficient in water use, as are other plants such as corn (Zea mays L.) and sorghum (Sorghum bicolor (L.) Moench) (as stated by Briggs and Schantz and cited by Ray et al. (50)). Nevertheless, they do suggest that cotton does have several special mechanisms which make it well adapted to semi-arid regions (as is commonly believed by farmers in those areas). Characters of the genus Gossypium which have definite importance in water-use efficiency are its relatively deep-penetrating and extensive root systems, leaves and fruits which can be shed when the plant is under stress, and a flexible fruiting period (50). Considerable variation is found in cotton for leaf area, thickness, and shape. Roark et al. (51) reported differences among cotton cultivars grown on the High Plains of Texas for stomatal behavior and distribution.

The water requirements for cotton from emergence to first square (according to Tharp as cited by Bilbro (7)) are approximately 0.10 inches (2.54 mm) per day. From first square to first bloom, it varies from 0.10 to 0.25 inches (2.54 to 6.35 mm) daily; and from first bloom to peak bloom, plants require 0.25 to 0.40 inches (6.35 to 10.16 mm) of water each day. After this stage, consumption of water declines until the plant stops growing.

Drought is one of the major limiting factors in cotton production in the state of Minas Gerais in Brazil (the author's home state and country) (2,48) and in the state of Oklahoma in the United States (the

state and country where this research was conducted). Thus, such studies are of mutual interest and concern.

The objectives of this study were to:

(a) Adapt a screening procedure previously used for seedling drought tolerance work in cereals (47, 61) for use in cotton;

(b) Identify genotypes of cotton more tolerant to drought from among a wide range of cultivars and race stocks; and

(c) Evaluate selected root and shoot characters for their possible use in predicting drought tolerance.

LITERATURE REVIEW

Definitions

The complexity of drought resistance begins with its definitions. In the classification suggested by Levitt (39), the xerophytes (plants adapted to arid zones) are described as:

(a) "drought escaping": plants which complete their life cycle in a short period of time when water deficits do not constitute a limiting factor for growth and reproduction; and

(b) "drought resisting": plants which can be further subdivided into "drought avoiding" ("savers" vs. "spenders") and "drought tolerant".

Water savers (according to Maximov as cited by Levitt (39)) are those plants which conserve water, as opposed to spenders which absorb water in quantities sufficient to supply their demand. The latter plants may lose water as much as 500,000 times as rapidly as the former. In this way, water savers are more efficient than water spenders under conditions of extreme water stress. Drought avoiding plants are resistant largely due to morphological and anatomical adaptations (Shields and Parker as cited by Levitt (39)). Those kinds of plants maintain a high internal water potential when exposed to an external water stress. Both types of adaptation are maintained in a state of high turgidity when exposed to water stress. Drought tolerance is usually a physiological type of adaptation and is highly specific.

A definition suggested by Wright (67) for range grasses in the southwestern United States is that drought-tolerant plants are those "which are able to establish, develop, and maintain themselves through drouth periods by efficient and economical use of moisture". Other authors such as May and Milthorpe (cited by Wright (67)) have reviewed the problem of definitions and have themselves defined drought resistance in terms of internal water content and tolerance to partial drying. According to Stocker (59), a resistant cultivar tends to maintain a high photosynthetic rate by restriction of transpiration which is attained by anatomical, morphological, or physiological adaptations.

A common problem in drought studies is the measurement of water stress. Soil water stress only indirectly controls plant growth and crop yield (37). Therefore, soil moisture data alone are not as reliable an indicator of water stress as is actual measurement of stress in the plants themselves. Several such methods are described in the literature. Sullivan (60) listed the characteristics desirable in such methods including that it be applicable to a wide range of plants and soils, require a minimum quantity of tissue, be simple and inexpensive, and be correlated with rates of physiological processes. The author regretted that the methods which have gained most recognition also require more sophistication in the manipulation and construction of equipment and that the expense and time involved are discouraging to many researchers.

Evaluation and Screening for Drought Resistance

Variation in drought resistance among and within species has been reported in the literature. For example, sorghum is grown in areas con-

sidered too dry for corn, but some sorghum genotypes are more suitable to drought conditions than are others (21). Field testing, although of much value, is difficult due to complex genotype by environment interactions and to the unpredictability of weather conditions.

According to Sullivan (60), it is presently doubtful whether any particular screening technique can be standardized to measure drought resistance because of the complexity inherent in the drought resistance problem, the wide variety of plant types, and the economy of the crops involved. Drought resistance could be measured as the amount of water withheld from the plant necessary to produce a specific irreversible strain, as for example, death of 50% of a plant (39). However, techniques for evaluating such strain are not precisely defined either.

Wilson and Sarles (65) presented a method for partitioning drought resistance and quantification of its components, i.e., drought tolerance and drought avoidance. According to the authors, when maximum leaf area is attained by blue grama ((Bouteloua gracilis (HBK.) Lag.) seedlings, all possible mechanisms of drought resistance are employed. At such a plateau stage, a quantification of drought tolerance may be estimated by tiller water potential; whereas, leaf diffusion resistance, maximum capacity of water uptake, and leaf area can be used to estimate drought avoidance. Therefore, drought tolerance is represented by that amount of plant drought sufficient to prevent seedling area expansion; and drought avoidance is the difference between plant drought and drought of the shoot environment.

Venkateswarlu and Rao (62) proposed a sand culture technique to screen sorghum cultivars for drought tolerance because field conditions were considered too variable. They recommended the criterion of yield

under stress (at the most critical stage of the plant's life cycle) compared to no stress, expressed as a percentage, as being a useful index for screening.

Todd and Webster (61) conducted survival studies on nine wheat (Triticum aestivum L.) cultivars in which the plants were subjected to weekly cycles of drought, followed by rewatering. They measured survival and turgor in each cycle through a total of eight cycles. Differences in survival were noted among cultivars, and the results correlated well with previous field information. This test was a modification of a single-stress test developed by Platt and Darroch (cited by Todd and Webster (61)). Using a similar technique, Wright (67) employed a growth chamber to evaluate six species of range grasses. Seedlings were grown in trays, and water stress was applied for several days. Significant differences among species for survival after rewatering were found. He concluded that selection using a program-controlled environment would be a good technique to isolate drought-tolerant plants.

Kilen and Andrew (34) found a high correlation between a greenhouse-conducted heat and atmospheric drought test and field drought resistance in corn. This test consisted of growing corn lines in flats arranged in a greenhouse. Plants at the four-leaf stage were subjected to a hot air flux to induce water stress, and classification was then made according to apparent injuries. Williams et al. (64), instead of rating injuries, measured recovery after exposure to the high temperatures.

Nour (47) screened sorghum cultivars in a growth chamber. At the age of nine days, the seedlings were left without water from seven to eight days and then rewatered. This cycle was repeated four times, and

mean percentage survival was recorded for each cycle (as counted two days after rewatering). He pointed out that this survival technique was a simple and effective method of screening for drought resistance among unknown genotypes; and he listed as advantages of the process that a large number of entries could be screened, environmental conditions could be fairly easily controlled, and the technique was relatively rapid. A similar technique was employed by Wright and Jordan (68) in testing boer lovegrass (Eragrostis curvula Nees) seedlings for drought tolerance. Progeny performance of superior selections was higher than the checks indicating the effectiveness of the method. Other screenings involving survival counts were described by Wood and Buckland (66) and McAlister (41).

Williams et al. (64) suggested two simple screening methods for drought resistance in corn; one, germinating seed in a mannitol solution at 15 atm osmotic pressure with further selection of genotypes showing high rates of germination, and two, exposure of plants to a 14-day wilting period in a greenhouse with additional wilt ratings. Solutions of mannitol, sodium chloride, and polyethylene glycol have been used in germination studies over a range of osmotic potentials. However, such substances have effects other than strictly on water potentials in the seed (26). Furthermore, these substances must be nontoxic as well as non-penetrating (39). All solutes now used in such studies are taken up to some extent by the developing seedling. Carbowax (polyethylene glycol) shows more promise in this regard because it is taken up very slowly and little or no injury has been reported as a consequence of its use. However, such methods cannot be used to investigate the extreme

water stresses that can occur in nature because extremely low water potentials cannot be obtained with them (39).

Nour (47) subjected seed of sorghum cultivars to several levels of water potential using a d-mannitol solution. Results from this experiment were not in close agreement with those from a survival screening test nor with a root study previously run with the same set of cultivars. At higher concentrations of the solution, he found differences among genotypes were more easily detectable. Powell and Pfeifer (49) subjected 670 single-plant selections to 7 and 11 atmospheres of d-mannitol solution to determine their germination and growth under low moisture conditions. The authors stated that the use of d-mannitol was a simple method which gave a relative measure of differences in drought hardiness among seedlings.

Root Studies

Survival of plants in dry habitats is related to the spread and depth of their root systems. According to Hurd (24), cultivars which have the greatest root masses under drought are important in breeding for drought resistance. Citing work by Belzakov and Danilchuk et al., he (24) commented that close relations have been found between growing root systems and grain production in the absence of moisture. Citing work by Townley-Smith and McBean, he (24) pointed out that several thousand plants can easily be screened for seedling root length in the greenhouse during a winter season. Relative root lengths of seedlings grown for five to seven days in sand were consistent with relative lengths at maturity.

In general, perennial grasses and shrubs of deserts or dry regions

have root/shoot ratios higher than those of similar species found in more humid areas (Fischer and Turner (16) citing Oppenheimer, Caldwell, and Evenari et al.). The same relation may or may not occur with annual plants and probably lower values for root/shoot ratios will be obtained in annuals because of the accumulation of assimilates in their seed.

Nass and Zuber (46) commented that plants grown in sand more nearly represented plants grown under field conditions than did those grown in solution culture and that large numbers of plants can be screened for root type in a greenhouse. An advantage for the use of sand instead of soil is that, in so doing, the removal of plants and intact roots is greatly simplified. In corn, a good correlation was found between fast-growing, large root systems in early-stage plants and massive root systems at maturity.

Klepper et al. (35) studied the response of 70-day-old cotton plants when subjected to a 26-day drying cycle in a rhizotron. The pattern of root distribution shifted from the majority of roots being in the upper part of the soil in well watered plants to an increase in the root density at greater depths. This shift was caused by the death of roots in the upper horizons and new growth in the lower ones. They also demonstrated that if the soil dried beyond -1 bar of water potential, roots will display preferential extension growth into wetter regions.

Salim et al. (53) grew cultivars of barley (Hordeum vulgare L.), oats (Avena sativa L.), and wheat in glass-fronted boxes and daily measured root growth under various soil moisture conditions. The extent of root growth was correlated with the availability of soil moisture.

More drought-hardy cultivars and species had longer seminal roots (and usually in greater number) than did the drought-susceptible ones. Sandhu and Laude (55) have shown in a root/top growth study in wheat that, in general, cultivars hardy to drouth and heat had greater dry weight of roots in proportion to top growth than non-hardy types. Nour (47) grew sorghum plants in sand pots for a three-week period after which root weights, lengths, and volume measurements were taken. The more drought-resistant cultivars in a previous screening test had greater root volumes, longer roots, higher root/shoot ratios, and heavier root weights.

Field measurements of soybean (Glycine max (L.) Merr.) roots showed that root length decreased when soil was drier than -2 bars (56). According to Bennett and Hsiao (cited by Fischer and Turner (16)), root/shoot ratios can be doubled by soil-water deficiencies. The same authors (now citing Stocker and Hoffmann) stated that increased air saturation deficits can also increase the ratio. They believe root/shoot ratios change in a manner such that plant-water potential is maintained within certain limits.

Water Relations and Other Studies

Dedio (15), working with wheat (Triticum durum Desf. and T. aestivum L.), regarded the use of characters concerned with water relations in the leaves as possible tests for drought resistance. Such relations include relative saturation deficit (RSD), water content (WC), and water retention ability (WRA). After submitting potted five-week-old plants to one week with no irrigation, a known drought-tolerant cultivar,

'Pitic 62', exhibited the lowest RSD at low moisture and the highest WC. Durum types, compared with spring cultivars, showed higher WRA at the three-week stage. Such behavior, suggested the author, explained the superior drought resistance of durum wheats. Ackerson et al. (1) showed that changes in relative water content (RWC) per unit change in leaf water potential are greater in cotton than in sorghum. Therefore, the authors concluded that cotton requires more water to recover from drought stress than does sorghum.

As cautioned by Barrs and Weatherley (3), errors may interfere with RWC measurement. Water can infiltrate into the cut edges and intercellular spaces during saturation; also, cell growth may occur during this period, and respiration may cause losses. These errors may be diminished by taking precautions such as shortening the saturation time and constant illumination (thereby compensating for weight loss due to respiration). Nour (47) reported that RWC measurements in his experiments with sorghum were not useful, but he did find that the parameter decreased gradually with progressive drying of the soil.

Kaloyereas (32) tested loblolly pine (Pinus taeda L.) strains known to be drought resistant and found a good correlation between chlorophyll stability and drought resistance.

Other Aspects of Drought Resistance

Pre-Conditioning. Considerable evidence exists that water stress can influence subsequent plant response to future water deficits. McCree (42) and Brown et al. (11), for example, studied the influence of water stress on stomatal response to subsequent stress in sorghum and cotton, respectively. In the latter case, eight water-stress cycles conditioned

stomata in the lower surface of cotton leaves to remain open at a leaf water potential about 14 bars lower than in non-stressed plants. In sorghum, a similar though less extreme reaction was observed after five moderate soil moisture stress cycles. These results suggest that no unique relationship exists in a plant between leaf water potential and stomatal closure (11).

Similar relations were described by Cutler and Rains (13) in cotton. Their results suggested that cotton plants grown in pots under controlled environments and subjected to water stress during development were less sensitive to subsequent drought. Their analyses of water potential to RWC of pre-stressed and irrigated plants suggested that the reduced sensitivity of the former, may be due to osmotic adjustment. Dehydration did not seem to play a role in the process since water potential at a given RWC is decreased in pre-stressed plants. The more likely explanation for such osmotic variation seemed to be solute accumulation (13).

Age of Tissues. Wright (67) divided the plant's life cycle into three stages:

(a) seedling - from the embryo, through germination, up to the exhaustion of seed reserves;

(b) young plant - from the start of independent life up to reproduction; and

(c) mature plant - from reproductive age until death.

To adequately study drought resistance, plant breeders must be aware of the most critical stages in their plant's life cycle. Citing Mueller and Weaver, the author (67) stated that for some perennial grasses the seedling stage is critical for drought tolerance studies and generally

seedling techniques have proved successful in applied breeding and basic genetic investigations.

Variations in the response of plants to stress stimuli are reported in the literature. According to Levitt (39), young plant tissues are always more resistant to drought than are older tissues. Jordan et al. (30) demonstrated that stomata of older leaves in cotton are more sensitive to closure caused by water stress than are those of younger leaves. Dedio (15) studied the water retention ability (WRA) of young vs. mature wheat plants and reported that WRA increased with advancing maturity in some cultivars.

Water Stress and Physiological Processes. Many physiological processes and responses are influenced by water deficits. Among the most important are reduction in cell expansion and reduction in carbon fixation, mediated partially by stomatal closure (23).

Changes in cell water content usually induce changes in protoplasmic properties as well as modifications in the rates of physiological processes (36). Those rates generally decrease with decreasing plant water content. Burstrom, cited by Kramer (37), stated that growth is particularly sensitive to lack of water because loss of turgidity stops cell enlargement resulting in smaller plants. Water deficits also change growth patterns. For example, root/shoot ratios are often increased by water stress, leaf area is reduced, and leaf thickness is enhanced (37).

Heatherly et al. (20) submitted 63-day-old soybean plants grown in moist soil in a greenhouse to a nine-day drying cycle to evaluate water relations and growth. When xylem pressure potential reached -4.5 bars,

a pronounced decline was observed in leaf enlargement with total cessation of enlargement between -10 and -12.9 bars.

Gates (17) applied moderate and severe levels of water shortage to tomato (Lycopersicon esculentum Mill.) plants, and growth responses were monitored. Dry weight of the entire plant at harvest was depressed by water deficits even at the moderate level. Reduction of root and shoot growth in cotton plants due to water deficits has also been reported (29, 35).

Stomata are recognized as the major pathway for transpiration and gaseous exchanges between the plant and its medium (33). In field-grown cotton, plant evaporation during periods of prolonged drought was sustained by extraction of water from layers of soil below the main root zone; and such extraction was only made possible by the failure of stomata to close in response to low leaf water potential (31).

Henzell et al. (21) stated the generally accepted relation between stomatal closure and drought resistance, i.e., the greater the stomatal sensitivity to water stress, the less drought resistant the plant is. Stomatal sensitivity is therefore an important component of drought resistance. However, he insisted that this hypothesis has not yet been directly proven. Sanchez-Diaz and Kramer (54) submitted 40-day-old corn and sorghum plants to a severe water-stress period and measured stomatal resistance during drying and after rewatering as well as leaf water potential and water saturation deficit. The average leaf water potential for corn was -4.5 bars and for sorghum -6.4 bars. The lowest leaf water potential for corn was -12.8 bars at a WSD of 45% and for sorghum -15.7 bars at a WSD of 29%. In summary, corn loses much more of

its water before its stomata close than does sorghum. These results may explain why sorghum is a more resistant plant to desiccation than is corn.

Kramer (37) stated that plant water stress reduces photosynthesis both directly and indirectly. Directly because dehydrated protoplasm has reduced ability for photosynthesis and indirectly, because water deficits reduce leaf area and cause stomata to close. Harris (19), working with 97-day-old cotton plants, concluded that a 4 bar polyethylene glycol osmotic pressure in a nutrient solution reduced photosynthesis, transpiration, and relative water content.

Todd and Webster (61) stated that differences in field hardiness to drought as exhibited by wheat and oat cultivars are not due to differences in rates of photosynthesis or ability to synthesize. However, after a single drought period, slightly higher rates were obtained at lower turgor for nearly all cereals. Furthermore, Brix (10) found almost no differences in the ability to photosynthesize, at a given diffusion pressure deficit, between loblolly pine and tomato, although there is a wide difference in drought resistance between the two species.

Jones (28) subjected cotton plants to one- or two-week periods of mild water stress and described effects of the water shortage on several photosynthetic parameters. Stressed plants had lower rates of photosynthesis than the controls, but most of the parameters studied showed recovery after 24 hours. He supported the position that the major factor causing reduced photosynthesis in stressed plants was stomatal closure. Other evidence for the influence of stomatal closure on the reduction of photosynthesis in cotton was given by Bielorai and Hopmans (6). However, stomatal closure is not the only explanation for reduction in

photosynthesis under water stress. Boyer (9) showed that cytoplasmic resistance to carbon dioxide diffusion caused a decrease of 25% or more in photosynthesis of cotton plants grown in a sodium chloride medium (-8.5 bars), even though the stomata were fully open.

Biochemical Effects of Water Stress. Kramer (37) believes the study of biochemical effects of water stress in plants, especially changes in enzyme activities, is one of the most promising fields for research in plant-water relations. Changes in mineral metabolism, rapid senescence of leaves, and disturbances in nitrogen metabolism are also caused by water deficits.

Blum and Ebercon (8) stated that recovery from water stress is probably related to free proline accumulation, as this amino acid constitutes a source of respiration energy to plants. Waldren and Teare (63) have also speculated that proline accumulation and drought resistance could be interrelated. This amino acid could be a source of nitrogen as well as energy, once the stress is over (43). Free proline accumulation in plant tissues during periods of water shortage has been widely reported in recent literature. Leaves of stressed cotton plants, for example, accumulated free proline up to a hundred times more than leaves of normally watered plants; the threshold leaf water potential for such accumulation was -15 to -17 bars (43). So proline accumulation may not be a good indicator at the outset of plant-water stress since it does not accumulate until water deficits are quite severe. However, it could play an important role in the process of hardening plants, thereby, causing stomata to close at a much lower water potential (43). Several other reports of water-stress-induced free proline accumulation

in plants were published by Chu et al. (12), Stewart et al. (58), and Routley (52) among others.

In barley, Hanson et al. (18) concluded that proline accumulating potential should not be utilized as an index for drought resistance in screening because very strong environmental effects influence the rate of water stress development and of proline accumulation.

Another important aspect of stress physiology is at the hormonal level. According to Darbyshire (14), auxin levels may be reduced by enzymatic degradation in water-stressed plants, and retardation of growth during stress may result from lack of auxin-induced wall loosening. Abscisic acid (ABA) content of plant tissues at several stages of water stress was determined in two species of Ambrosia (69). In both, ABA content increased sharply when water potential decreased from -8 to -9 atm. His observations indicated that a water potential threshold existed at which ABA concentration began to increase; but over a relatively wide range of potentials (0 to -8 atm), no additional increments of that hormone were detected. Several other studies in the literature confirm these findings in other species (4, 38, 40).

Hiron and Wright (22) directed a continuous stream of warm air (38 C) on the leaves of dwarf bean (Phaseolus vulgaris L.) seedlings and observed that the leaves wilted and then gradually regained turgor. They measured endogenous ABA levels from the beginning of the experiment and found an increase in ABA levels in the leaves and also an increase in leaf resistance. This hormone increase, triggered by the treatment, was associated with stomatal closure which enabled the plants to regain full turgor.

Little (40) has also implicated the role of ABA in closure of

stomata and its interference with and inhibition of auxin-induced cambial activity and movement. Beardsell and Cohen (4) found that the actual amounts of ABA produced in leaves of maize and sorghum during high negative water potentials are in excess of that actually required to cause stomatal closure. Still, Hiron and Wright (22) suggested that ABA appears to be part of a mechanism by which the effects of water stress on plants are alleviated so that plants are able to pass through stress periods with little harm.

Not only abscisic acid and proline are accumulated during water stress, but other substances as well (5, 25, 45). The study of these effects, as well as their causes, may shed additional light on the problem of drought resistance.

MATERIALS AND METHODS

Two types of studies were conducted in this research. First, a screening procedure previously used for seedling drought tolerance work in cereals (47, 61) was adapted for use in cotton. Then, the modified procedure was used to identify those genotypes which were more tolerant to drought from among a wide range of cultivars and race stocks. Second, selected root-shoot characteristics were evaluated for their possible use in predicting drought tolerance.

These tests were conducted in a walk-in Horblit[2] growth chamber with an internal area of 127 X 210 cm and with automatic temperature and light controls. This chamber was located in the Controlled Environment Research Laboratory of Oklahoma State University, and the experiments were conducted from spring 1977 through fall 1978. A mixture of fluorescent and incandescent bulbs was used in the chamber with the average light flood being approximately 150 watts/m^2 at 76 cm below the light source. Controls were set for a 14-hour light period with an average temperature of 32 C followed by a 10-hour dark period with an average temperature of 18 C. Relative humidities were approximately 45% and 90% during the day and night, respectively. Air in the chamber was kept in

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[2]Reference to commercial products or trade names is made with the understanding that no discrimination is intended and that no endorsement is implied.

motion by means of six large fans mounted in two groups of three on opposite ends of the chamber and by six small fans located above the light source.

From direct observations and from additional information obtained from the literature (44), environmental conditions within the growth chamber were judged to be non-uniform. Two initial experiments were conducted to characterize the chamber. Ten metal trays (50 cm long X 35 cm wide X 9 cm deep) were filled with a 1:1:1 mixture by volume of soil, vermiculite, and peat moss. Two cotton cultivars, 'Westburn M' and 'Stoneville 213', were planted in alternate 35 cm-long rows with rows spaced four cm apart and plants spaced three cm apart within the rows (after thinning). Twelve plants were left per row, 12 rows per tray, making a total of 144 plants in each tray. One plant on each extremity of each row and one row on each side of the tray were disregarded in an attempt to negate possible border effects. Fifteen days after planting, four-day drought cycles separated by an irrigation were applied up to four times, and survival counts were made two days after each irrigation to allow stressed plants a chance to recover.

After the onset of the first drought cycle, several sections within the chamber were identified as having plants which displayed more severe drought symptoms than other sections. After the two preliminary experiments, those sections were identified as shown in Fig. 1. By those same patterns, it appeared that the outside plants in the trays in general suffered less, which was probably caused by water accumulation on the bottom of the trays around the outside edges (as illustrated by the non-shaded areas in the same figure). As a consequence, holes were punched in all outside bottom edges of each tray thereby reducing this type of variation. Also based on these results, a randomized

complete-block design with four replications was chosen as the design of the experiment. Replication one consisted of trays II and III, two of I and IV, three of VII and X, and four of V and VIII. Trays VI and IX were judged to be inadequate for screening compared to other positions in the chamber. Thus, they were not used in subsequent experiments.

Each of the following experiments consisted of 16 entries (cultivars, race stocks, or both) distributed at random in each of the four blocks. Each block consisted of two trays planted with eight entries. Each entry was planted with 30 to 35 seeds; and after thinning, 12 seedlings were left per row. Plants were spaced approximately three cm apart, and rows were spaced four cm apart. Two plants on each end of each row and two rows on each side of each tray were disregarded to counteract any possible border effects. Seed were treated with fungicide to avoid seedling disease. The two trays in each block were rotated daily in an attempt to reduce possible variation existing within the blocks due to position of the trays in the chamber. Up to the start of the first drought cycle, plants were irrigated on alternated days with 1.5 liters of water per tray. At about 15 days after planting, irrigation was suspended for four days; and then, plants were watered again with 1.25 liters of water per tray. (These quantities of water had been previously determined by means of subjective observations in the preliminary trials used to characterize the chamber.)

Two days after rewatering, the number of surviving plants were counted in each plot; and four such cycles, on the average, were used in each experiment. A total of seven experiments were conducted, and 90 germplasms were studied. In the sixth experiment, two of the better and two of the poorer performing entries from most of the previous ex-

periments were selected, and a "confirmation" test was run. In the seventh experiment, 11 primitive race stocks from the Texas collection were tested together with five selected cultivars. The complete list of entries and their origins is provided in Table 1.

The data, originally expressed as percentage survival, were transformed to arcsin of the square root, as recommended by Steel and Torrie (57). A further correction suggested by Bartlett (cited by the same authors (57)) was employed for percentages of 100 and zero. Statistical analyses were conducted as split-plots in time on the transformed data.

In the root-shoot study, five seed of an entry were planted in plastic pots 15 cm in diameter and 15 cm deep (standard six inch pots) filled with washed sand. A randomized complete-block design with the same four replications utilized previously was also used in this study. Fifteen entries (Table 2) selected from the seven previous screening tests comprised this study. This selection was based on seedling survival of the entries after the last cycle of the survival tests. Seed were treated with fungicide before planting and one week after germination, they were thinned to the most vigorous plant per pot. Due to differences in rate of germination, pots were rearranged in blocks grouping the same size of young plants as close together as possible. Within each block, pots were rotated daily so that every pot occupied every position within the block several times. Alternate irrigations with 150 ml per day of nutrient solution (27, Table 3) one day and of tap water the next day prevented the plants from suffering water stress.

After 35 days, plants were harvested. Roots were washed free of sand; root lengths of the bulk of the roots and to the very tip of the roots as well as shoot lengths were measured in cm. Number of leaves

was recorded, and leaf area was measured for each plant in cm^2 with a portable area meter model Li Cor LI-3000. Stem, leaves, and roots were placed in an electric oven at 85 C to dry for 72 hours. Dry weights in grams were determined for each component on a 0.0001 g precision scale, and root/shoot ratios were calculated based on the dry weights obtained.

RESULTS AND DISCUSSION

Screening for Seedling Survival Under Drought Conditions

Ninety cotton genotypes (primarily cultivars and race stocks) were screened for drought tolerance in a total of seven experiments. Fig. 2 illustrates more-or-less typical behavior of entries in these experiments over successive drought cycles. For its construction, four cultivars (two entries with the highest and two with the lowest percent survival after the last drought cycle) were chosen from among the entries in experiment 1. Differences in survival among some entries are obvious after only the first cycle while others appear more dramatically after the third. The pronounced effects in many entries in the third and later cycles may indicate a threshold effect, i.e., some stress can be tolerated, but prolonged stress overcomes what tolerance some entries may have.

The drastic shifts in performance for some entries from one cycle to the next suggests interaction between entries and cycles. To study the matter, two types of statistical analyses were conducted. In the first type, all cycles of an experiment were analyzed together, and those results are provided in the Appendix (Tables 12 and 13). As expected, some of those experiments (Nos. 3, 4, 5, and 6) did exhibit significant entry X cycle interaction at the 0.05 or at the 0.01 probability levels for the entry X cycle source of variation. This interaction

was significant at the 0.10 level in experiment 1. Based on these results and on subjective observations that the last two drought cycles invariably displayed more severe symptoms and were generally similar within each experiment, statistical analyses based only in those two cycles were performed. Those results appear in Tables 9 and 10 in the Appendix. None of these analyses detected a significant entry X cycle interaction at the 0.05 or higher levels. However, this interaction was significant at the 0.10 probability level in experiment 5. These results would also imply that the last drought cycle was unnecessary. Omitting it would permit greater efficiency in screening by saving almost a week per experiment.

All experiments except No. 2 exhibited significant differences among entries over the last two cycles at the 0.10 probability level or lower. Tables 4 through 7 present mean percent survival data for each germplasm over the last two drought cycles in each experiment. Means from those experiments with significant differences among entries at the 0.05 probability level were compared according to Duncan's New Multiple Range Test. Those results are largely self evident for experiment 1 through 5 and for experiment 7. The "confirmation" test (experiment 6) presented somewhat tenuous results. Some entries (e.g., 'IAC-13-1', 'IAC-RM_4-SM_5', 'Minas Sertaneja', and 'Acala 1517-75') appeared resistant as they had previously; others (e.g., 'Stoneville 213' and 'Minas Dona Beja') again appeared susceptible. Yet others (e.g., 'AC 307', 'Allen 333-61', 'Deltapine Land 61', and 'M4') reacted completely opposite to their earlier performance.

Little unequivocal information on the performance of cotton cultivars under drought conditions in the field is available; however, sub-

jective observations of several of the above cultivars over time suggest some correlation between these results and field performance. For example, the cultivars 'Paymaster Dwarf', 'Stoneville 213' (Table 4, Fig. 2), and 'Minas Dona Beja' (Table 4) generally produce relatively low yields under dryland or drought conditions - though all do very well under irrigation. On the other hand, some indication of drought tolerance in the field exists for the cultivar 'Minas Sertaneja' (Table 4).

Generally speaking, this technique is probably of practical value for screening a large number of genotypes for their drought tolerance especially if confirmation tests are run to verify earlier estimates. Mistakes in classification will be made, but all selections made by breeders are subject to such errors. Confirmation tests should reduce the number of mistakes. The results of such tests should be coupled with field experiments and observations, and every possible source of information must be pooled so that final decisions and selections may be made with some confidence.

Of paramount importance before such studies begin is an exhaustive study of the environmental characteristics within the growth chambers to be used. Some control of the variation present in them before starting actual tests can be attained by grouping like areas into replications. Rotation of trays within a block each day, in spite of the labor, is an effective means of minimizing variation within blocks.

These tests involved screening cotton seedlings for drought tolerance. It would be useful to the breeder to know how highly correlated are a seedling's vs. a mature plant's performance for this trait in cotton.

Root-Shoot Study

Fifteen cotton cultivars and race stocks, selected from the previous experiments, were grown in this study. Thirty-five days after germination, plants were collected and measurements taken. Results are summarized in Table 8. Corresponding analyses of variance are found in the Appendix in Table 11.

For all characters studied, except the two measures of root length, significant differences at the 0.05 or 0.01 levels were detected among entries. One of the measures of root length was significant at the 0.10 level. Some characters such as leaf area, number of leaves per plant, and plant height may be related with initial speed of germination and rate of growth.

Although entries differed widely for root/shoot ratio, no obvious correlations were found between these results and the previous screenings for drought tolerance.

The technique of culturing plants in pots filled with sand is a simple, easy procedure. A similar experiment, but submitting the plants to both treatments (drought versus non-drought conditions), would probably be of value in providing information about characters which would be differentially expressed under drought vs. non-drought conditions.

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Fig. 2. Histogram displaying the differential survival of four selected cotton genotypes in experiment 1 across four cycles of water stress.

Table 1. List of the 90 entries screened for drought tolerance and their countries of origin.

Identification	Origin	Identification	Origin	Identification	Origin
G002-7-1	Australia	T-461	Mexico	Deltapine Land 61	U.S.A.
G077-2	Australia	AC 134	Pakistan	Dunn 120	U.S.A.
IAC-13-1	Brazil	AC 307	Pakistan	GSA-71	U.S.A.
IAC-RM_4-SM_5	Brazil	Lasani 11	Pakistan	Gregg 35W	U.S.A.
Minas Dona Beja	Brazil	LSS	Pakistan	Gregg 45E	U.S.A.
Minas Sertaneja	Brazil	M4	Pakistan	HyBee 200A	U.S.A.
SL-23-6879	Brazil	Pak 51	Pakistan	Lankart 57	U.S.A.
SU 0450/8909	Brazil	Del Cerro	Peru	Lankart LX 571	U.S.A.
73	Bulgaria	SK 14	Thailand	Lockett 77	U.S.A.
3279	Bulgaria	SK 32	Thailand	Lockett BXL	U.S.A.
3996	Bulgaria	AH(67)M	Uganda	Lockett 4789-A	U.S.A.
4521	Bulgaria	BP 52/NC 63	Uganda	Mo-Del	U.S.A.
6111	Bulgaria	BPA 68	Uganda	Paymaster 202	U.S.A.
HL-1	Cameroon	CA(68)36	Uganda	Paymaster 303	U.S.A.
BJA 592	Chad	SATU 65	Uganda	Paymaster Dwarf	U.S.A.
HG 9	Chad	Acala SJ-4	U.S.A.	Stoneville 213	U.S.A.
4S 180	Greece	Acala SJ-5	U.S.A.	Stoneville 256	U.S.A.
10E	Greece	Acala 1517-75	U.S.A.	Tamcot 788	U.S.A.
T-102	Guatemala	Acala 1517E-1	U.S.A.	Tamcot SP-37	U.S.A.
T-111	Guatemala	Broadcot	U.S.A.	Thorpe	U.S.A.
T-141	Guatemala	Blightmaster A-5	U.S.A.	TPSA-110	U.S.A.
T-169	Guatemala	Cascot B-2	U.S.A.	Westburn M	U.S.A.
Laxmi	India	Coker 310	U.S.A.	Western Prolific 44	U.S.A.
Allen 333-61	Mali	Coker 312	U.S.A.	C-1211	U.S.S.R.
T-1	Mexico	Coker 348	U.S.A.	CX 349	U.S.S.R.
T-25	Mexico	Coker 5110	U.S.A.	108-F	U.S.S.R.
T-133	Mexico	Delcot 277	U.S.A.	137-F	U.S.S.R.
T-147	Mexico	Deltapine Land SR-2	U.S.A.	138-F	U.S.S.R.
T-254	Mexico	Deltapine Land SR-4	U.S.A.	152-F	U.S.S.R.
T-295	Mexico	Deltapine Land 16	U.S.A.	Albar 627	Zambia

Table 2. List of the 15 entries used in the root-shoot study and their countries of origin.

Identification	Origin
IAC-13-1	Brazil
IAC-RM_4-SM_5	Brazil
Minas Dona Beja	Brazil
Minas Sertaneja	Brazil
4521	Bulgaria
T-169	Guatemala
T-1	Mexico
T-133	Mexico
T-254	Mexico
T-295	Mexico
Acala 1517-75	U.S.A.
Blightmaster A-5	U.S.A.
Paymaster 303	U.S.A.
Stoneville 213	U.S.A.
Stoneville 256	U.S.A.

Table 3. Chemical composition of the modified Hoagland's solution used in the root-shoot study.

Chemical	Concentration (mg/liter)
$Ca(NO_3)_2 \cdot 4H_2O$	472.00
$MgSO_4 \cdot 7H_2O$	246.00
KNO_3	505.00
KH_2PO_4	68.00
H_3BO_3	2.40
$MnSO_4 \cdot H_2O$	1.20
$ZnSO_4 \cdot 7H_2O$	0.36
$CuSO_4 \cdot 5H_2O$	0.03
$CoCl_2 \cdot 6H_2O$	0.06
$Na_2MoO_4 \cdot 2H_2O$	0.20
FeEDTA†	(3.5 ppm Fe)

†Ten g $FeCl_3$ and 10.5 g EDTA were dissolved in one liter of water.

Table 4. Mean percent seedling survival for 32 cotton germplasms in experiments 1 and 2 over the last two drought cycles.

Experiment 1		Experiment 2	
Entry	Mean percent survival	Entry	Mean percent survival
Deltapine Land 61	71.2 a*	IAC-13-1	66.0 a*
Coker 348	47.0 ab	IAC-RM_4-SM_5	60.5 a
Paymaster 303	33.7 bc	Minas Sertaneja	59.6 a
GSA-71	32.3 bc	Dunn 120	53.1 a
Gregg 45E	25.9 bc	Westburn M	48.4 a
Paymaster 202	25.4 bc	SU 0450/8909	39.9 a
Broadcot	21.8 bcd	Tamcot 788	31.8 a
Deltapine Land SR-2	19.1 bcd	Gregg 35W	30.4 a
Tamcot SP-37	17.2 bcd	Del Cerro	26.3 a
Lankart LX 571	15.6 bcd	Coker 5110	22.3 a
Delcot 277	15.3 bcd	Lankart 57	20.5 a
Lockett 77	10.2 cd	Deltapine Land 16	19.2 a
Lockett 4789-A	7.8 cd	Coker 312	19.2 a
Paymaster Dwarf	5.2 cd	SL-23-6879	16.9 a
Coker 310	4.1 cd	Lockett BXL	10.9 a
Stoneville 213	1.2 d	Minas Dona Beja	8.1 a

*Means followed by the same letter were not significantly different at the 0.05 probability level according to Duncan's New Multiple Range Test.

Table 5. Mean percent seedling survival for 32 cotton germplasms in experiments 3 and 4 over the last two drought cycles.

Experiment 3		Experiment 4	
Entry	Mean percent survival	Entry	Mean percent survival
Lasani 11	73.6 a*†	Allen 333-61	99.6a*†
G077-2	63.6 a	4521	84.7 a
Pak 51	55.3 a	Albar 627	81.5 a
SATU 65	53.2 a	C-1211	79.9 a
SK 32	52.8 a	BPA 68	79.9 a
BJA 592	39.5 a	M4	79.7 a
Laxmi	37.8 a	CA(68)36	79.7 a
138-F	29.8 a	137-F	79.1 a
G002-7-1	28.4 a	73	77.0 a
BP 52/NC 63	24.0 a	10E	76.8 a
HyBee 200A	21.0 a	LSS	75.0 a
152-F	20.6 a	4S 180	74.9 a
108-F	11.0 a	AH(67)M	73.5 a
3996	10.9 a	3279	68.7 a
HG 9	10.9 a	AC 134	64.7 a
AC 307	4.6 a	HL-1	62.5 a

*Means followed by the same letter were not significantly different at the 0.05 probability level according to Duncan's New Multiple Range Test.

†Significant differences among entries at the 0.10 probability level.

Table 6. Mean percent seedling survival for 30 cotton germplasms in experiments 5 and 6 over the last two drought cycles.

Experiment 5†		Experiment 6	
Entry	Mean percent survival	Entry	Mean Percent survival
Acala 1517E-1	82.4 a*	IAC-13-1	97.2 a*
Deltapine Land SR-4	77.3 a	IAC-RM_4-SM_5	95.7 a
Cascot B-2	75.4 a	AC 307	93.5 ab
6111	74.5 a	Minas Sertaneja	84.0 abc
Acala 1517-75	71.6 a	AH(67)M	81.1 abc
Acala SJ-4	71.2 a	Acala 1517-75	75.1 abc
SK 14	63.2 ab	Paymaster 303	66.8 abcd
Western Prolific 44	58.0 ab	Stoneville 256	66.2 abcd
CX 349	57.5 ab	G077-2	64.7 abcd
Mo-Del	53.2 ab	Minas Dona Beja	62.0 abcd
Thorpe	51.7 ab	4521	56.1 bcd
Acala SJ-5	50.0 abc	Deltapine Land SR-4	53.6 bcd
Stoneville 256	28.8 bc	Stoneville 213	51.6 dc
TPSA-110	19.3 c	Allen 333-61	46.3 dc
---	---	Deltapine Land 61	24.3 d
---	---	M4	23.1 d

*Means followed by the same letter were not significantly different at the 0.05 probability level according to Duncan's New Multiple Range Test.

†Seed of two entries originally included in this experiment failed to germinate and thus are not listed here.

Table 7. Mean percent seedling survival for 16 cotton germplasms in experiment 7 over the last two drought cycles.

Experiment 7	
Entry	Mean percent survival
4521	67.2 a*
IAC-RM_4-SM_5	63.7 ab
IAC-13-1	60.9 ab
T-133	58.1 ab
T-254	54.7 ab
T-169	51.9 abc
Blightmaster A-5	49.0 abc
T-141	48.1 abc
T-461	45.2 abc
T-25	43.6 abcd
Stoneville 213	38.4 abcd
T-111	37.3 abcd
T-102	35.5 bcd
T-147	35.1 bcd
T-1	23.9 cd
T-295	17.2 d

*Means followed by the same letter were were not significantly different at the 0.05 probability level according to Duncan's New Multiple Range Test.

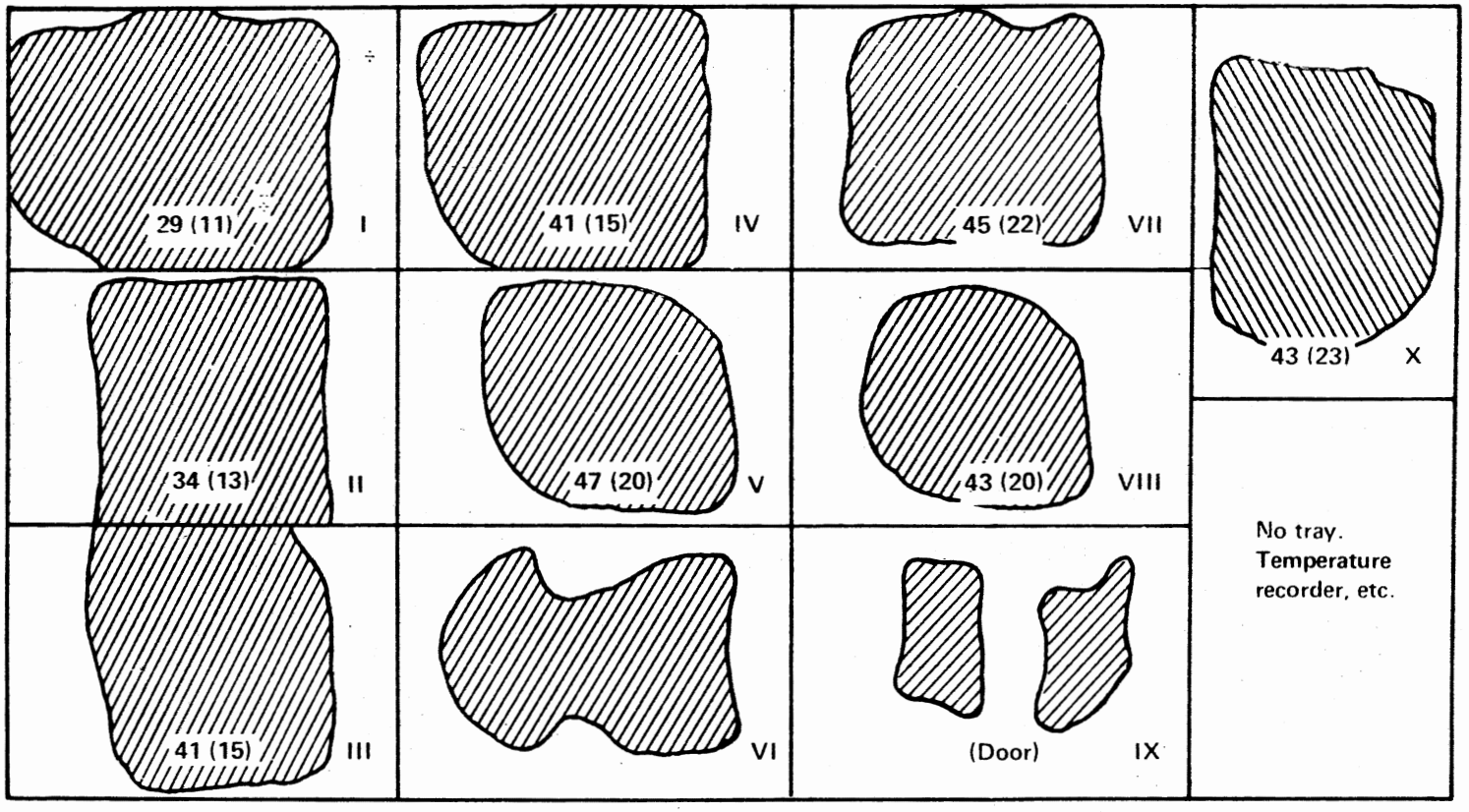
Table 8. Root and shoot characteristics of 15 cotton germplasms.

Entries	Leaf area	No. leaves/ plant	Plant height	Dry weights		Root/shoot ratio	Root length 1†	Root length 2†
				Shoot	Root			
	cm^2		cm	g			cm	
T-133	67.5 cde*	7.5 ab*	11.9 bc*	0.737 cde*	0.323 bcdef*	0.473 a*	33.5 a*	22.2 a*‡
IAC-RM_4-SM_5	96.8 abc	5.7 ced	12.0 bc	1.109 abc	0.470 ab	0.460 ab	26.4 a	19.7 a
Stoneville 256	112.0 ab	6.2 bcd	10.9 cd	1.224 ab	0.537 a	0.452 ab	22.9 a	18.5 a
T-169	69.7 cde	5.2 cde	7.9 fg	0.787 cde	0.351 bcdef	0.441 abc	25.4 a	18.0 a
4521	108.1 ab	7.7 a	13.7 ab	1.246 ab	0.481 ab	0.402 abcd	27.7 a	20.5 a
Stoneville 213	90.9 bcd	5.7 cde	8.3 efg	1.026 abcd	0.403 abcde	0.389 abcd	23.6 a	17.7 a
T-254	79.7 bcde	6.7 abc	7.9 fg	0.781 cde	0.284 def	0.384 bcd	31.8 a	17.0 a
T-1	90.2 bcd	7.5 ab	7.2 g	0.923 bcde	0.331 bcdef	0.362 cde	31.2 a	20.2 a
IAC-13-1	56.0 de	4.5 e	9.4 def	0.709 de	0.249 ef	0.362 cde	24.2 a	18.0 a
T-295	53.3 e	6.0 cd	10.0 de	0.540 e	0.195 f	0.359 cde	24.5 a	16.7 a
Minas Sertaneja	128.5 a	6.5 abc	14.0 a	1.335 a	0.459 abc	0.339 de	27.5 a	21.0 a
Acala 1517-75	106.2 ab	6.7 abc	13.0 ab	1.282 ab	0.416 abcd	0.333 de	25.5 a	19.2 a
Minas Dona Beja	84.3 bcde	5.7 cde	8.7 efg	0.917 bcde	0.303 cdef	0.325 de	24.8 a	17.0 a
Paymaster 303	60.8 de	5.0 de	7.9 fg	0.762 cde	0.241 f	0.322 de	25.0 a	17.0 a
Blightmaster A-5	69.9 cde	5.5 cde	9.7 def	0.947 bcd	0.270 def	0.282 e	28.4 a	16.2 a

*Means followed by the same letter were not significantly different at the 0.05 probability level according to Duncan's New Multiple Range Test.

†Measure 1 represents root length measured from the crown region to the tip of the root; measure 2, the length containing the greater concentration of roots.

‡Significant differences among entries at the 0.10 probability level.



÷Shaded areas represent areas in the trays (numbered I through X) exhibiting more intense drought stress.

⁘Numbers outside and inside parentheses represent mean number of surviving seedlings at the end of the experiments in the tray as a whole and in the experimental area (no border plants), respectively.

Fig. 1. Patterns exhibited by water-stressed cotton seedlings in preliminary growth chamber experiments (data are averages based on two experiments).

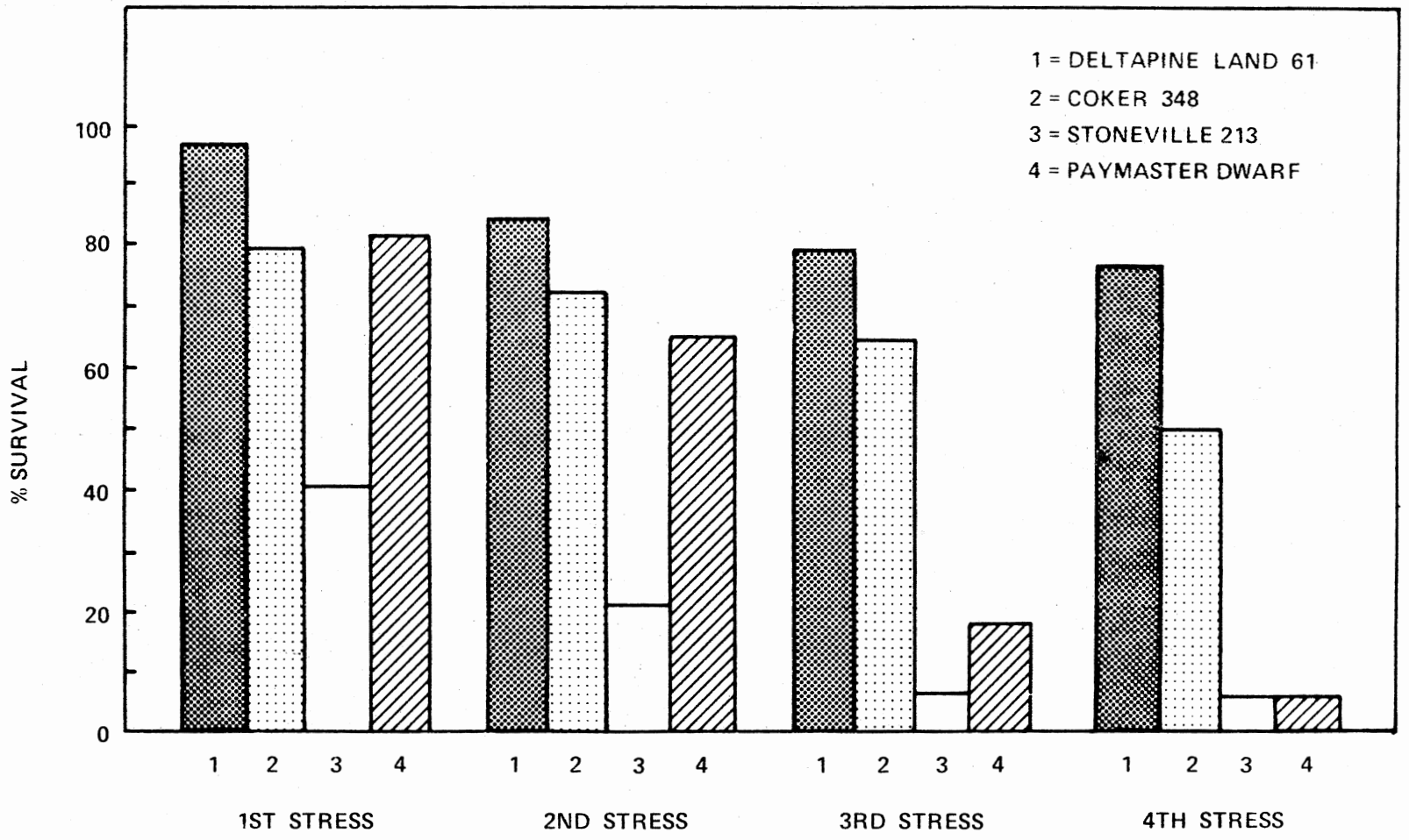


Fig. 2. Histogram displaying the differential survival of four selected cotton genotypes in experiment 1 across four cycles of water stress.

APPENDIX

(Tables 9 to 13)

Table 9. Analyses of variance for survival data presented in Tables 4 and 5.

Source	df	Mean squares			
		Experiment 1	Experiment 2	Experiment 3	Experiment 4
Rep	3	0.352**	0.058**	0.833**	8.401**
Entry	15	0.394**	0.342	0.452†	0.140†
Rep X Entry	45	0.121**	0.229**	0.246**	0.085
Cycle	1	2.147**	0.297**	0.039	3.157
Rep X Cycle	3	0.054*	0.005	0.014	1.086**
Entry X Cycle	15	0.018	0.010	0.007	0.128
Rep X Entry X Cycle	45(37)‡	0.021	0.011	0.007	0.082

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

†Significant at the 0.10 probability level.

‡Number in parentheses is the error degrees of freedom (corrected for missing values) in experiment 1.

Table 10. Analyses of variance for survival data presented in Tables 6 and 7.

Source	df	Mean squares		
		Experiment 5	Experiment 6	Experiment 7
Rep	3 (3)[§]	1.094**	2.315**	1.316**
Entry	13 (15)	0.302**	0.571**	0.172**
Rep X Entry	39 (45)	0.099**	0.177**	0.069
Cycle	1 (1)	9.822*	3.625**	4.142*
Rep X Cycle	3 (3)	0.480**	0.102	0.269**
Entry X Cycle	13 (15)	0.108[†]	0.059	0.033
Rep X Entry X Cycle	37[‡](45)	0.058	0.042	0.044

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

[†]Significant at the 0.10 probability level.

[‡]Number indicated is the error degrees of freedom (corrected for missing values) in experiment 5.

[§]Numbers in parentheses are the degrees of freedom for experiments 6 and 7.

Table 11. Analyses of variance for data presented in Table 8.

Source	df	Mean squares							
		Leaf area	No. leaves/ plant	Plant height	Dry weight		Root/shoot ratio	Root length 1	Root length 2
					Shoot	Root			
Entries	14	2008.8**	3.67**	20.73**	0.233**	0.041**	0.013**	40.48	12.92†
Blocks	3	5755.4**	5.53**	13.77**	1.010**	0.097**	0.050**	50.01†	25.93*
Entries X Blocks	42	448.2	0.68	1.53	0.054	0.009	0.003	29.45	7.22

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

†Significant at the 0.10 probability level.

Table 12. Analyses of variance for experiments 1 through 4 over all stress cycles.

Source	df	Mean squares			
		Experiment 1	Experiment 2	Experiment 3	Experiment 4
Rep	3	1.199**	0.566**	1.073**	9.822**
Entry	15	0.695**	0.518**	0.579†	0.317
Rep X Entry	45	0.208**	0.210**	0.348**	0.217**
Cycle	3	6.964**	8.126**	10.016**	2.202
Rep X Cycle	9	0.097**	0.211**	0.083*	0.729**
Entry X Cycle	45	0.041†	0.034	0.056*	0.070*
Rep X Entry X Cycle	135(119)‡	0.029	0.043	0.037	0.046

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

†Significant at the 0.10 probability level.

‡Number in parentheses is the error degrees of freedom (corrected for missing values) in experiment 1.

Table 13. Analyses of variance for experiments 5 through 7 over all stress cycles.

Source	df	Mean squares		
		Experiment 5	Experiment 6	Experiment 7
Rep	3 (3)[§]	2.288**	2.665**	2.810**
Entry	13 (15)	0.553**	0.450**	0.238[†]
Rep X Entry	39 (45)	0.162**	0.169**	0.126**
Cycle	4 (3)	5.718**	5.694**	6.194**
Rep X Cycle	12 (9)	0.204**	0.152**	0.172**
Entry X Cycle	52 (45)	0.058**	0.096**	0.031
Rep X Entry X Cycle	151[‡] (135)	0.032	0.045	0.032

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

[†]Significant at the 0.10 probability level.

[‡]Number indicated is the error degrees of freedom (corrected for missing values) in experiment 5. This experiment had

[§]Numbers in parentheses are the degrees of freedom for experiments 6 and 7.

VITA[2]

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