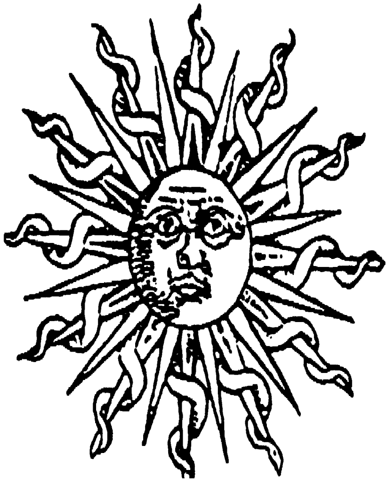

Arid Lands

Today and Tomorrow

Proceedings of an International Research
and Development Conference

Tucson, Arizona U.S.A.
October 20-25, 1985

edited by Emily E. Whitehead,
Charles F. Hutchinson, Barbara N. Timmermann,
and Robert G. Varady

copyeditor
Susan C. Fansler

Westview Press • Boulder, Colorado
Belhaven Press • London

An Approach to Screening for Resistance to Water and Heat Stress in Sorghum (*Sorghum bicolor* (L.) Moench)

J.M. Peacock
Cereals Program
International Crops Research Institute for the Semi-Arid Tropics
Patancheru, Andhra Pradesh, India

S.N. Azam-Ali
Groundnut Improvement Program
International Crops Research Institute for the Semi-Arid Tropics
Patancheru, Andhra Pradesh, India

R.B. Matthews
Department of Physiology and Environmental Science
School of Agriculture
Sutton Bonington, Loughborough, U.K.

ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is an important food crop of the arid and semiarid tropics but average yields are still below 1,000 kilograms per hectare, and under drought conditions these may fall below 100 kilograms per hectare or there may even be total crop failure.

Breeding for drought resistance in sorghum is a complex problem. The objective of this paper is to describe the development of a screening technique, based on a "physiological approach." Initially, a sample of genotypes were selected from the sorghum genetic resources accessions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center to represent material collected from many countries over a range of altitudes (0 to 2,000 meters) and mean annual rainfall (250 to 2,500 millimeters), covering most of the taxonomic groups. Some advanced breeding materials were also included in this sample of over 700 lines. They were screened under severe drought conditions at ICRISAT in the relatively rain-free summers from 1983 to 1985.

Considerable genetic variation was shown in the response of these genotypes to water and heat stress. Visually observed differences in "resistant" and "susceptible" genotypes in terms of desiccation tolerance or avoidance, recovery resistance and ability to produce grain after the onset of rains was then shown to be associated with specific physiological traits. Selected physiological data from a few contrasting genotypes were shown on relative leaf-water content (RLWC), leaf-water potential (ψ_l) and leaf stomatal conductance (g_l). These results verify that the visual "resistance" traits identified earlier were based on measurable physiological responses.

In conclusion, it is argued that the approach described has proved to be successful in identifying drought-resistant germplasm. With more basic research on the same contrasting genotypes, the problem of identifying drought-resistant sorghums could be largely overcome.

INTRODUCTION

A major objective of this conference is to discuss better ways of utilizing the biological resources of the arid and semiarid zones. If irrigation is not available, which is often the case in many arid and semi-arid areas, the only possible solutions are through cultural practices that increase the availability of stored soil moisture, or by the development of crop varieties that can more efficiently avoid or tolerate drought and the high temperatures often associated with it.

Sorghum is recognized as an important crop for arid and semiarid regions (1) but despite this, average yields in the developing world are only 1,000 kilograms per hectare. In Africa, average yields are less than 600 kilograms per hectare, falling to as low as 100 kilograms per hectare in Botswana in 1983 (2). These low yields often reflect the severe drought conditions that exist in farmers' fields.

The physiological and genetic aspects of crop improvement in response to drought and high temperature stress have been comprehensively reviewed in recent years (3, 4, 5, 6, 7, 8). These reviews indicate that ample genetic variation for physiological components of drought resistance exists in several crops.

This paper outlines the development of a method to systematically screen sorghum germplasm and breeders' lines and to identify the important physiological traits that impart resistance to high temperature and soil water shortage, with particular emphasis on midseason stress.

DEVELOPMENT OF SCREENING METHOD

A knowledge of the physical environment in which sorghum is grown is required before any attempt can be made to develop improved genotypes. The primary climatic variables affecting crop growth in the arid and semiarid regions are annual precipitation and its variability and distribution, relative humidity, temperature, and type and depth of soils. As there are many possible combinations of climates, crop scientists charged with the prime responsibility of developing drought-resistant genotypes have a nearly impossible task. This is further complicated by the effect of timing of any set of conditions on the stage of development of the sorghum crop, for example, low soil moisture may be more critical at the grain-filling stage than in the early vegetative phase.

The first step of our research was to define a minimum number of critical stages of growth to be examined. The second was to select a set of environmental conditions that represent the drier and hotter parts of the semiarid regions where sorghum is grown. The third was to obtain a representative sample of the germplasm for initial screening. The fourth was to attempt to identify physiological traits that might impart "drought resistance." Our final objective was, in collaboration with other scientists around the world, to attempt to understand the underlying mechanisms associated with "drought resistance" observed under these field conditions.

Growth Stages

Three important developmental stages in sorghum were defined: i) sowing to floral initiation (or the seedling-stress stage), ii) floral initiation to flowering (midseason stress), and iii) and flowering to physiological maturity (terminal stress). The approach to all three growth stages with respect to response to drought was essentially the same and only the midseason stage was described here. Some findings on the effects of heat stress at the seedling stage are used to illustrate some of the basic collaborative research.

Environment

The severest environmental conditions at ICRISAT Center, Patancheru, India, occur during the summer (March through June), which is characterized by low soil moisture, high temperature (> 40°C) and low relative humidity (< 10 percent). This season was selected for experimentation because it was under similar conditions that the severe droughts, in eastern and southern Africa, occur. Under milder, rainy season conditions many higher yielding sorghums have already been identified.

Germplasm

It would be extremely difficult to systematically screen the 26,000 accessions of sorghum that have been collected so far. Thus, a representative sample was selected based on morphological and physiological traits that might or might not impart drought resistance.

In 1983, a total of 700 selected germplasm accessions and advanced breeding lines were screened during the summer (March through June) at ICRISAT Center (9). The material was divided into three groups; the first two comprised germplasm lines selected from a wide range of taxonomic groups (for example, durra, caudatum, etc.), geographical locations (countries where sorghum was collected) and climates (range of altitudes and mean annual rainfall). The third group included both germplasm and 70 advanced breeding stocks developed at ICRISAT or by national programs. The three groups were sown at monthly intervals, from early February until April, and were established with irrigation for 15 to 18 days. Irrigation was then discontinued and the midseason stress imposed.

Morphological and Physiological Traits

Many physiological traits that affect crop adaptation to drought and high temperatures have been identified and a "physiological approach" to breeding for drought resistance has been described by Morgan (10), Bidinger (11), Jordan and Miller (12) and Steponkus et al. (13). However, in the early stages of our screening program it was essential to examine only those traits that could be visually recognized. The two traits examined were

1. Desiccation tolerance or avoidance, that is, a measure of the amount of leaf area that remained unscorched or "fired." We scored leaf firing at regular intervals during the stress period on a 1 to 5 scale, where 1 = less than 20 percent of leaf-area fired, and 5 = over 80 percent leaf-area fired.
2. Recovery resistance, that is, ability of a line to produce new leaves and grain after rain. We scored recovery resistance on a 1 to 5 scale where 1 = over 80 percent of the plants in a row recovered, and 5 = less than 20 percent recovered.

We examined the results of the 1983 screenings and retained lines that had a leaf firing score between 1 and 2.99 (most resistant) as well as those between 3.99 and 5 (most susceptible). Figure 1 shows the effects of stresses due to heat and lack of water on a typically "resistant" and "susceptible" line. From the 1983 experiments, we concluded that a March planting gave the most suitable and reliable selection pressure. We selected 266 lines for further screening in 1984.

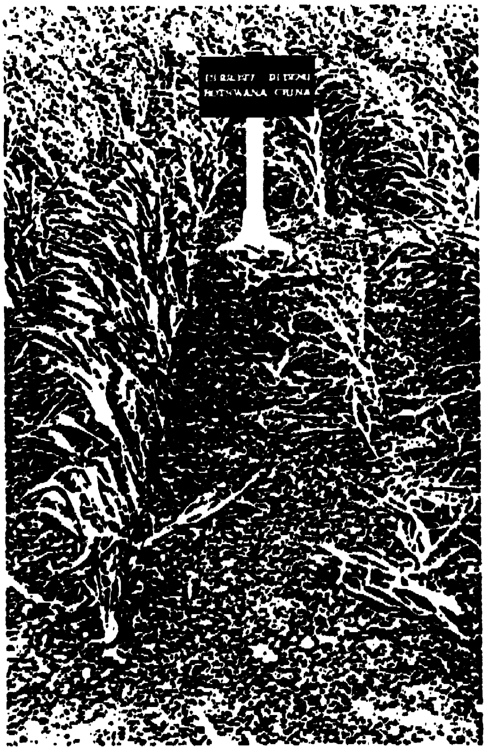

Figure 1. Effects of stresses due to heat and lack of water on two sorghum cultivars, IS 22327 (left), a resistant line from Botswana, and IS 12741 (right), a susceptible line from China, ICRISAT Center, 1984.

These selected lines were sown on 16 March 1984 in an Alfisol at ICRISAT Center in four replications. The crop was established with irrigation and a midseason stress imposed after 20 days when all further irrigation was stopped. All the lines experienced stresses from heat and lack of water for a period of 66 days. During the stress period only 4.5 millimeters of rain fell, and the mean maximum temperatures were close to 40°C. The stress ended 91 days after sowing (DAS), following 21.6 millimeters of rain. We scored the material for leaf firing at 48, 61, 70 and 83 (DAS), for recovery resistance at 89, 94, 102 and 117 (DAS), and for agronomic scores when the lines reached physiological maturity.

We observed 28 lines to be both firing-resistant (FR) and able to recover from severe stress (RR) (Table 1). Three of these lines also had good grain yields. The group included two germplasm accessions, IS 1347 from Egypt and IS 13441 from Zimbabwe (Figure 2), and an ICRISAT breeding line SPH 225.

The visual screenings in 1983 and 1984 had clearly demonstrated that there were marked differences in the response of these sorghum genotypes to high temperature and water deficit. It was argued that our screening approach could be simplified even further if the underlying mechanisms associated with these striking differences (see Figure 1) were understood.

Survival is ultimately determined by the plant's ability to maintain an internal water status that will allow it to sustain a minimum of essential metabolic processes such as photosynthesis and respiration, and to facilitate transpirational cooling of leaves. The apparent failure of some of the genotypes in the earlier screenings could be a consequence of one or more of the following:

1. An inadequate root system, which is unable to extract water to sustain atmospheric demand.
2. Stomatal behavior, which is extremely sensitive to plant water deficit and high evaporative demand.
3. Inability of the cell and leaf tissue to survive at temperatures above 40°C.

From the 266 lines sown in 1984 we selected only the 157 that flowered during the period of stress and recovery. Of these we selected five "susceptible" lines (FS) and four "resistant" lines (FR) (Table 2) for a detailed experiment to examine the physiological bases of resistance to midseason heat and water stress. The lines were sown on March 12, 1985, in a RBD design of 60-centimeter rows. There were four replicates in the stressed treatment and two in the "control." In the four stress plots no irrigation was applied from 20 DAS until the onset of the rains, in June. Irrigation was applied weekly in the control plots, at a rate sufficient to replace evaporation. After emergence, the plots were thinned to a plant population of 120,000 plants per hectare.

Instrumentation was installed in two replicates of each of five genotypes in stress and control treatments. Further replication was restricted by the channels available on the two data-logging units. Also, comparable physiological measurements could only be made on a maximum of five lines per day. The lines measured are marked with an asterisk in Table 2 and represent an early- and a late-maturing "susceptible" line and two early and one late maturing "resistant" lines.

Measurements of solar radiation, dry- and wet-bulb temperature, leaf and soil temperature and wind speed were scanned every minute and readings were averaged for each hour. Measurements of wet- and dry-bulb temperature and leaf temperature were made at heights corresponding to the top and middle of the canopy in each plot.

Leaf temperature was measured at the tip, middle and base of the underside of the first fully expanded leaf using copper-constantan (38 standard wire gauge) thermocouples. Soil thermocouples (20 standard wire gauge) were positioned at 30 centimeters depth. Solar radiation was measured using tube solarimeters. The fraction of total radiation intercepted by each stand was calculated by comparing the output of tubes placed beneath the canopy with that of a "standard" tube positioned above the crop. Wind speed was measured at canopy height using cup anemometers.

Detailed physiological measurements were made twice weekly throughout the stress period on each of the five genotypes in the stress and control plots. Measurements were made daily at 0830 hours, 1230 hours and 1530 hours and were made at the midpoint of the youngest fully expanded leaf and on a leaf in the middle of the canopy (usually three leaves lower). Two days later, measurements were made only on the youngest fully expanded leaf but in three positions: at its tip, middle and base.

Table 1. Sorghum lines identified as having firing resistance (FR) and recovery resistance (RR) traits at ICRISAT Center, summer 1984.

Sorghum line	Origin	Elevation[a] (m)	Rainfall[b] (mm)	Taxonomic group
IS 8564	Chad	--	--	Caudatum
IS 1347	Egypt	--	--	Caudatum bicolor
IS 1096	India	500	800	Durra
IS 22064	India	500	500	Durra
M 35-1	India	500	700	Durra
SPH 225	India	--	--	--
SPV 138	India	--	--	--
SPV 386	India	--	--	--
SPV 394	India	--	--	--
IS 18463	India	--	--	Kafir caudatum
IS 18465	India	560	800	Durra
IS 20965	Kenya	1,100	1,500	Drummondii
IS 20969	Kenya	1,100	1,500	Caudatum
IS 21479	Malawi	70	800	Guinea
IS 3898	Mali	--	--	Guinea bicolor
IS 23687	Mozambique	250	900	Guinea
IS 8344	Pakistan	--	--	Durra
IS 22380	Sudan	600	450	Caudatum
IS 3511	Sudan	--	--	Kafir caudatum
IS 9708	Sudan	--	--	Caudatum
IS 12737	Taiwan	--	--	Caudatum
IS 113	USA	--	--	Kafir caudatum
IS 121	USA	--	--	Kafir caudatum
IS 13441	Zimbabwe	--	--	Caudatum
IS 13446	Zimbabwe	--	--	Caudatum
CSH 5	India	--	--	--
CSH 8	India	--	--	--
CSH 9	India	--	--	--

[a] Point identified as nearest to area of collection.

[b] Mean annual rainfall in area of collection.

Figure 2. Sorghum cultivar IS 13441 from Zimbabwe with firing resistance and ability to recover from severe stress. This line has also produced good grain yields on large panicles (left).

Table 2. Sorghum lines used in detailed physiology experiment at ICRISAT Center, summer 1985.

Sorghum line	Origin	Elevation (m)	Rainfall (mm)	Taxonomic group	Maturity
<u>Susceptible (FS)</u>					
*IS 17605	Yemen	1,970	600	Durra	Late
*IS 12739	China	--	--	Caudatum bicolor	Early
IS 12744	Taiwan	--	--	Guinea caudatum	Early
IS 21436	Malawi	75	800	Durra	Early
IS 22253	Botswana	1,250	514	Kafir	Early
<u>Resistant (FR)</u>					
*IS 20969	Kenya	1,100	1,500	Caudatum	Late
*IS 1347	Egypt	--	--	Caudatum bicolor	Early
*IS 13441	Zimbabwe	--	--	Caudatum	Early
IS 22380	Sudan	600	450	Caudatum	Late

*Lines on which measurements were made (see text).

The following measurements were made on these leaves: relative leaf water content (RLWC--defined as the ratio of leaf water content at sampling to that at full turgor), leaf water potential (ψ_l--as measured with a pressure chamber) and stomatal conductance (g_l-- measured with a diffusion porometer). Measurements of light incident on the leaves (S_l) and the leaf temperature (t_l) at the time and site of measurement of conductance were made with a quantum sensor and an infrared thermometer, respectively.

Measurements of ψ_l, g_l, S_l and t_l were made on the same leaves. Immediately after the measurements of g_l, S_l and t_l, the leaf was excised and returned to a field laboratory for measurements of ψ_l. Soil water content was measured in these plots, using a neutron probe. Detailed measurements continued until the onset of the rains at 84 DAS after which only dry matter production and grain yield were measured.

Dry matter production of shoots and roots and leaf areas was measured weekly throughout the experiment. Full details of this study are being published separately.

DISCUSSION

As indicated earlier the purpose of this paper was not to examine in detail or interpret the data from this physiological experiment but to illustrate that it is possible to systematically screen the germplasm and breeding lines. Also, by setting the correct "selection pressure," it was possible to rapidly identify material from which it may be possible to impart drought "resistance" genes to more agronomically elite material. To do this we selected three sets of data.

Results shown in Figures 3a and 3b clearly demonstrate that, after a critical level of stress is reached (56 DAS), the "resistant" lines have a very different plant water status to the susceptible lines, in terms of RLWC and ψ_l, under both soil and atmospheric water stress. Noticeably, the trend for both traits is the same. A similar response under atmospheric water stress only is shown in Figure 3c for stomatal behavior in terms of individual leaf conductance, g_l. The terminology requiring phrases such as "resistant" and "susceptible" was obviously subjective, based on the earlier visual scorings. Yet these data verify that these visual differences are based on measurable physiological traits and could be used effectively in an improvement program to identify "resistant" genes.

In addition to quantifying these visual differences, it is also possible to identify which traits are more suitable for field screening. From these data alone, it is clear that RLWC is a more sensitive measurement than either ψ_l or g_l. These initial results are very encouraging and researchers in the United Kingdom and now in Australia, the United States and Italy, will look at additional factors that also appear to be consistently different among these very contrasting genotypes.

Collaboration

An example of this collaborative research is a Ministry of Overseas Development Project (R3801) being conducted at the Welsh Plant Breeding Station (WPBS), in the United Kingdom. Earlier, using techniques developed at ICRISAT Center (14, 15), we had shown considerable genetic variation in the ability of sorghum seedlings to emerge at high temperatures. An example, showing differences between a few selected hybrids and varieties, is shown in Table 3. The biochemists at the WPBS, working with the same genotypes, showed clearly (Figure 4) that the differences in seedling emergence were closely related to the rates of embryo protein synthesis (16, 17). Such collaborative research has not only enabled us to jointly develop a more rapid screening method, but has led to an understanding of some of the underlying mechanisms influencing crop establishment at high temperatures.

In accepting that the problem of arid land research is one of the most important that the world has to tackle in the next decade, we believe that an international approach involving many scientists from different countries and directed from an international center should be more effective than single groups working in isolation.

We are still some way from our goal, as these promising traits have yet to be incorporated by conventional breeding methods into better agronomic backgrounds. But as can be seen from Figure 2, some of the so-called germplasm accessions, such as IS 13441 from Zimbabwe, not only are a source of these useful traits but also can have a relatively high yield.

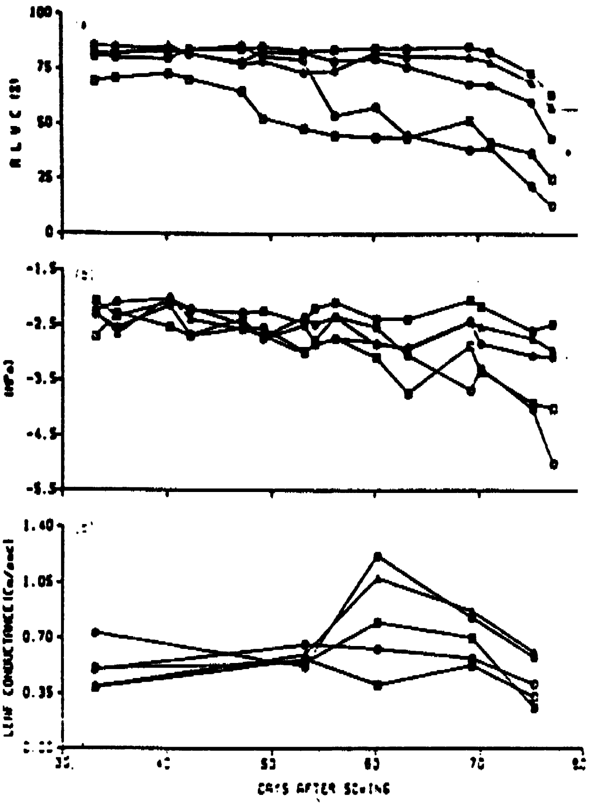

Figure 3. The RLWC (a), ψ_1 (b) and g_1 (c) measured at 12.30 h on the mid-portion of the youngest expanded sorghum leaf of IS 17605 (□), IS 12739 (○), IS 20969 (■), IS 1347 (●) and IS 13441 (▲) in the stress (a, b) and control (c) plots.

Table 3. Effects of four soil temperatures (°C) on sorghum seedling emergence (%) in a laboratory test, ICRISAT Center, 1982.

Entry	Seedling emergence (%)			
	35°C	40°C	45°C	50°C
Hybrids				
CSH 1	46	27	20	0
CSH 5	70	35	--[a]	--[a]
CSH 6	70	40	--[a]	--[a]
Varieties				
SPV 354	85	70	67	0
SPV 386	90	83	15	0
SPV 387	90	65	50	0
CSV 5	73	93	80	0
SE	±6.2	±10.1	±13.0	
Mean	74.9	59.0	46.4	0
CV%	21	44	61	

[a] Not tested at these temperatures.

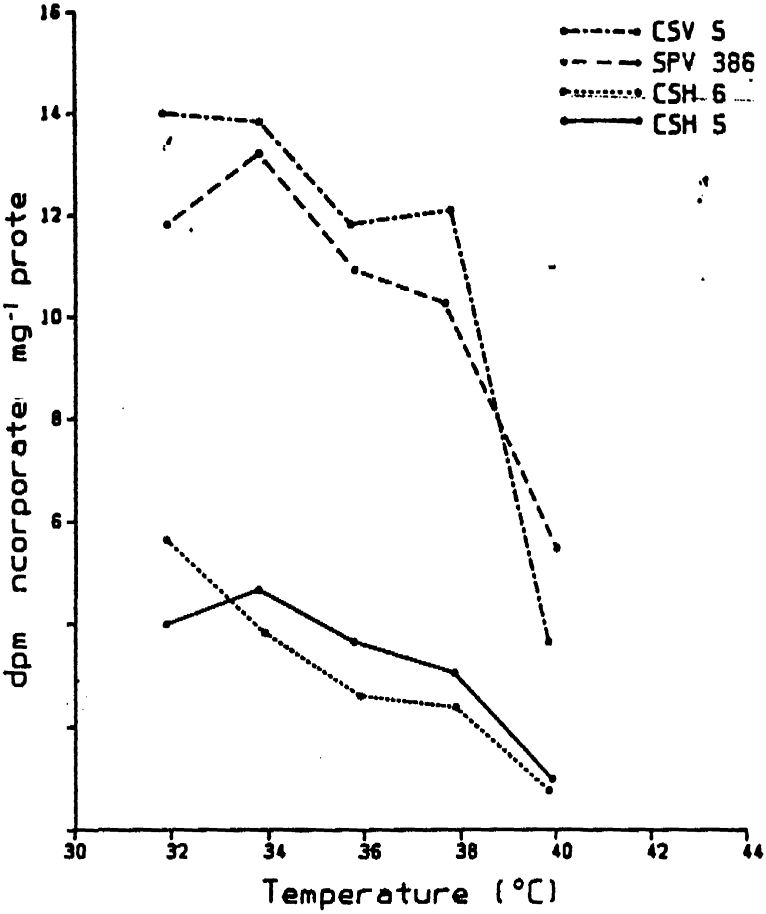

Figure 4. Embryo protein synthesis expressed as radioactive counts min^{-1} (dpm) incorporated mg^{-1} protein of sorghum varieties CSV 5 and SPV 386, and hybrids CSH 5 and CSH 6, during 16 h imbibition at different temperatures. Welsh Plant Breeding Station, 1982.

Some of the techniques now being actively researched under the heading of "biotechnology," such as anther culture, tissue culture and gametophytic selection, may enable us to make the large number of crosses necessary to find those one or two lines that will provide life-saving grain in extremely dry and hot years.

Looking to the future, we will continue to systematically screen the germplasm both in India and Africa, but an increased amount of basic research on the physiology, biochemistry and genetics must also continue in parallel with this field screening.

CONCLUSIONS

Bidinger (11), in summing up the papers presented on breeding for drought resistance at a workshop held in the United States in 1978, indicated that physiologists should investigate this approach despite the difficulties. We believe our findings must give support and encouragement to the physiologists who have persevered with this approach. It is hoped that the plant breeders who have had reservations about the approach (11) will now be encouraged to accept it, and with the new methods of plant breeding being developed using biotechnology techniques, perhaps more drought- and heat-tolerant sorghums will become a reality for the farmers living off the meager resources of the world's arid lands.

REFERENCES

1. Peacock, J.M. and G.L. Wilson. 1984. Sorghum. Pages 249-279 *in* P.R. Goldsworthy and N.M. Fisher, eds., The physiology of tropical field crops. John Wiley and Sons. 664 p.

2. FAO (Food and Agricultural Organization of the United Nations). 1984. Production year book 1983, pp. 119-120. FAO, Rome.

3. Mussell, H. and R.C. Staples. 1979. Stress physiology in crop plants. Wiley Interscience. New York. 510 p.

4. Raper, C.D., Jr., and P.J. Kramer. 1983. Crop reactions to water and temperature stresses in humid temperate climate. Westview Press, Boulder, Colorado. 373 p.

5. Turner, N.C. and P.J. Kramer. 1980. Adaptation of plants to water and high temperature stress. John Wiley and Sons. 482 p.

6. Blum, A. 1983. Genetic and physiological relationships in plant breeding for drought resistance. Agricultural Water Management 7:195-205.

7. Christiansen, M.N. and C.F. Lewis. 1982. Breeding plants for less favourable environments. Wiley Interscience, New York. 459 p.

8. Paleg, L.G. and D.D. Aspinall. 1981. The physiology and biochemistry of drought resistance in plants. Academic Press, New York. 492 p.

9. ICRISAT (International Crops Research Institute for Semi-Arid Tropics). 1984. Annual report 1983. ICRISAT, Patancheru, Andhra Pradesh, India. 371 p.

10. Morgan, J.M. 1980. Differences in adaptation to water stress within crop species. Pages 369-382 *in* N.C. Turner and P.J. Kramer, eds., Adaptation of plants to water and light temperature stress. Wiley Interscience, New York. 482 p.

11. Bidinger, F.R. 1980. Breeding for drought resistance. Pages 452-454 *in* N.C. Turner and P.J. Kramer, eds. Adaptation of plants to water and high temperature stress. Wiley Interscience, New York. 482 p.

12. Jordan, W.R. and F.R. Miller. 1980. Genetic variability in sorghum root systems: Implications for drought tolerance. Pages 383-399 *in* N.C. Turner and P.J. Kramer, eds., Adaptation of plants to water and high temperature stress. Wiley Interscience, New York. 482 p.

13. Steponkus, P.L., J.M. Cuttler and J.C. O'Toole. 1980. Adaptation to water stress in rice. Pages 401-418 *in* N.C. Turner and P.J. Kramer, eds., Adaptation of plants to water and high temperature stress. Wiley Interscience, New York. 482 p.

14. Wilson, G.L., P.S. Raju and J.M. Peacock. 1982. Effect of soil temperature on seedling emergence in sorghum. Indian Journal of Agricultural Science 52:848-851.

15. Soman, P. and J.M. Peacock. 1985. A laboratory technique to screen seedling emergence and pearl millet at high soil temperature. Experimental Agriculture 21:335-341.

16. Ougham, H.J. and J.L. Stoddart. 1985. Development of a laboratory screening technique, based on embryo protein synthesis, for the assessment of high temperature susceptibility during germination of *Sorghum bicolor*. Experimental Agriculture 21:343-355.

17. Ougham, H.J., J.M. Peacock, J.L. Stoddart and P. Soman. Effect of high temperature on the seedling emergence and embryo protein synthesis of sorghum. Crop Science. (In press)