Evaluation of Techniques to Screen for Drought Resistance in Sorghum Seedlings[1]

R.K. Maiti, P.S. Raju**, B.V.S. Reddy**, J.M. Peacock***

ABSTRACT

Different techniques for inducing water stress were evaluated in semi-controlled conditions in a glass house and the field to study the effects of increasing soil moisture deficits on seedling growth of sorghum (*Sorghum bicolor* (L.) Moench). Drought resistance indices, such as degrees of wilting, recovery and number of recovered plants, were measured and genotypic differences were observed. Correlations among these seedling indices for the different techniques were high ($r = 0.90^{**}$, $P < 0.01$).

COMPENDIO

Differentes técnicas por inducción de stress de humedad fueron evaluadas en condiciones semi-controladas en un invernadero y en el campo para estudiar los efectos de la incrementación del déficit de humedad del suelo sobre el crecimiento de la plántula de sorgo. Indices de resistencia a la sequía como grado de marchitez, recuperación y plantas recuperadas fueron medidas y fueron observadas diferencias genotípicas. Las correlaciones entre esos índices de la plántula en las diferentes técnicas fueron altas ($f = 0.90^{**}$, $P < 0.01$).

INTRODUCTION

In the semi-arid tropics, sorghum seedlings are frequently subjected to long periods without rain. Seedling establishment is a critical stage in the development of the crop and it is important to select lines tolerant to severe water deficits at this stage. Numerous attempts have been made in different crops to develop simple techniques for assessing resistance to water deficits at the seedling stage (1, 5, 6, 7, 8, 9, 10). Using simple techniques, Heyne and Laude (2) demonstrated that it was possible to distinguish maize cultivars with drought resistance by screening controlled growth room conditions. Attempts to screen soybean in a growth chamber for drought resistance were not successful (7), but screening in small containers for recovery from drought showed promising results (8). Similarly, growing sorghum cultivars in metal trays with limited water in a glass house and determining the percentage of seedlings surviving after imposing a severe water deficit was found to be useful in selecting genotypes with seedling drought resistance (5). Sammons *et al.* (7, 8) suggested that the relatively sophisticated instrumentation and apparatus used for evaluating cultivars for drought resistance in a growth chamber were cumbersome for mass screening of a large number of genotypes. Screening in the field is often difficult due to uncertainty of weather conditions, difficulty in the quantification of soil water deficit and the variability of soil moisture with soil type, season and the phenological stage of the crop (4).

The purposes of this study were to evaluate some simple techniques designed to screen sorghum germplasm and breeder's lines for ability to emerge and survive under severe shortage of water stress, and to examine the genetic variation of resistance to water stress at the seedling stage of these same genotypes.

MATERIALS AND METHODS

Three different techniques for screening sorghum for resistance to water stress at the seedling stage are described.

Screening in brick containers. The screening equipment is similar to that of Sammons *et al.*, (8) and Wilson *et al.* (11). Low brick containers (160 cm long, 70 cm wide and 22 cm high) in duplicate rows were constructed on levelled ground. Alfisol (classified as fine, clayed mixed udic Rhodustalf) was thoroughly mixed before filling the brick containers and levelled to 5 cm from the top. The soil was separated from the bricks by polyvinyl chloride film to

1 Received for publication 16 December 1987.

* Professor in Botany, Division de Post-Grado, Facultad de Ciencias Biológicas, Universidad Autonoma de Nuevo León, Ciudad Universitaria, Apartado Postal 2790, Monterrey, N.L., México.

** International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O.; Andhra Pradesh 502 324, India.

minimize the edge moisture effects, but was laid directly on the ground, allowing a free and even drainage after watering.

A randomized complete block (RCB) design in three replications was used with test entries and two checks repeated twice in each container. The cultivars CSH-1, (hybrid) and IS-1037 (local cultivar) were used as check entries. Seeds were sown at a 35 mm depth. The soil in each container was brought to field capacity by applying water uniformly at a rate equivalent to 40 mm of rainfall. No further irrigation was applied until the seedlings showed severe wilting. Two or three days after emergence, plants were thinned to 15 per entry, 35 mm apart in a row length of 50 cm. Seedlings were allowed to grow under an increasing soil moisture deficit.

Plant traits recorded were visual scores for wilting of leaves (before release of stress) and recovery from stress, and percent of plants recovered (after release of stress). The visual score range was determined on the basis of an initial survey of all the plots. For severity of wilting of seedlings, the score was given prior to rewatering and was on a 1-5 scale (1 = least wilted, 3 = intermediate, 5 = most wilted). Scoring was on the basis of number of leaves of an entry in a plot showing complete wilting and the degree of dessication. Stress was released when many of the lines had wilted and 50% had little chance of recovery (judged visually). Twenty-four hours after the release of stress, a visual score was given for the ability to recover, based on the extent of unrolling and level of regained turgidity of leaves (1 = leaves fully expanded, 3 = intermediate 5 = leaves least expanded). A second score of recovery ability was given five days later. Finally, the survival percentage of the initial stands for each entry was calculated after the release of stress. The entries showing minimum wilting, maximum recovery and maximum survival percentage were considered resistant genotypes.

Effects of the container on these plant indices were estimated as the difference between the mean of the checks within a container and the mean of checks across all containers. The test entries were adjusted by subtracting these container effects. The adjusted values were then subject to analysis of variance to assess the resistance of susceptibility of the test entries (breeding or germplasm lines).

Experiment 1: One hundred three breeding lines (F4 – F6s) were sown in each brick container on 2 February 1981. Only 10 test entries together with two checks were sown in single row plots. The plants were rewatered to field capacity, 25 days after sowing.

Experiment 2: One hundred germplasm lines (IS lines-international sorghum germplasm) with two checks were sown in a brick container on 7 March 1981. Three days after emergence, plants were thinned to 15 per plot. The plants were rewatered to field capacity 43 days after sowing.

Screening in PVC cylinders. Screening was carried out in a temperature-controlled glass house (temperature ranging from 35-40°C). Polyvinyl chloride cylinders (PVC) (400 mm length with an internal diameter of 105 mm), closed at the bottom with acrylic sheet in which six holes were made for drainage, were used. The tubes were filled with 440 g of soil, type Alfisol. The experimental design used was a randomised complete block design (RCB) with three replicates. Twelve seeds of each genotype were sown at a depth of 35 mm in each cylinder, and water equivalent to 50 mm of rainfall was applied to each cylinder, forming a descending gradient of moisture content down the cylinder. No further water was applied until the test entries showed severe wilting symptoms. Twenty-four hours after emergence, seedlings were thinned to six in each cylinder. Data on visual scores for wilting and recovery and the percentage of recovered plants were recorded using methods described earlier. Each line was represented by six seedlings in each cylinder. Two experiments were conducted:

Experiment 1: Seventy-four advanced breeding lines used in drought screening experiments at ICRISAT were grown with three replications on 13 September 1980. The stress was released 13 days after emergence when the plants in all plots showed severe wilting.

Experiment 2: Sixty-five germplasm lines were sown in three replications on 22 January 1981. The stress was released 13 days after emergence.

Field trials. Two field trials were conducted on a flat field, soil type Alfisol, prepared to a fine tilth. A randomised block design was used with two checks in each block and with three replications. Water was applied equivalent to 60 mm rainfall with sprinkler irrigations (perfo-sprayers) the day after sowing and there was no further irrigation. Visual scores for wilting and recovery and plant height and seedling dry weight (10 seedlings) were recorded. The plants grew in a regime of declining soil moisture over time.

Experiment 1: One hundred three germplasm lines were sown in a flat seed bed with two replications. The cultivars CSH1 and IS 1037, a released hybrid and a local cultivar respectively, were used as checks in each block. Single row plots of 100 cm length with 30 cm between row spacing were used. Each row had

15 plants with 6.5 cm spacing. Soil samples were collected for gravimetric soil moisture determination with a soil sampling tube at 29 and 54 days after sowing from depths of 0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm. Plant height (at 39 days after planting), wilting score and recovery score were recorded.

Experiment 2: One hundred nine germplasm lines were planted in three replications on a flat seed bed of soil, type Alfisol, on 14 February 1980. The lines were sown in a randomised complete block design with three replications. Single row plots of 1.4 m length with a 30 cm between-row spacing were used. Plants were spaced at 5.5 cm within the row. Plant height (at 35 days after planting), seedling dry weight (at 35 days after planting), and visual scores for wilting and recovery were recorded.

Although the genotypes evaluated by different methods were different sets of genotypes, comparison among methods have been made on the basis of responses of common genotypes (16) used in different techniques.

RESULTS AND DISCUSSION

Genetic variation

Analysis of variance revealed that genotypes showed significant differences in visual scores for wilting, recovery score and percent recovery, both in brick containers and PVC cylinders (Tables 1 and 2). In the field experiments, genotypes also showed significant differences in visual scores for wilting and recovery, and in plant height and dry weight. The present study establishes genetic variability for resistance to water stress in sorghum and thus resembles findings of other researchers in sorghum and other crops (3, 8, 11) (Tables 1 and 2).

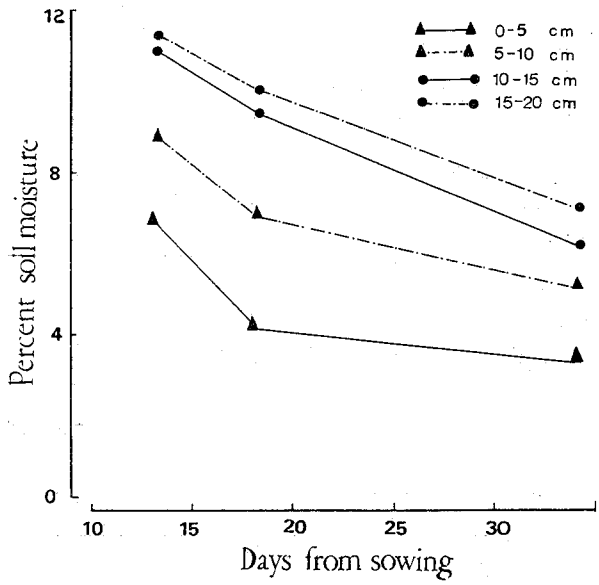

Fig. 1. Soil moisture depletion pattern at different depths.

Table 1. Mean, range and coefficient of variation (CV) for different seedling drought parameters measured in various experiments on sorghum breeding lines (Experiment 1) and germplasm lines (Experiment 2).

Experiments		Mean	Range	CV%
1. Brick containers				
Expet. 1.	a) Wilt score	3.0	1–5	23.0
	b) Recovery score	3.3	1–5	21.6
	c) Number of recovered plants	6.0	0.7–12.3	40.8
Expt. 2.	a) Wilt score	3.4	1.3–4.7	18.0
	b) Recovery score	3.2	1–5	17.8
2. PVC cylinders				
Expt. 1.	a) Wilt score	3.0	1.7–4.7	12.8
	b) Recovery score	3.0	1–5	14.4
	c) Number of recovered plants	3.1	0–6	20.9
Expt. 2.	a) Wilt score	2.8	1–5	17.4
	b) Recovery score	2.8	1–5	26.3
	c) Number of recovered plants	3.4	0–6	30.4
3. Field				
Expt. 1.	a) Plant height cm	11.7	3.8–23.5	23.2
	b) Wilt score	3.5	1–5	22.3
	c) Recovery score	3.2	1–5	21.7
Expt. 2.	a) Plant height, cm	25.9	15.5–37.3	8.3
	b) Wilt score, 45 days	3.3	1.3–5.0	18.5
	c) Recovery score	3.2	1–5	19.0
	d) Seedling dry wt, g	1.9	0.8–4.2	22.8

Table 2. Variance ratios for genotypic differences among sorghum breeding lines (Experiment 1) and germplasm lines (Experiment 2) for different drought parameters on seedings in various experiments conducted.

	Expt. 1	Expt. 2
1. Brick containers		
a) Wilt score	1.17(NS)	1.43**
b) Recovery score	1.65**	1.96**
c) Recovered plants (number)	1.68**	–
2. PVC cylinders		
a) Wilt score	1.98**	3.10**
b) Recovery score	1.73**	6.25**
c) Recovered plants (number)	1.94**	6.70**
3. Field		
a) Plant height (cm)	1.99**	2.69**
b) Wilt score	1.95**	1.98**
c) Recovery score	1.68**	2.12**
d) Seedling dry weight (g)	–	1.81**

NS Not significant.

** Singificant at 1% probability level.

Comparison of different techniques in screening sorghum for drought resistance

Brick containers and PVC cylinders have the advantage of being reasonably controlled environments, unlike the field, where weather conditions vary and may affect the experiment.

Inter-cultivar competition was eliminated in PVC cylinders, while in the brick containers individual lines were grown in competition with neighbouring lines. In this system, susceptible lines wilt at an early stage compared to resistant lines, and this may be accented by competitive interaction among neighbouring lines. Therefore, it is difficult to assess drought tolerance in brick containers due to competition, where as in PVC cylinders the lines are isolated and the true capability of a line to tolerate water stress and recover with the release of stress can be easily assessed. The coefficient of variation (CV%) was higher in brick containers and field techniques probably because of competition and field variability in soil water distribution, but lower in PVC cylinders with less scope for variation in soil water and no intercultivar competition (Table 1). Susceptible sorghum lines can easily be rejected using the PVC cylinder technique. Uniform levelling of soil in the brick containers and field to prevent localized water accumulation and the use of checks in each plot are useful ways of reducing local effects. It is necessary to ensure uniform application of water in field experiments by perfo-sprayers with suitable adjustament of perfo-lines, or by other suitable methods.

Relationship between drought response traits and probable selection criteria

When eight common lines have been selected from different techniques and correlations have been made among the drought parameters (Table 3) observed within an experiment, the seedling drought parameters generally showed highly significant correlations among themselves ($P < 0.01$) (Table 3). For example, in the PVC cylinders the correlations between the visual score for wilting and recovery score and recovery percent of plants were high ($r = 0.90^{**}$ and 0.90^{**} respectively; $P < 0.01$). In the brick container method, visual scores for wilting showed a positive correlation with recovery scores ($r = 0.87^{**}$). Unfortunately, the sets of genotypes used in different techniques are different, thus not allowing us to compare their response in different techniques. However, rank correlations were calculated among eight lines common to all screening methods. Recovery scores of lines in PVC cylinders showed a significant correlation with the recovery score of these lines in the field ($r = 0.80^{**}$, $P < 0.01$), but recovery scores of lines in brick containers did not show a significant correlation with those observed in the field ($r = 0.40^{*}$) Table 3).

The high degree of association among recovery scores and number of recovered plants can be considered as reliable indices in selecting genotypes for seedling drought resistance in the field techniques, brick containers and the PVC cylinder ($r = 0.99^{**}$, 0.99^{**}).

Table 3. Significant correlations between different drought resistance parameters ($P < 0.01$) observed in sorghum seedlings.

	Brick container	PVC cylinder	Field
Wilting score vs. recovery score	0.87	0.90	0.61
Wilting score vs. recovered plants (number)	0.66	0.90	
Recovery score vs. recovered plants (number)	0.95	0.99	

During the course of the experiments, several germplasm and breeder lines were selected for a high level of drought resistance at the seedling stage. These were: (germplasm) IS 2146, IS 5604, IS 3962, IS 1096, IS 4664 and IS 5642; (breeding) D-71914 m, D-71914, D-71939, IS 7389, IS 6834, D-71873, D-71824 and BG-74.

CONCLUSION

The techniques described, although they have room for improvement, are simple and capable of being used to screen a large number of gentypes for resistance to water stress at the seedling stage. Genotypes differed significantly in resistance to water stress at the seedling stage. Screening in PVC cylinders, with their uniform soil water distribution, seemed more efficient than screening in brick containers and in the field. Germplasm selected either on the basis of good agronomic traits or selected at random could be screened first in brick containers and then a final assessment of different lines could be made in PVC cylinders. The performance of the lines selected needs to be further tested under drought-prone situations in the field. Eventually, lines with good agronomic traits will be available to the researchers as sources for these traits.

LITERATURE CITED

1. DOUGLAS, A.J.; ASSAY, R.H. 1978. A technique in assessing seedling emergence under drought stress. Crop Science 18:520-522.

2. HEYNE, E.G.; BRUNSON, A.M. 1940. Genetic studies of heat and drought tolerance in maize. Journal of the American Society of Agronomy 32:803-814.

3. HEYNE, E.G.; LAUDE, H.H. 1940. Resistance of corn seedlings to high temperatures in laboratory tests. Journal of the American Society of Agronomy 32:116-126.

4. HSIAO, T.C.; ACEVEDO, E. 1974. Plant responses to water deficits, water use efficiency and drought resistance. Agricultural Meteorology 14:59-84.

5. NOUR, A.E.M.; WEIBEL, E.E.; TODD, G.W. 1978. Effect of repeated drought periods on the survival of sorghum seedlings. Agronomy Journal 70:509-510.

6. POWELL, L.M.; PFEIFER, R.P. 1956. The effect of controlled limited moisture on seedling growth of Cheyenne winter wheat selections. Agronomy Journal 48:555-557.

7. SAMMONS, D.J.; PETERS, D.B.; HYMOWITZ, T. 1978. Screening soybeans for drought resistance. 1. Growth chamber procedure. Crop Science 18: 1050-1055.

8. SAMMONS, D.J.; PETERS, D.B.; HYMOWITZ, T. 1979. Screening soybeans for drought resistance; Drought box procedure. Crop Science 19:719-721.

9. SHARMA, M.L. 1976. Interaction of water potential and temperature effects on germination of three semi-arid plant species. Agronomy Journal 68: 390-394.

10. WILLIAMS, T.V.; SNELL, R.S.; ELLIS, J.F. 1967. Methods of measuring drough tolerance in corn. Crop Science 7:278-285.

11. WILSON, G.L.; RAJU, P.S.; PEACOCK, J.M. 1982. Effect of soil temperature on seedling emergence in sorghum. Journal of Agricultural Science 52: 848-851.