Published September, 1984

Stress Tolerance in Soybeans. I. Evaluation of Three Screening Techniques for Heat and Drought Tolerance[1]

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ABSTRACT

Three screening techniques for characterizing drought tolerance were evaluated using 20 soybean [*Glycine max* (L.) Merr.] genotypes. These techniques involved: 1) germinating seed in polyethylene glycol-600 (PEG) at −0.6 MPa osmotic pressure, 2) subjecting seedlings to PEG-600 at −0.6 MPa osmotic pressure in hydroponic solution for 14 days, and 3) a heat tolerance test based on the cellular membrane thermostability. Genotypic variability associated with drought tolerance was demonstrated by the evaluation techniques. Based on these procedures, 2 of the 20 cultivars tested were relatively drought tolerant, 2 were relatively susceptible, and the remaining cultivars fell into an intermediate group. Highly significant correlations were found between the hydroponic seedling and heat tolerance tests, indicating that both drought and heat tolerance were identified in the same cultivars under the conditions of this study. It is doubtful that the seed germination test could be used as a reliable procedure for identifying drought tolerant cultivars because it tended to reflect differences in seed quality and had no relation to field performance. Although reproducibility and consistency of genotypic differences did exist using the heat tolerance tests, comparisons with more yield data are needed to evaluate this technique. Of the three procedures evaluated in this study, the hydroponic seedling test seemed to be the most reliable and potentially useful as a means for screening for drought tolerance in soybeans.

***Additional index words:* *Glycine max* (L.) Merr., Drought tolerance, Genetic variability, Polyethylene glycol, Hydroponics, Cellular membrane thermostability.**

WATER deficits and high temperatures are among the most important environmental factors that limit crop productivity in many areas of the world. The lack of stability in soybean production because of variable climatic conditions has indicated a need to develop methods to improve this crop genetically through breeding cultivars capable of withstanding environmental stress.

Breeding for drought resistance has been accomplished by selecting for seed yield under field conditions (12, 14), but since such procedures require full season field data it is not always an efficient approach, especially in mesic locations (14). An alternative may be to screen material under laboratory or greenhouse conditions using seedlings as test material (14). Evidence has been presented that alfalfa (*Medicago sativa* L.) accessions that emerge at −0.65 MPa osmotic pressure (O.P.) in the laboratory show a better field emergence and survival under drought stress than do accessions that cannot emerge under stress in the laboratory (13). Several physiological characteristics in crops have been reported as being reliable indicators for the selection of plant germplasm possessing drought and heat tolerance. These characteristics include seed germination and seedling growth in hydroponic solutions of low osmotic potential (2, 11, 15, 16, 17, 19) and heat tolerance measured by the degree of electrolyte leakage from heat damaged leaf cells after exposure to elevated temperatures (1, 10, 17, 18). Heat and drought tolerance are frequently combined into one term without distinctions between the two traits (18). Kilen and Andrew (8) concluded that heat testing of seedlings may be of value in screening for drought resistance in corn (*Zea mays* L.), and several other researchers have used heat stress to select for drought resistance (6, 7, 8, 17). Plants with good drought resistance are usually heat tolerant (9). Correlation between heat and desiccation tolerance has been found, but not always (18).

The success of these approaches will require evidence that the drought tolerance of cultivars tested under laboratory conditions reflects drought tolerance under field conditions (14). It has been found that significant correlations existed between field and laboratory data in corn (8).

The objectives of this study were to: 1) evaluate the genetic variability among 20 soybean cultivars subjected to drought stress and 2) determine whether these parameters are effective criteria to select for drought and heat tolerance.

MATERIALS AND METHODS

Twenty soybean cultivars, differing in growth habits, in Maturity Groups III, IV and V were chosen for this study (Table 1).

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Table 1. Soybean cultivars tested for heat and moisture tolerance.

	Maturity group		
Growth habit	III	IV	V
Indeterminate	Calland Cumberland Williams 79	C1573 Crawford Cutler 71 DeSoto Douglas	
Determinate	Elf Hobbit K74-108	K1048 K1049 Pixie V76-398 V76-482	Bedford Dare Essex Forrest

I. Seed Germination Test

Twenty seeds of each soybean cultivar were germinated in polyethylene glycol with a molecular weight of 600 (PEG-600) at −0.6 MPa O.P. Aqueous solutions of 0 and 0.6 MPa O.P. were obtained by dissolving 0 and 75 mL of polyethylene glycol with a molecular weight of 600 (PEG-600) at −0.6 MPa O.P. Aqueous solutions of 0 and −0.6 MPa O.P. were obtained by dissolving 0 and 75 mL of polyethylene glycol 600 in 1 L of nutrient solution, respectively (C.Y. Sullivan, unpublished data). Seeds were placed on two layers of Whatman no. 2 filter paper in 20 × 140 mm glass petri dishes and 25 mL of 0 or −0.6 MPa O.P. of solution were added. Seeds used were produced during 1980 and 1981 at the Kansas State University Ashland Agronomy Farm, Manhattan, KS. Normal temperature and rainfall based on records for 30 years from May to September are 22.9°C and 56.0 cm, respectively. The 1980 average temperature from May to September was 25.2°C and ranged from 38.5 to 11.1°C with a rainfall of 28.2 cm while in 1981 the average temperature was 22.7°C and ranged from 31.2 to 11.0°C with a rainfall of 57 cm. Four replicates (petri dishes) of each treatment were placed at random in a growth chamber for 8 days at day/night temperatures of 25/20°C at 50% relative humidity. Artificial light of approximately 260 μmol $m^{-2}s^{-1}$ provided a 12-h photoperiod. Germination was recorded when the radicle reached 3 mm in length. Results were expressed in terms of a promptness index (P.I.) as described by George (5). To consider the slow germination of some seeds, the P.I. was modified for soybeans and calculated as follows:

$$\text{P.I.} = nd_2\,(1.00) + nd_4\,(0.75) + nd_6\,(0.50) + nd_8\,(0.25)\,,$$

where:

P.I. = promptness index

nd_2, nd_4, nd_6, and nd_8 = percent of seeds observed to germinate on the 2nd, 4th, 6th, and 8th day of observation, respectively. A germination stress index (GSI) was expressed in percent as follows:

$$\frac{\text{Promptness index of stressed seeds (PIS)}}{\text{Promptness index of control seeds (PIC)}} \times 100$$

These data were analyzed as a 20 × 2 factorial in a completely randomized design with 4 replications.

II. Hydroponic Seedling Test

Seedlings at the cotyledon (VC) stage of development (4) were transferred to 2-L black plastic pots 16 cm in diameter where they could be grown hydroponically in a Conviron Model E15 growth chamber with a growth height of 150 cm. Growth chamber day/night temperatures were maintained at 25/20°C with a 14-h photoperiod of light from fluorescent and incandescent bulbs mounted 75 cm above the plant canopy, resulting in a photosynthetic photon flux density of 260 μmol $m^{-2}s^{-1}$. Relative humidity was maintained at 50%. The pots were covered with plastic tops having six holes, 1.2 cm in diameter and spaced 3.5 cm apart. The radicle was placed in the hole when the seedling was transplanted. Each plant was supported by a cork and glass wool. All seedlings were grown in Hoagland's nutrient solution for 1 week for a preconditioning period. After 1 week the −0.6 MPa osmotic potential treatment was initiated using PEG-600 as the osmoticum. Solutions were changed every seven days. The pH (5.8–6.0) was checked frequently and distilled water was added daily when needed. The seedlings were subjected to PEG for 14 days. Control plants were maintained in Hoagland's nutrient solution for the same period of time and aerated throughout the duration of the experiment. Six plants (two stress levels × three replications) of each cultivar were treated for a total of 3 weeks. Plants were then removed from the growth chamber for the following measurements: 1) plant height (cm) and 2) weight of shoots and leaves (g) dried at 70°C for 48 h. Stress indices were calculated as follows:

$$\frac{\text{Dry matter of stressed seedlings (DMS)}}{\text{Dry matter of control seedlings (DMC)}} \times 100$$
$$= \text{Dry matter stress index (DMSI)}$$

or

$$\frac{\text{Plant height of stressed seedlings (PHS)}}{\text{Plant height of control seedlings (PHC)}} \times 100$$
$$= \text{Plant height stress index (PHSI)}$$

Dry matter and plant height data were analyzed as 20 × 2 factorials in a completely randomized design with three replications, repeated three times.

Heat Tolerance

The technique used for measuring heat tolerance of leaf tissue was as described for sorghum (17) and soybeans (10) as modified by Dexter (3). The leaf tissue sampled in the experiment came from plants grown at the Kansas State University, Ashland Agronomy Farm. Twenty genotypes were planted randomly in 4-row plots, 6.1 m long with a 76 cm row spacing. Plots were grown under dryland conditions in 1980 and 1981. Leaf samples were taken at weekly intervals during the season from the vegetative growth stage, V5, to reproductive growth stage R2 (4). However, because of the consistency and the significant cultivar difference found at the R_1 stage for the 2 years, only data collected at the R_1 stage has been considered. Twelve leaflets were obtained from fully expanded trifoliolates in the upper portion of the canopy from random plants in adjacent plots of twenty soybean cultivars. For each cultivar the 12 leaflet samples were randomly divided into three 4-leaflet groups. Leaf discs were cut from 12 leaflets of each cultivar with a 12 mm diam cork borer. From each 4-leaflet group, 14 leaf discs were placed into a 16 × 150 mm test tube. Two of the test tubes or samples were used for duplicate temperature treatments and the third one served as the control. Leaf discs were washed several times with deionized distilled water, distributed to a series of test tubes, then placed in a temperature regulated water bath at 50°C for 15 min while the control was maintained at 25°C. The tubes were then removed from the water bath and 30 mL of deionized distilled water were added to each and then they were incubated at 10°C for 18 h. Then, initial conductance readings were made at 25°C with the use of a Yellow Springs Instrument Company Model 31 conductivity bridge and

Model 3403 conductivity cell. The tubes were then autoclaved at 110°C, with 1.4 kg pressure for 10 min. Final conductance measurements were taken after all tubes were cooled to 25°C. Percent thermoinstability was calculated by the following formula:

$$\text{Percent Thermoinstability} = 1 - \frac{1 - (T_1/T_2)}{1 - (C_1/C_2)} \times 100\,,$$

where T and C refer to the conductivity of mean treatment and control tubes, respectively, and the subscripts one and two of T and C refer to initial and final conductance, respectively. The experimental design consisted of 20 cultivars completely randomized with three replications, with duplicate samples per lot.

To evaluate these parameters as selection criteria they were compared to seed yield performance data of seven cultivars summarized from the Kansas soybean performance tests from 1978 through 1981 at 10 locations, 5 of which were irrigated tests and 5 dryland tests. Only 7 of the 20 cultivars were used in this comparison because they were tested in all 10 environments. The remaining 13 cultivars were not tested at 1 or more of the 10 environments. To establish a stress index or yield stability estimate based on yield data, a dryland to irrigation yield ratio was calculated for each cultivar.

RESULTS AND DISCUSSION

Seed Germination Test

The cultivars differed significantly in the germination stress index (Table 2). A significant interaction between years and cultivars was not observed in the germination stress index (GSI), thus results are presented as 2-year averages (Table 2). A highly significant year effect on GSI was identified due to the climatic conditions experienced in 1980 (hot and dry summer) and 1981 (wet and relatively cool summer), which resulted in the seed harvested in 1981 to be superior in quality to the 1980 seedlots. The GSI over the 2 years ranged from a high of 87% (for 'Forrest') to a low of 51% (for 'Douglas').

Hydroponic Seedling Test

Cultivar differences for both dry matter accumulation and plant height were detected when seedlings were treated with PEG-600. No date by cultivar interaction was seen across the three dates of testing, so results were presented as averages (Table 3). Dry matter stress indices ranged from 92% in 'Essex' to 34% in 'Cutler 71' with dry matter of the stressed plants ranging from 1.48 g in K1049 to 0.87 g in 'Pixie' (data for latter not shown). Plant height stress indices ranged from 74% in both Essex and Forrest to 39% in Cutler 71 with plant height of the stressed plants ranging from 45 cm in Forrest to 23 cm in Cutler 71 (data not shown).

This technique identified the cultivars Essex and Forrest as relatively stress tolerant, while Cutler 71, K74–108 and 'Crawford' were stress susceptible. The remaining cultivars fell into an intermediate group.

Heat Tolerance

A preliminary test subjecting leaf discs from two soybean cultivars, Essex and Cutler 71, to various levels of temperatures was performed to determine the standard heat treatment to be used to evaluate all 20 cultivars. A sigmoidal response curve was found in these two cultivars with a maximum cultivar separation at 50°C (Fig. 1). Similar findings have been reported in sorghum (17), soybean (10), and wheat (1). Although weekly samples were taken from V5 to R2, the evaluation of the effect of plant stage of development on injury indicated that the maximum significant cultivar separation occurred at the flowering initiation stage of R_1 (4). Consequently, this stage was used to assess the relative heat tolerance in both growing seasons.

No year by cultivar interaction was found in percent thermoinstability, so results are presented as averages of the 2 years (Table 4). For the 20 cultivars tested by this technique cultivar thermoinstability

Table 2. Germination stress index of soybean seedlings germinated for 8 days in PEG-600 at −0.6 MPa osmotic pressure.

Cultivar	Germination stress index 1980–1981	Rank
	%	
Forrest	87 a†	1
Essex	85 ab	2
Williams 79	82 a-c	3
Elf	81 a-d	4
DeSoto	80 a-d	5
Pixie	80 a-d	5
Bedford	74 a-e	7
Dare	73 a-e	8
Crawford	72 a-e	9
Cumberland	71 a-f	10
V76-398	68 a-f	11
K1048	68 a-f	11
Hobbit	66 b-f	13
V76-482	64 c-f	14
K74-108	62 d-f	15
Calland	60 ef	16
C1573	56 ef	17
Cutler 71	56 ef	17
K1049	55 ef	19
Douglas	51 f	20

† Means followed by the same letter are not significantly different at the probability level of 0.05 according to Duncan's Multiple Range Test.

Table 3. Dry matter and plant height stress indices of 20 soybean cultivars subjected to 14 days of water stress in PEG-600 at −0.6 MPa osmotic pressure, averaged over 3 experiments.

Cultivar	Dry matter stress index		Plant height stress index	
	%	Rank	%	Rank
Essex	92 a†	1	74 a	1
Forrest	73 b	2	74 a	1
Elf	68 bc	3	59 b	3
V76-482	61 b-d	4	54 b	6
K1049	59 b-d	5	55 b	4
Bedford	59 b-d	5	52 bc	12
Douglas	56 cd	7	54 b	6
C1573	55 cd	8	51 bc	13
Williams 79	55 cd	8	49 bc	15
K1048	47 de	10	55 b	4
Cumberland	47 de	10	47 bc	17
DeSoto	46 de	12	51 bc	13
Calland	46 de	12	48 bc	16
Dare	46 de	12	53 b	9
V76-398	46 de	12	53 b	9
Hobbit	40 e	16	54 b	6
Crawford	39 e	17	47 bc	17
Pixie	38 e	18	53 bc	9
K74-108	36 e	19	47 bc	17
Cutler 71	34 e	20	39 c	20

† Means followed by the same letter within a column are not significantly different at the probability level of 0.05 according to Duncan's Multiple Range Test.

Table 4. Leaf tissue injury induced by heat in 20 soybean cultivars.

Cultivar	Thermoinstability 1980–1981	
	%	Rank
Essex	44 a†	1
Forrest	46 a	2
Elf	64 b	3
V76-398	66 bc	4
Calland	66 b-d	4
DeSoto	67 b-e	6
Williams 79	67 b-e	6
Dare	69 b-e	8
Hobbit	70 b-e	9
C1573	71 b-e	10
V76-482	71 b-e	10
K1048	71 b-e	10
K74-108	72 c-e	13
Douglas	74 c-e	14
Crawford	74 c-e	14
K1049	75 de	16
Cutler 71	75 e	16
Pixie	75 e	16
Cumberland	75 e	16
Bedford	75 e	16

† Means followed by the same letter are not significantly different at the probability level of 0.05 according to Duncan's Multiple Range Test.

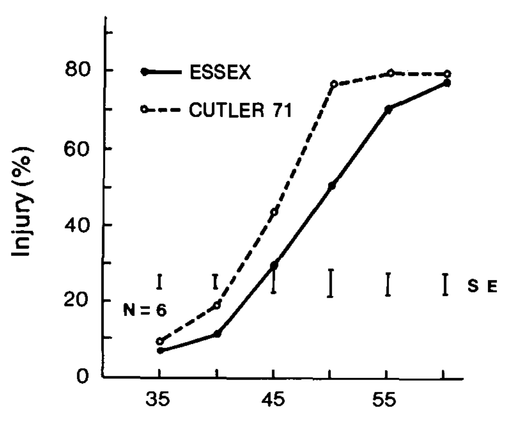

Fig. 1. Effect of temperature on inducing injury on leaf tissue of two soybean cultivars.

Table 5. Correlations among four stress indices in soybeans.

	Dry matter stress index	Plant height stress index	Heat tolerance
Germination stress index	0.41	0.53*	−0.59**
Dry matter stress index		0.85**	−0.74**
Plant height stress index			−0.85**

*,** Significant at 0.05 and 0.01 level of probability, respectively.

Table 6. Correlations between stress indices and stress parameters in soybeans.

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Germination stress index vs. promptness index	0.91**
Dry matter stress index vs. dry matter stress	0.60**
Plant height stress index vs. plant height stress	0.87**

** Indicates significance at the 0.01 level of probability.

Table 7. Yield and stability (dryland/irrigated) ratio of 7 cultivars from 10 locations in Kansas from 1978 through 1981.

Cultivar	Yield Dryland	Yield Irrigated	Stability ratio	Rank
	kg/ha	kg/ha	%	
Elf	1888	2406	78	1
Douglas	2426	3333	73	2
Williams	2137	2997	71	3
Cumberland	2238	3158	71	3
Crawford	2043	2910	70	5
Calland	2137	3165	67	6
DeSoto	2164	3320	65	7

Table 8. Correlations of the stress criteria with seed yield and the dryland/irrigated stability ratio of seven cultivars.

	Dryland/ irrigated yield	Yield Dry	Yield Irrigated	Yield Mean
Germination stress index	0.07	−0.70	−0.52	−0.59
Dry matter stress index	0.79*	−0.21	−0.55	−0.46
Dry matter stress	0.56	−0.14	−0.41	−0.32
Plant height stress index	0.70†	−0.22	−0.50	−0.42
Plant height stress	0.27	−0.62	−0.55	−0.59
Heat tolerance	−0.03	0.56	0.40	0.42

†,* Indicates significance at 0.1 and 0.05 levels of probability, respectively.

ranged from a low of 44% in Essex to a high of 75% in K1049, Cutler 71, Pixie, 'Cumberland', and 'Bedford' (Table 4). There was a highly significant year effect, as was observed with seed germination test.

Correlations among the stress parameters were significant, except for the relationship between GSI and DMSI (Table 5). The significant correlations between heat tolerance and drought stress noted here have been observed in sorghum [*Sorghum bicolor* (L.) Moench.] (17), but not in wheat (*Triticum aestivum* L.) (1). It is possible that the heat tolerance test could be used in soybeans to select for drought and moisture stress tolerance, as was the case for corn, pasture grasses and sorghum (6, 7, 8, 17). Correlations between the stress indices and the actual stress measurements were found to be highly significant for each characteristic (Table 6), indicating that the control treatments could possibly be eliminated and permit a larger number of cultivars to be evaluated.

To examine the relationships between the stress indices and grain yield in different environments, a yield stability index was calculated for seven cultivars (Table 7). The index was calculated by dividing the mean dryland yield by the irrigated yield for each cultivar from 10 test environments (5 irrigated and 5 dryland trials). 'Elf' had the lowest yield under dry and irrigated conditions, but it had the greatest yield stability. 'DeSoto' had the largest difference between seed yield under dry and irrigated conditions, and hence the lowest yield index for stability (Table 7). The yield ratio was significantly correlated with the DMSI and PHSI (Table 8). None of the other stress indices were correlated with the yield stability estimates. Thus, the seedling growth procedure used under the conditions of this experiment appeared to be useful to screen a larger number of cultivars for relative drought or moisture stress tolerance. No significant correlations existed between the stress parameters and either dryland, irrigated, or mean yield (Table 8).

According to the data collected in this study, cultivar differences in germination, seedling growth and heat tolerance tests were evident. However, not all drought stress treatments appeared to be equally useful for screening soybean cultivars for sensitivity to stress. The germination test did not seem to reflect stress tolerance response in soybeans, but rather seed quality differences, nor did this procedure reflect the yield stability of the cultivars. Similar findings for wheat (2) confirm that tolerance to water stress cannot be predicted from germination tests under moisture stress. However, there are other reports (11, 19) indicating that this procedure could be useful in screening for drought tolerance.

Although several factors influence seed quality, such as the age of the seed, environmental conditions during growth and development, harvest and storage conditions, etc., the genotypes may respond differently to these factors which could be reflected in their respective seed quality scores. Thus, the seed germination test may be useful to identify vigorous seedlots and genotypes capable of establishing adequate populations under low soil moisture conditions but these genetic differences may be unrelated to subsequent seedling growth and seed yield.

The repeatability of cultivar differences revealed by the heat tolerance test implied that such a technique may be effective in screening soybean germplasm for relative moisture stress tolerance, even though the results of such a test and the yield stability data were not correlated. The low correlation might be due to the lack of available yield data for all 20 cultivars tested. The low correlation might also have resulted from differences in avoidance mechanisms the plants used in reaction to the laboratory tests vs. the field environment.

The reproducibility and consistency of the cultivar differences determined by the hydroponic seedling procedure, plus the positive correlation between dry matter accumulation, plant height and the yield stability data suggest that the hydroponic seedling test could be a reliable and efficient procedure for screening for moisture stress tolerant soybean germplasm. This finding was in agreement with Blum et al. (2) who concluded that PEG-containing solutions can be used to screen for tolerance to water stress in wheat seedlings.

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