CHAPTER VI

Use of a Heat-Shock Stress Test to Predict Field Emergence of Sorghum

Introduction

The poor ability of a standard germination test to predict field emergence of sorghum has been demonstrated by Camargo and Vaughan (1973), Vanderlip et al. (1973), and Ahmed (1977) and in Chapter IV. Such laboratory germination tests apparently do not (by design) place on the seeds stresses that they will likely encounter in the field. Stress (or vigor) tests are intended to do this.

A brief high-temperature stress (heat shock) could be useful as a practical index of seed vigor according to Van de Venter and Lock (1992). The heat shock presumably creates a greater strain on less vigorous seeds and reduces their performance more than it does for more vigorous seeds. The objective of this research is to study the effect of heat-shock treatment on seeds of sorghum of several hybrids. Parameters to be examined following heat shock will be germination, radicle length, and fresh weight of seedlings. Data from the laboratory studies will be compared with field emergence to determine if they can be used to predict performance of the seeds in the field.

Materials and Methods

The same hybrids used for the osmotic stress (Chapter V) and standard germination tests (Chapter IV, Table 4.1) were assigned to the heat-shock test. The same procedures were used as in the former tests to compose the seedlots and run the imbibition period. After the 24-hr imbibition period in distilled water in a Petri dish, the seeds were exposed to 50°C for 2 hr according to procedures of Van de Venter and Lock (1992). Following the heat shock, the seeds were spread on germination toweling moistened with 10 ml of distilled water. The toweling was folded and rolled. The ragdolls thus created were placed in zip-clousure plastic bags to retain their moisture, and the seeds were incubated for another 46 hr at 25°C in darkness. Thus there was a total of 70 hr of incubation at 25°C plus 2 hr of exposure to 50°C.

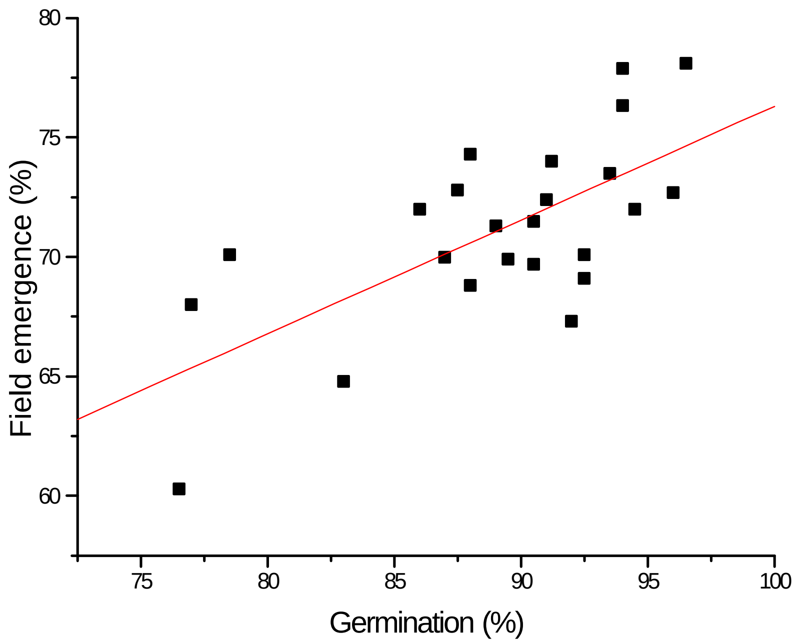
Three days (72 hr) after the initial incubation, radicle length, seedling fresh weight, and germination percentage were determined using procedures described previously (Chapter IV). Variance analyses were performed to test differences among hybrids in germination, radicle length, and seedling fresh weight. Seedling fresh weight was determined only for hybrids in the 1996 Virginia Sorghum Trials. Germination percentages were arcsine transformed prior to the ANOVA (Sokal and Rohlf, 1995). The three parameters from heat-shocked seeds were each regressed against arcsine transformed field emergence data (two locations) for each hybrid within each year. To determine if seedlots showed significant differences between years, the ANOVA contrasts were applied using SAS software (SAS Institute,1993). The sensitivity of each hybrid to the heat shock was estimated by calculating the percent reduction of each parameter in relation to the standard germination test, i.e., percent reduction = (standard - heat-shock / standard) x 100. The arcsine-transformed percent reduction was then regressed with field emergence. All arcsine-transformed data were back-transformed for reporting results.

Results and Discussion

Germination following heat-shock treatment for hybrids in the 1995 Virginia Sorghum Trials

Germination of the seeds varied among hybrids following the heat-shock treatment ($P< 0.001$). The top eleven hybrids were more highly germinable than KS936, XS345, and KS604 (Table 6.1). The general mean of this treatment was 78.5%, and the CV was 9.3%.

The field emergence was regressed on germination following heat shock. There was a linear association between variables, with a P value of 0.0002 and an $r^2= 0.476$. The distribution of the data is shown in a scatter diagram (Figure 6.1), and the emergence (y) can be predicted from arcsine transformed data by using the empirical equation $y = 0.8011 + 0.1871x$. For back-transformed data the predictive equation is $y = 28.68 + 0.476x$.



P< 0.0002 **r^2= 0.476** **y = 28.68 + 0.476x**.

Figure 6.1. Linear regression of field emergence and germination following heat-shock treatment for hybrids in the 1995 Virginia Sorghum Trials.

Radicle length of the seedlings following heat-shock treatment for hybrids in the 1995 Virginia Sorghum Trials.

Radicle length of the seedlings varied among hybrids (P = 0.02). The hybrid CAR775 produced longer radicles than P8446, KS936, X604, and XS345. The hybrid XS345 also had shorter radicles than several other hybrids (Table 6.2). The general mean of this treatment was 34.9 mm, and the CV was 21.7%.

There was not a linear association between field emergence and radicle length ($P = 0.059$ and $r^2 = 0.151$). The distribution of the data is shown in a scatter diagram (Figure 6.2).

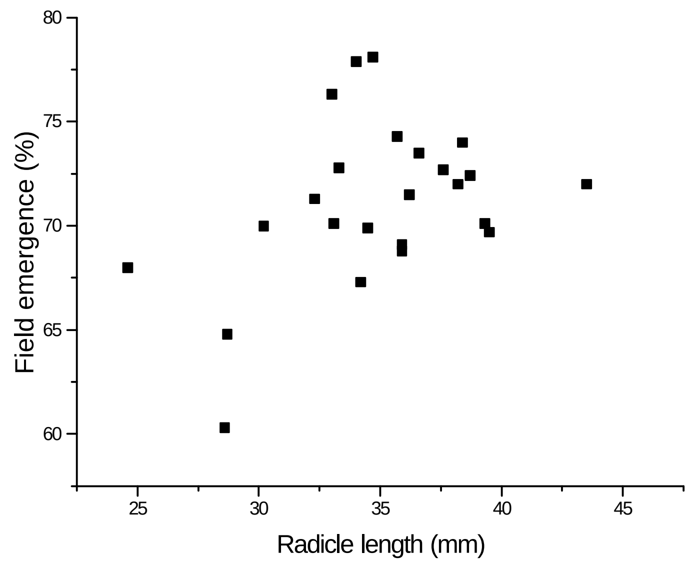


Figure 6.2. Linear regression (ns) of field emergence and average radicle length of 3-day-old seedlings following heat-shock treatment for hybrids in the 1995 Virginia Sorghum Trials.

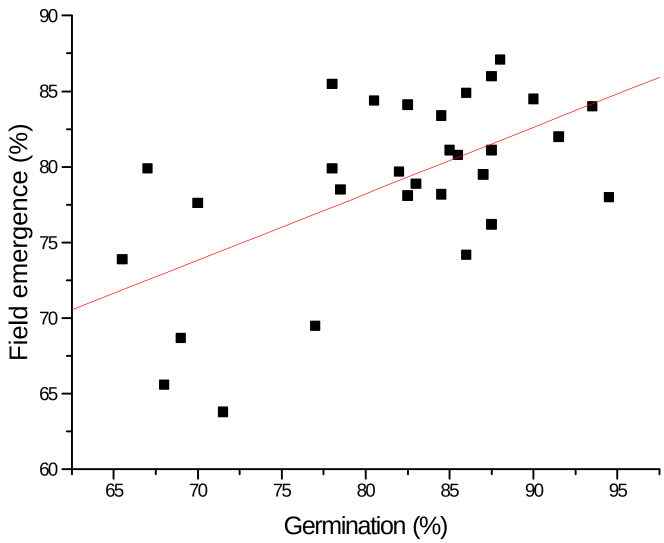
Table 6.1. Germination and radicle length of 3-day-old seedlings following heat-shock treatment of hybrids in the 1995 Virginia Sorghum Trials.

Hybrid	Germination		Radicle length	
	Value	Rank	Value	Rank
	%		mm	
CAR775	93.5 [a *]	1	43.5 [a]	1
SS160	89.0 [a b]	2	37.6 [a b]	7
GW1114	88.5 [a b]	3	36.2 [a b c]	9
SS115	86.5 [a b]	4	39.3 [a b]	3
CAR577	84.5 [a b]	5	38.2 [a b]	6
CAR837	84.0 [a b]	6	34.0 [a b c]	16
GW8046	83.5 [a b]	7	35.9 [a b c]	10
P8118	83.5 [a b]	8	33.3 [a b c]	17
P8212	82.5 [a b]	9	35.9 [a b c]	11
FFR321	82.0 [a b]	10	34.7 [a b c]	13
CAR1992	81.5 [a b]	11	39.5 [a b]	2
P8699	80.5 [a b c]	12	32.3 [a b c]	20
P8446	80.5 [a b c]	13	30.2 [b c]	21
CAR630	79.5 [a b c]	14	34.2 [a b]	15
KS714	79.5 [a b c d]	15	33.1 [a b c]	17
SS1211	78.0 [a b c d]	16	38.4 [a b]	5
SS1313	78.0 [a b c d]	17	36.6 [a b]	8
GW6089	77.5 [a b c d]	18	34.5 [a b c]	14
P8310	75.5 [b c d]	19	35.7 [a b c]	12
P8305	75.0 [b c d]	20	38.7 [a b]	4
CAR737	73.0 [b c d]	21	33.0 [a b c]	19
KS936	60.5 [c d e]	22	28.7 [b c]	22
XS345	58.0 [d e]	23	24.6 [c]	24
X604	50.5 [e]	24	28.6 [b c]	23
Average	78.5	-	34.9	-

* Means within a column followed by the same letter are not significantly different at 0.05 probability level.

Germination following heat-shock treatment for hybrids in the 1996 Virginia Sorghum Trials

A significant germination difference ($P<0.001$) between hybrids was observed following heat shock. The top three hybrids germinated at a higher percentage than the bottom six (Table 6.2). The general mean of this treatment was 80.7%, and the CV was 10.9%. The field emergence was regressed on percent of germination, and there was a linear association between these two variables ($P = 0.0005$ and $r^2 = 0.350$). A field emergence value can be estimated from arcsine-transformed data by using the equation $y = 0.600 + 0.443x$ (Figure 6.3). The predictive equation using percentage data is $y = 43.09 + 0.439x$.

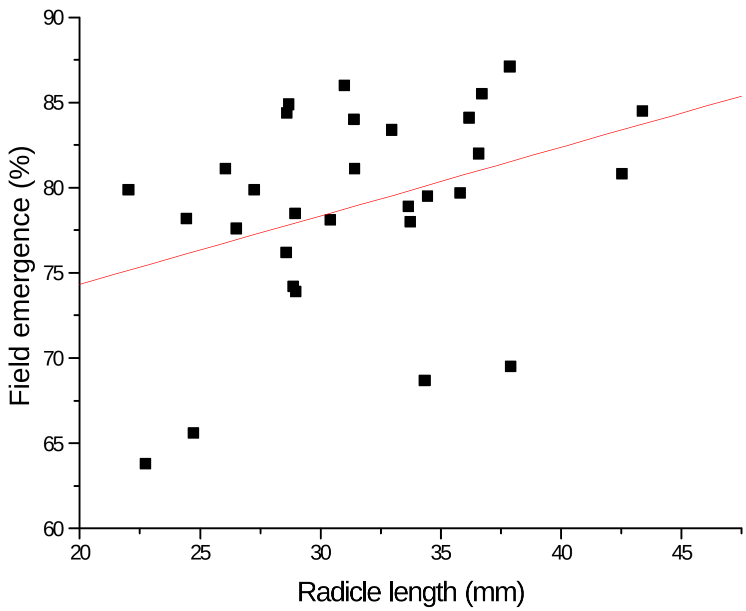


P< 0.0005 **$r^2 = 0.350$** **y = 43.09 + 0.439x**

Figure 6.3 Linear regression of field emergence and germination following heat-shock treatment for hybrids in the 1996 Virginia Sorghum Trials.

Radicle length of the seedlings following heat-shock treatment for hybrids in the 1996 Virginia Sorghum Trials

Radicle length of the seedlings varied among hybrids ($P = 0.042$), however significant differences among means could not be detected by Tukey's test. The general mean of this treatment was 31.0 mm, and the CV was 24.7%. When field emergence was regressed on radicle length, there was a significant linear association between variables,($P = 0.045$ and $r^2 = 0.134$) (Figure 6.4). A field emergence value can be estimated by using the equation $y = 66.29 + 0.401x$. A prediction of field emergence with arcsine-transformed data for field emergence can be made using the equation $y = 0.944 + 0.005x$.

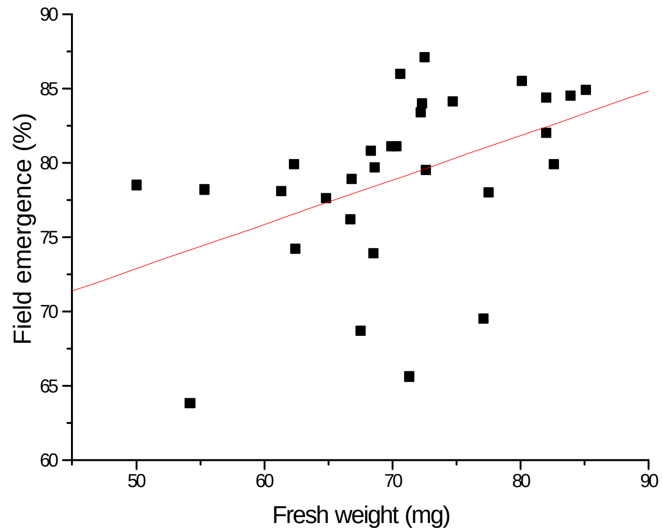


P = 0.045 **r^2 = 0.134** **y = 66.29 + 0.401x**

Figure 6.4 Linear regression of field emergence and radicle length of 3-day-old seedlings following heat-shock treatment for hybrids in the 1996 Virginia Sorghum Trials.

Fresh weight of seedlings following heat-shock treatment for hybrids in the 1996 Virginia Sorghum Trials

A significant difference ($P < 0.001$) was observed between hybrids in fresh weight of the seedlings following the heat-shock treatment. The hybrids SS1211, CAR647, FFR321, SS1313, and GW9089 were heavier than DK45, DK55, KS735, CAR730, XS345, P8118, KS711 and DK40 (Table 6.2). The general mean of this treatment was 70.7 mg, and the CV was 8.2%. There was a linear association between field emergence and average weight of the seedlings, with a P value of 0.014 and an $r^2 = 0.196$ (Figure 6.5). A predicted emergence (y) can be estimated by using the equation $y = 57.93 + 0.298x$. The predictive equation using arcsine-transformed data is $y = 0.828 + 0.0039x$.



P = 0.014 **$r^2 = 0.196$** **y = 57.93 + 0.298x**

Figure 6.5 Linear regression of field emergence percent and fresh weight of 3-day-old seedlings following heat-shock treatment of hybrids in the 1996 Virginia Sorghum Trials.

Table 6.2. Germination, radicle length, and fresh weight of 3-day-old seedlings following heat-shock treatment for hybrids in the 1996 Virginia Sorghum Trials.

Hybrid	Germination		Radicle length		Seedling weight	
	Value	Rank	Value	Rank	Value	Rank
	%		mm		mg	
CAR627	94.5 [a *]	1	33.74 [a]	10	77.5 [a b c d e]	7
CAR775	93.5 [a]	2	31.40 [a]	14	72.3 [a b c d e f]	12
GW9089	91.5 [a]	3	36.58 [a]	4	82.0 [a b c]	5
CAR647	90.0 [a b]	4	43.38 [a]	1	83.9 [a b]	2
P8305	88.0 [a b c]	5	37.87 [a]	3	72.0 [a b c d e f]	11
X12027	87.5 [a b c]	6	26.05 [a]	26	69.9 [b c d e f g]	18
DK55	87.5 [a b c]	7	28.58 [a]	22	66.7 [d e f g h]	24
SS160	87.5 [a b c]	8	31.00 [a]	15	70.6 [a b c d e f]	16
P8282	87.0 [a b c d]	9	34.44 [a]	8	72.0 [a b c d e f]	10
CAR730	86.0 [a b c d]	10	28.86 [a]	19	62.4 [f g h]	26
SS1211	85.5 [a b c d]	11	28.69 [a]	20	85.1 [a]	1
P8414	85.5 [a b c d]	12	27.12 [a]	24	68.3 [c d e f g h]	21
CAR837	85.0 [a b c d]	13	31.42 [a]	13	70.3 [b c d e f g]	17
FFR321	85.0 [a b c d]	14	22.02 [a]	30	82.6 [a b]	3
KS711	84.5 [a b c d]	15	24.42 [a]	28	55.3 [g h]	29
P8310	84.5 [a b c d]	16	32.95 [a]	12	72.2 [a b c d e f]	13
DK45	83.0 [a b c d]	27	33.65 [a]	11	66.8 [d e f g h]	23
P8118	82.5 [a b c d]	18	30.40 [a]	16	61.3 [f g h]	28
DK47	82.5 [a b c d]	19	36.17 [a]	5	74.4 [a b c d e f]	9
P8212	82.0 [a b c d]	20	35.81 [a]	7	68.6 [c d e f g h]	19
SS1313	80.5 [a b c d]	21	28.61 [a]	21	82.0 [a b c]	4
P8446	78.5 [a b c d]	22	28.94 [a]	18	70.7 [a b c d e f]	15
SS115	78.0 [a b c d]	23	36.17 [a]	6	80.1 [a b c d]	6
DK18	77.0 [a b c d]	24	37.92 [a]	2	77.1 [a b c d e]	8
DK40	71.5 [b c d]	25	22.73 [a]	29	54.2 [h]	30
KS735	70.0 [b c d]	26	26.50 [a]	25	64.8 [e f g h]	25
DK36	69.0 [b c d]	27	34.33 [a]	9	67.5 [c d e f g h]	22
KS714	68.0 [c d]	28	24.72 [a]	27	71.3 [a b c d e f]	14
XS345	67.0 [c d]	29	27.23 [a]	23	62.3 [f g h]	27
DK54	65.5 [d]	30	28.96 [a]	17	68.5 [c d e f g h]	20
Average	80.7	-	31.0	-	70.7	-

*Means within a column followed by the same letter are not significantly different at 0.05 probability level.

Germination following heat-shock treatment

Sorghum tends to have poor germination at either high or low temperatures. Mortlock and Vanderlip (1989) exposed sorghum seeds to a heat-shock treatment of 40 to 45°C for 1, 2, 4, or 8 hr. Their results point out the importance of the size of seeds when exposed to high temperatures; larger and denser seeds had more germination following heat shock than those that were lighter and smaller. With 8 hr of heat stress, all sizes had zero germination. In our work, a correlation between seed size and post-heat-shock germination was not found ($P = 0.35$ and $r^2 = 0.031$) (data not shown). Perhaps the time of heat exposure of 2 hr was not sufficient, and/or the 24 hr imbibition period before heat shock may have altered the seeds' sensitivity.

Van de Venter and Lock (1992) used a heat-shock test on six genotypes to try to predict the field emergence of sorghum. They found differences among genotypes in germination rate following the heat-shock but did not find any correlation with field emergence. Baskin et al. (1993) also used a temperature stress to try to predict field emergence. They used both cold and heat stress and found different rates of germination for the 40 genotypes tested. The cold treatment gave the highest correlation with field emergence. Working with 24 genotypes, Somman and Peacock (1985) found different germination rates among genotypes and also showed that increasing temperatures decreased germination. Their mean germination ranged from 86.8% at 35°C to 5% at 50°C.

In my work, the heat shock reduced germination when this test was compared with the standard germination test in both years. Even though the difference between standard germination and heat-shock germination was less than 7% for both years, the difference was significant at $P<0.001$ within years (Table 6.3). These data are in general agreement with Baskin et al. (1993), who found up to 36% reduction in germination when they used a heat-shock-like treatment. The 24-hr imbibition period at 25°C before the heat-shock treatment used in this experiment may have had some influence on protecting germination processes from heat shock.

Table 6.3 . Comparison between germination in a standard germination test and in a heat shock test for the hybrids in the 1995 and 1996 Virginia Sorghum Trials.

Year	Test	Germination	Difference
		----------------%	-----------------
1995	standard	87.9 [a*]	
	heat-shock	78.5 [b]	-10.6
1996	standard	87.3 [a]	
	heat-shock	81.7 [b]	-6.4

* Values followed by the same letter are not significantly different at 0.05 probability level.

When the germination data from the heat-shock test were regressed with field emergence for both years, the F test was significant at P values of less than 0.05, however the r^2 values were very low. This weak correlation suggests that this test cannot be used as a consistent predictor of field emergence. The correlations with nonstressed (standard) germination and field emergence were, in fact, better in 1996.

Radicle length following heat-shock treatment

Differences among hybrids were observed in both years for radicle length, and the radicle length was reduced by the heat-shock treatment when comparisons were made with the standard test. A reduction of 28% in 1995 and 24% in 1996 for average radicle length was observed, when the heat-shock treatment was compared with standard germination test. The radicles of heat-shocked seedlings were shorter by more than 25% in both years, and there were significant differences between treatments (standard versus heat-shock) within years (Table 6.4). Obviously the seeds were stressed by the 2-hr exposure to 50°C. There was, however no significant correlation between radicle length of stressed seeds and field emergence. Correlations between primary root length following heat-shock and field emergence were performed by Van de Venter and Lock (1992). Their results also showed no correlation between root length and field emergence.

Table 6.4. Comparison between heat-shock and standard germination tests for radicle length of sorghum seedlings.

Year	Treatment	Radicle length	Difference
		mm	%
1995	standard	49.3 [a *]	
	heat-shock	34.9 [b]	-29.2
1996	standard	41.9 [a]	
	heat-shock	31.0 [b]	-26.0

* Values followed by the same letter are not significantly different at 0.05 probability level.

Seedling fresh weight

Seedling fresh weight measurements following heat shock were made only for hybrids used in the 1996 Virginia Sorghum Trials. The hybrids were different in seedling weight after heat shock. When this treatment was compared with the standard, the difference between the means was 9% (Table 6.5). The correlation between seedling fresh weight and field emergence was significant (P = 0.014) but the r^2 was rather low (0.196) No reference to use of the variable as a predictor of field emergence was found in the literature.

Table 6.5. Comparison between heat-shock and standard germination tests for fresh weight of 3-day-old seedlings for hybrids in the 1996 Virginia Sorghum Trials.

Test	Seedling weight	Difference
	mg	%
standard	77.3 [a *]	
heat shock	70.8 [b]	-9.1

***** Values followed by the same letter are not significantly different at 0.05 probability level

Contrasts of the hybrids used in both years trials

The 14 hybrids that occurred in both years of the Virginia Sorghum Trials were analyzed and contrasted one year against the other. The objective of this analysis was to determine if seedlots showed significant differences between years. Any differences would presumably reflect variations in vigor or in laboratory procedures. The contrast of years did not reveal differences for germination. Only hybrid P8305 had a P value lower than 0.05 (Table 6.6). Radicle length showed significant differences between years for FFR321 and CAR775 (Table 6.6).

These results suggest that the hybrids generally maintained their performance across years as measured by germination and radicle length following heat shock. If the heat-shock test is an indicator of vigor, we can conclude the seedlots did not vary greatly in vigor from year to year.

Table 6.6. Contrasts of 1995 vs 1996 seedlots for germination and radicle length following heat-shock treatment.

	Germination		Radicle length	
Hybrids	F value	P value	F value	P value
CAR775	0.01	0.999	5.09	0.026
CAR837	0.03	0.872	0.23	0.630
FFR321	0.28	0.596	9.21	0.003
KS714	3.50	0.065	2.45	0.121
P8118	0.03	0.871	0.30	0.586
P8212	0.01	0.935	0.00	0.983
P8305	4.47	0.037	0.02	0.876
P8310	2.14	0.147	0.27	0.605
P8446	0.11	0.745	0.06	0.809
SS115	1.91	0.170	0.35	0.556
SS160	1.17	0.685	2.19	0.142
SS1211	0.06	0.807	0.23	0.635
SS1313	1.69	0.196	3.22	0.076
XS345	2.11	0.150	1.50	0.224

Sensitivity of hybrids to heat-shock treatment

The variables percent germination and radicle length under heat-shock treatment were evaluated in comparison with the same parameter in the standard germination test. A delta (Δ) value was calculated following the same procedures used in the osmotic test (Chapter V).

Germination showed significant differences between hybrids in germination reduction following heat shock for the 1995 Trials ($P = 0.02$) but not for 1996 Trials (Table 6.7). Radicle length was significantly affected in 1995 but not in 1996 (Tables 6.8 and 6.9), and seedling fresh weight also was not affected in 1996. The linear regression of sensitivity of all three parameters against field emergence did not show significant correlations. P values were not significant, and the r^2 values were very low (data not shown).

Table 6.7. ANOVA for percent of reduction in germination, radicle length, and 3-day- old seedling fresh weight for the hybrids in the 1995 and 1996 Virginia Sorghum Trials.

Parameter	**1995**		**1996**	
	F value	P value	F value	P value
Germination	1.91	0.02	1.14	0.31
Radicle length	1.33	0.18	1.34	0.14
Seedling fresh weigth	**-**	**-**	1.40	0.12

Some hybrids used in 1995 were more heat sensitive than the others as seen by reduction of germination. The hybrids KS604 and XS345 were more affected with a reduction of more than 26%, while the bottom 12 hybrids were affected less than 10% (Table 6.8).

In 1996, hybrids were not differentially affected by heat shock (Table 6.9). No correlation of this variable with field emergence percent was observed. Radicle length reduction did not show significant differences among hybrids in either year (Tables 6.8 and 6.9); also correlations with field emergence percent were not significant. Fresh weight of 3-day-old seedlings in 1996 also did not show differences among hybrids and either correlation was not significant.

The 24-hr imbibition period could have affected these results, and perhaps temperature or time used in this test was not adequate to stress the seedlings to produce a sensitivity that could differentiate hybrids and evaluate the vigor of the seeds. Taken together, the data suggest a heat-shock test as performed here provides a moderately good predicative stress test at best for sorghum emergence.

Table 6.8. Sensitivity of the sorghum hybrids to heat shock [(standard - heat shock / standard) x 100] for the hybrids in 1995 Virginia Sorghum Trials.

Hybrid	Δ germination		Δ radicle length	
	%	Rank	%	Rank
KS604	37.5 [a]	1	38.2 [a]	2
XS345	26.8 [a b]	2	38.0 [a]	3
KS936	21.7 [a b c]	3	40.5 [a]	1
P8305	17.6 [a b c d]	4	10.7 [a]	20
P8310	14.5 [b c d]	5	6.6 [a]	21
CAR737	13.9 [b c d]	6	33.2 [a]	6
FFR321	12.9 [b c d]	7	15.1 [a]	17
P8446	11.0 [b c d]	8	25.0 [a]	12
CAR630	10.8 [b c d]	9	32.2 [a]	7
P8699	10.5 [b c d]	10	28.6 [a]	9
CAR837	10.5 [b c d]	11	26.8 [a]	10
GW6089	8.8 [b c d]	12	26.0 [a]	11
SS115	8.1 [b c d]	13	23.7 [a]	13
SS1211	6.9 [b c d]	14	4.0 [a]	22
P8118	5.3 [b c d]	15	37.3 [a]	4
CAR577	5.2 [b c d]	16	-7.1 [a]	23
SS160	3.7 [c d]	17	21.9 [a]	14
GW8046	3.7 [c d]	18	18.7 [a]	15
CAR19225	3.5 [c d]	19	12.1 [a]	19
P8212	3.4 [c d]	20	18.0 [a]	16
CAR775	1.9 [c d]	21	13.6 [a]	18
GW1114	-0.3 [c d]	22	33.7 [a]	5
KS714	-4.5 [d]	23	30.7 [a]	8
Average	10.1		22.9	

* Means within a column followed by the same letter are not significantly different at 0.05 probability level.

Table 6.9. Difference between standard germination and heat shock stress test (Δ) in percent germination, radicle length, and fresh weight of the 1996 Virginia Sorghum Trials hybrids.

Hybrid	Δ germination		Δ radicle length		Δ fresh weight	
	%	Rank	%	Rank	%	Rank
XS345	28.5 [a]	1	5.0 [a]	27	2.3 [a]	27
KS735	25.0 [a]	2	40.1 [a]	4	9.5 [a]	13
DK54	22.4 [a]	3	39.0 [a]	7	11.9 [a]	6
DK36	20.0 [a]	4	22.1 [a]	17	6.6 [a]	18
SS115	17.5 [a]	5	30.9 [a]	10	9.9 [a]	12
KS714	13.9 [a]	6	29.0 [a]	11	6.5 [a]	19
SS1313	13.8 [a]	7	39.6 [a]	5	10.6 [a]	9
DK45	10.9 [a]	8	26.2 [a]	14	11.4 [a]	7
P8446	9.7 [a]	9	27.3 [a]	12	10.0 [a]	11
X12027	9.2 [a]	10	37.4 [a]	8	14.9 [a]	5
DK47	8.9 [a]	11	13.6 [a]	25	2.5 [a]	25
KS711	8.3 [a]	12	39.5 [a]	6	15.7 [a]	2
P8282	8.1 [a]	13	24.1 [a]	16	8.4 [a]	14
P8212	7.9 [a]	14	13.5 [a]	26	0.5 [a]	28
DK18	7.9 [a]	15	14.4 [a]	22	7.4 [a]	17
SS1211	7.4 [a]	16	42.6 [a]	2	11.0 [a]	8
CAR837	7.2 [a]	17	-3.9 [a]	30	2.3 [a]	26
CAR647	6.2 [a]	18	3.03 [a]	28	6.1 [a]	21
SS160	6.1 [a]	19	31.8 [a]	18	4.7 [a]	22
P8118	4.62 [a]	20	26.8 [a]	13	7.8 [a]	16
P8414	3.9 [a]	21	32.7 [a]	9	15.0 [a]	4
CAR730	3.1 [a]	22	25.7 [a]	15	10.0 [a]	10
P8305	1.9 [a]	23	14.1 [a]	24	6.2 [a]	20
CAR775	1.7 [a]	24	0.5 [a]	29	-1.8 [a]	30
DK55	1.6 [a]	25	41.2 [a]	3	17.2 [a]	1
P8310	-1.2 [a]	26	14.3 [a]	23	0.6. [a]	29
DK40	-1.7 [a]	27	19.3 [a]	20	4.3 [a]	23
GW9089	-2.26 [a]	28	19.5 [a]	19	8.1 [a]	15
FFR321	-2.58 [a]	29	51.18 [a]	1	15.7 [a]	3
CAR627	-4.8 [a]	30	19.0 [a]	21	4.3 [a]	24
Average	8.1		24.3		8.0	

* Means within a column followed by the same letter are not significantly different at 0.05 probability level.