Chapter V

Use of an Osmotic Stress Test to Predict Field Emergence of Sorghum

Introduction

Exposing seeds to stress in the laboratory is a technique that theoretically mimics the stresses experienced in field conditions. Such stress tests are often used to evaluate vigor (Hadas,1977). Vigor is a subjective concept that attempts to quantify the ability of seedlots to perform, especially under adverse or stressful conditions (McDonald, 1975). Stress tests, therefore, are attempts to assess vigor and predict field performance. Macromolecular substances such as polyethylene glycol (PEG) have been used to reduce water potential without being absorbed by tissues. Because PEG is a nonpenetrating, inert osmoticum and forms a colloidal solution, its effect is similar to matric properties of soil particles; and it has been used to simulate drought stress in seeds (Dart, 1992; Gurmu and Naylor, 1991; Garcia and Lasa, 1991).

The objective of this work was to study the effect of reduced water potential on sorghum germination and early seedling growth. The variables measured were percent germination, radicle length, and fresh weight of seedlings. These data will then be regressed with field emergence values for the same seedlots to test the predictive value of the laboratory tests.

Materials and Methods

Polyethylene glycol stress test

Twenty-three hybrids from the 1995 Virginia Sorghum Trials (the hybrid SS1313, which was tested in other laboratory vigor tests, did not have enough seeds from the same seedlot to run this test) and 30 from 1996 (Table 4.1) were examined in this test. This experiment was composed of four, 50-seed replications of each hybrid. The 50 seeds were placed in a Petri dish on blotter paper with 7 ml of distilled water for an "imbibition period" of 24 hours at 25°C. After the imbibition period, the seeds were transferred to moistened germination paper. Twelve ml of a 21% solution of polyethylene glycol (PEG) (8,000 MW) (Fisher Biotech Laboratory) were used to soak the paper towel. This concentration was chosen based on the - 0.50 MPa osmotic potential desired and by using the Michel equation (Michel, 1983). Seeds were rolled in the moistened paper to form ragdolls as described previously, placed in plastic bags to retain their moisture, and incubated for 48 hours at 25°C in darkness.

The water potential of the PEG solution for each replication was measured with a Wescor 5500 Vapor Pressure Osmometer model M2448-4 and calculated using the Michel (1983) equation.

$$\Psi = 8.3141 \times 10^{-5} \times (273.15 + °C) \times \text{mosmol/kg} \times 10^{-1} = \text{MPa}$$

This value, which was determined for solutions at 37°C, was then corrected for the experimental (incubation) conditions of 25 °C, using the following Michel (1983) equations:

$$[PEG] = 4 - (5.16 \times T \times \psi) - (560 \times \psi + 16)^{0.5} / 2.58 \times T - 280$$

$$\psi = 1.29 \times [PEG]^2 - 140 \times [PEG]^2 - 4 [PEG]$$

The PEG solution was prepared at the same concentration for each replication. Three osmometer readings of each prepared solution (one preparation per replication) were taken, and three discs of paper were randomized in the ragdolls inside each replication plastic bag to test the osmotic potential experienced by the seedlings at the end of the incubation period.

After 48 hours in the PEG-soaked ragdolls, the seedlings were counted and weighed (fresh weight), and the radicle lengths were measured using procedures previously described (see Chapter IV). Seeds were considered germinated if the radicle length was 3 mm or more. Coleoptiles were not considered in this case because they were strongly affected by PEG solutions. Most of the seedlings did not develop coleoptiles within the 3-days incubation period.

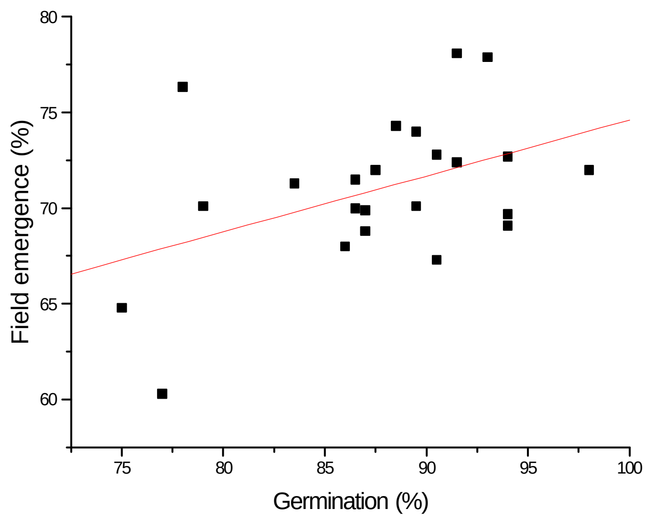
Data analysis

Analysis of variance was done as appropriate using the SAS software package (SAS Institute, 1993) to test for differences between hybrids in germination, radicle length, and fresh weight. Mean separation was performed by Tukey's test, if the ANOVA F-statistic indicated significant effects at the 0.05 probability level (SAS Institute, 1993). Percentage data were arcsine transformed (Sokal and Rohlf, 1995) prior to analysis and back transformed for reporting. Regression of laboratory test parameters with field emergence was done by using SAS software (SAS institute, 1993). The scatter-plot figures were generated using software from Microcal Origin (1995). The variation of the hybrids within years was calculated by using the one-way ANOVA, contrasts, and multiple comparisons (SAS Institute,1993).

Results and Discussion

Germination under osmotic stress of hybrids in the 1995 Virginia Sorghum Trials

Germination of the seeds under PEG-induced osmotic stress varied among hybrids (P< 0.001). The hybrids CAR775, GW8046, CAR1922, and SS160 were superior in germination to X604 and KS936 (Table 5.1). The general mean of this treatment was 87.7%, with a CV of 7.2%. The dependent variable field emergence was regressed on the independent (predictor) variable germination in PEG. There was a linear association between arcsine variables (P = 0.035 and r^2 = 0.194) (Figure 5.1). A predicted field emergence value (y) can be estimated from a germination percent (x) by using the arcsine transformed values as y = 0.762 + 0.197x. The equation for nontransformed percentage values is y = 45.35 + 0.292x.



P = 0.035 **r^2 = 0.194** **y = 45.35 + 0.292x**

Figure 5.1. Linear regression of germination of the seeds under osmotic stress and field emergence of hybrids in the 1995 Virginia Sorghum Trials.

Radicle length of the seedlings under osmotic stress for hybrids in the 1995 Virginia Sorghum Trials

Variance analysis showed that average radicle length differed among hybrids (P< 0.0001). The top five ranked hybrids had significantly longer radicles than the bottom eleven (Table 5.1).

The general mean of this treatment was 24.4 mm, and the CV was 11.8%. No significant linear association was observed between field emergence and radicle length (P = 0.47) (Figure 5.2).

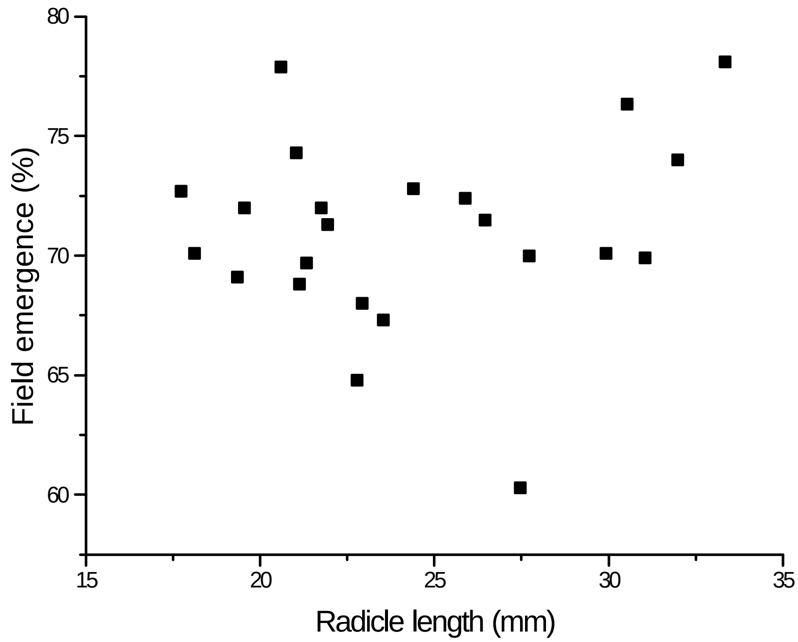


Figure 5.2. Linear regression (ns) of radicle length of 3-day-old seedlings under osmotic stress and field emergence of hybrids in the 1995 Virginia Sorghum Trials.

Fresh weight of the seedlings grown under osmotic stress for the hybrids used in the 1995 Virginia Sorghum Trial

Fresh weight of the seedlings varied among hybrids (P < 0.001). The top six hybrids were significantly heavier than all but two of the others (Table5.1). The general mean of this treatment was 48.5 mg, and the CV was 4.3%. When field emergence was regressed on fresh weight of the seedlings, there was no significant linear association between variables (P = 0.86) (Figure 5.3).

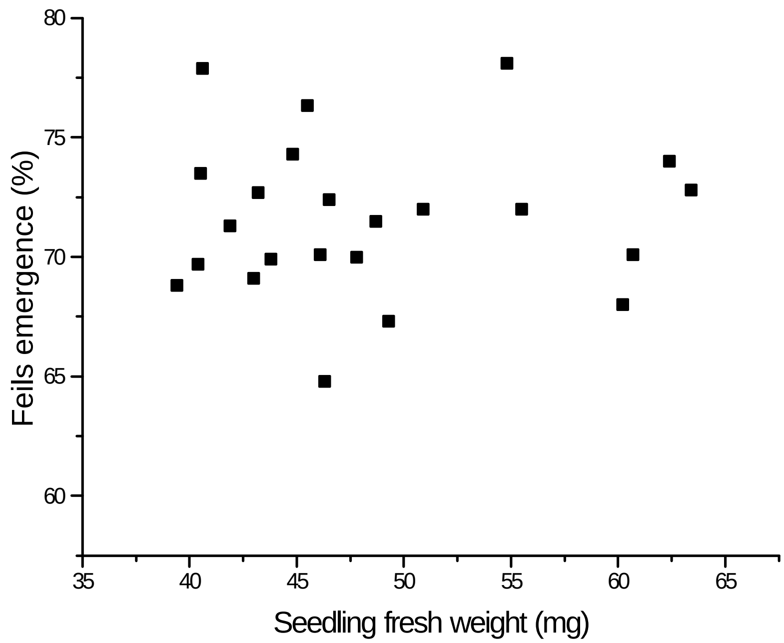


Figure 5.3. Linear regression (ns) of fresh weight of 3-day-old seedlings under osmotic stress and field emergence of hybrids in the 1995 Virginia Sorghum Trials.

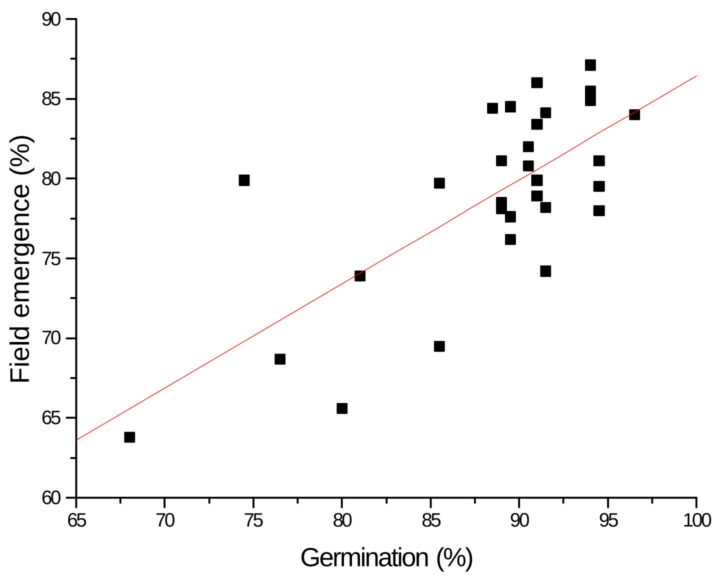
Table 5.1. Germination, radicle length, and fresh weight of 3-day-old seedlings grown under osmotic stress for hybrids used in the 1995 Virginia Sorghum Trials.

Hybrid	Germination		Radicle length		Fresh weight	
	Value	Rank	Value	Rank	Value	Rank
	%		mm		mg	
CAR775	98.0 ^{a*}	1	19.5 ^{gh}	20	50.9 ^{def}	7
GW8046	94.0 ^{ab}	2	19.3 ^{gh}	21	47.8 ^{fghi}	10
CAR1992	94.0 ^{ab}	3	21.3 ^{fgh}	16	43.0 ^{ijklmn}	18
SS160	94.0 ^{ab}	4	17.7 ^{h}	23	43.2 ^{hijklm}	17
CAR837	93.0 ^{abc}	5	20.6 ^{fgh}	19	40.6 ^{klm}	20
FFR321	91.5 ^{abc}	6	33.4 ^{a}	1	54.8 ^{cde}	6
P8305	91.5 ^{abc}	7	25.8 ^{abcdefg}	9	46.5 ^{fghij}	11
CAR630	90.5 ^{abc}	8	23.5 ^{cdefgh}	11	39.4 ^{n}	23
P8118	90.5 ^{abc}	9	24.4 ^{bcdefgh}	10	40.5 ^{lmn}	21
SS1211	89.5 ^{abc}	10	31.9 ^{ab}	2	62.4 ^{a}	2
SS115	89.5 ^{abc}	11	29.9 ^{abcde}	5	60.7 ^{ab}	3
P8310	88.5 ^{abc}	12	21.0 ^{fgh}	18	44.8 ^{ghijklmn}	15
CAR577	87.5 ^{abc}	13	21.7 ^{fgh}	15	55.5 ^{bcd}	5
P8212	87.0 ^{abc}	14	21.1 ^{fgh}	17	40.4 ^{mn}	22
GW6089	87.0 ^{abc}	15	31.0 ^{abc}	3	63.4 ^{a}	1
GW1114	86.5 ^{abc}	16	26.4 ^{abcdefg}	8	60.2 ^{abc}	4
P8446	86.5 ^{abc}	17	27.7 ^{abcdef}	6	48.7 ^{fgh}	9
XS345	86.0 ^{abc}	18	22.9 ^{defgh}	12	43.2 ^{hijklm}	16
P8699	83.5 ^{abc}	19	21.9 ^{fgh}	14	41.9 ^{jklmn}	19
KS714	70.0 ^{bc}	20	18.1 ^{h}	22	49.3 ^{efg}	8
CAR737	78.0 ^{bc}	21	30.5 ^{abcd}	4	45.5 ^{fghijkl}	14
X604	77.0 ^{c}	22	27.4 ^{abcdef}	7	46.3 ^{fghijk}	12
KS936	75.1 ^{c}	23	22.7 ^{fgh}	13	46.1 ^{fghijkl}	13
Average	87.7	-	24.4	-	48.5	-

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

Germination under osmotic stress for hybrids in the 1996 Virginia Sorghum Trials

A significant difference (P<0.001) in germination of the seeds under osmotic stress was observed between hybrids in 1996. The top seven ranked hybrids were more germinable than DK36, XS345, and DK40 (Table 5.2). The general mean of this treatment was 88.5%, and the CV was 6.0%. When the dependent variable arcsine field emergence was regressed on arcsine germination in PEG, a linear association was found ($P < 0.0001$) with an $r^2 = 0.533$. The distribution of the data is shown in the scatter diagram Figure 5.4. An arcsine-based prediction of field emergence (y) for a given percentage of germination (x) can be made employing the empirical equation $y = 0.3919 + 0.5763x$. The predictive equation using nontransformed data is $y = 21.25 + 0.651x$.



$P<0.0001$ $r^2 = 0.533$ $y = 21.25 + 0.651x$

Figure 5.4. Linear regression of germination under osmotic stress and field emergence of hybrids in the 1996 Virginia Sorghum Trials.

Radicle length of the seedlings grown under osmotic stress for hybrids in the 1996 Virginia Sorghum Trials

Radicle length in PEG-soaked ragdolls varied among hybrids (P< 0.001). The top five ranked hybrids had longer radicles than the lowest five. The general mean of this treatment was 23.2 mm, and the CV 17.6%. When field emergence was regressed on radicle length under osmotic stress, there was no significant linear association (P = 0.103) (Figure 5.5).

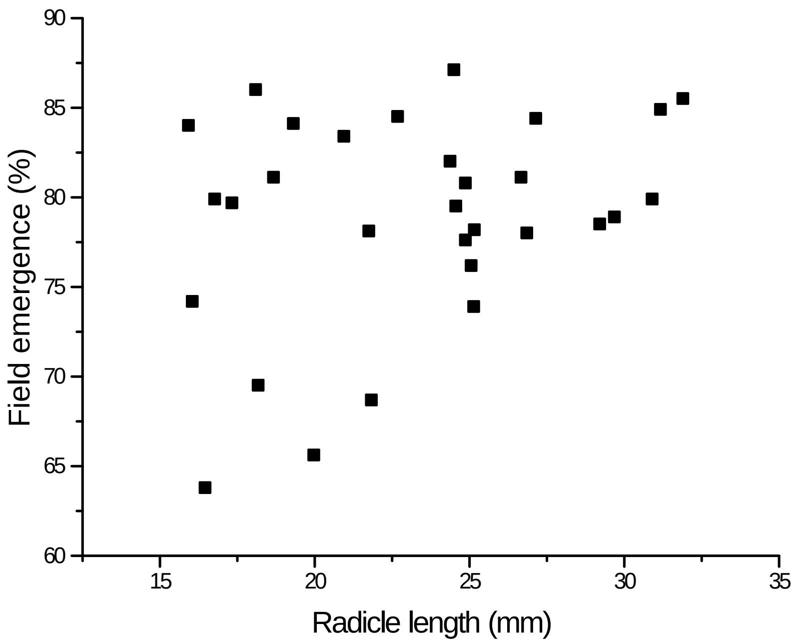
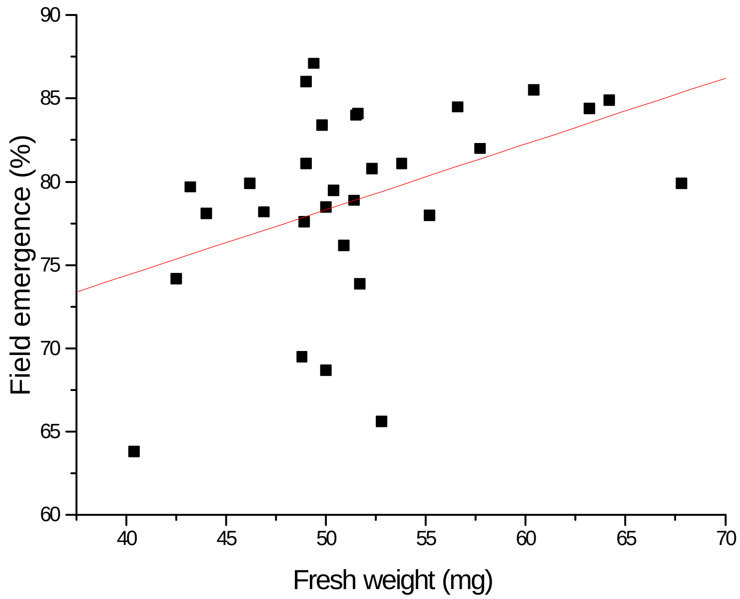


Figure 5.5. Linear regression (ns) of radicle length of 3-day-old seedlings under osmotic stress and field emergence of the hybrids in the 1996 Virginia Sorghum Trials.

Fresh weight of the seedlings grown under osmotic stress for hybrids in the 1996 Virginia Sorghum Trials

Fresh weight of the seedlings varied among hybrids ($P < 0.001$). The top five ranked hybrids were heavier than 12 of the remaining ones (Table 5.2). The general mean of this treatment was 51.4 mg, and the CV was 5.5%. There was a linear association between field emergence and fresh weight of the osmotically stressed seedlings ($P = 0.021$ and $r^2 = 0.176$). The distribution of the data is shown in the scatter diagram (Figure 5.6), and their dispersion in relation to an empirical linear equation $y = 58.55 + 0.3956x$, where x is fresh weight and y is field emergence. The predictive equation using arcsine transformed data is $y = 0.847 + 0.005x$.



P<0.021 r^2 = 0.176 y = 58.55 + 0.3956x

Figure 5.6. Linear regression of fresh weight of 3-day-old seedlings under osmotic stress and field emergence of hybrids in the 1996 Virginia Sorghum Trials.

Table 5.2. Germination, radicle length, and fresh weight of 3-day-old seedlings grown under osmotic stress for the hybrids in the 1996 Virginia Sorghum Trials.

Hybrid	Germination		Radicle length		Fresh weight	
	Value	Rank	Value	Rank	Value	Rank
	%		mm		mg	
CAR775	96.5 [a*]	1	15.9 [f]	30	51.5 [defg]	13
P8282	95.0 [ab]	2	24.5 [abcdef]	14	50.4 [defgh]	16
CAR627	94.5 [ab]	3	26.8 [abcdef]	7	55.2 [cde]	7
CAR12027	94.5 [ab]	4	26.6 [abcdef]	8	53.8 [cdef]	8
SS115	94.0 [ab]	5	31.8 [a]	1	60.4 [abc]	4
SS1211	94.0 [ab]	6	31.1 [a]	2	64.2 [ab]	2
P8305	94.0 [ab]	7	24.4 [abcdef]	15	49.4 [efghi]	20
DK47	91.5 [abc]	8	19.3 [cdef]	22	51.6 [defg]	12
CAR730	91.5 [abc]	9	16.0 [ef]	29	42.5 [ij]	29
KS711	91.5 [abc]	10	25.1 [abcdef]	9	46.9 [fg hi]	25
P8310	91.0 [abcd]	11	20.9 [abcdef]	20	49.4 [efghi]	19
SS160	91.0 [abcd]	12	18.0 [def]	25	49.0 [efghi]	21
DK45	91.0 [a bcd]	13	25.1 [abcdef]	10	51.4 [defg]	14
FFR321	91.0 [abcd]	14	30.9 [ab]	3	67.2 [a]	1
GW9089	90.5 [abcd]	15	24.3 [abcdef]	16	57.7 [bcd]	5
P8414	90.5 [abcd]	16	24.8 [abcdef]	13	52.3 [def]	10
DK55	89.5 [abcd]	17	25.0 [abcdef]	11	50.9 [defgh]	15
CAR647	89.5 [abcd]	18	22.6 [abcdef]	17	56.6 [bcde]	6
KS735	89.5 [abcd]	19	24.8 [abcdef]	12	48.9 [efghI]	23
P8446	89.0 [abcd]	20	29.2 [abcd]	5	50.0 [defghi]	17
SS1313	88.5 [abcde]	21	27.1 [abcde]	6	63.2 [ab]	3
P8118	87.0 [abcde]	22	21.7 [abcdef]	19	44.0 [ghij]	27
CAR837	87.0 [abcd e]	23	18.6 [cdef]	23	49.0 [efghij]	22
DK18	85.5 [abcde]	24	18.1 [def]	24	48.8 [efghi]	24
P8212	85.5 [abcde]	25	17.3 [ef]	26	43.2 [hij]	28
DK54	83.0 [abcde]	26	29.6 [abc]	4	51.7 [defg]	11
KS714	80.0 [bcde]	27	19.9 [bcdef]	21	52.8 [cdef]	9
DK36	76.5 [cde]	28	21.8 [abcdef]	18	50.0 [adefgh]	18
XS345	74.5 [de]	29	16.7 [ef]	27	46.2 [fghij]	26
DK40	68.0 [e]	30	16.4 [ef]	28	40.4 [j]	30
Average	88.5	-	23.2	-	51.4	-

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

Germination under osmotic stress test

Significant germination differences among sorghum genotypes were found by Garcia and Lasa (1991) when they used PEG solutions at an osmotic potential of -0.2MPa. In their results, the best predictor of field emergence was time to appearance of the first seedling leaf. Saint Clair (1976) found marked differences in germination among cultivars in PEG solutions ranging in concentration from 0 to 12%. Increasing PEG concentrations resulted in poorer germination. There was no significant difference between the effect of PEG 6,000 and 20,000 at the same osmotic potential according to Stout et al. (1980). They found little difference in germination between 0 and -0.4 MPa for one hybrid, while another was strongly affected. The hybrids used in our research showed similar variability to Garcia and Lasa (1991) and Saint-Clair (1976), i.e., significant differences in percent germination under PEG treatment were found between hybrids in both years. However we note here that the differences in PEG were similar to those seen when germinated in water. We will discuss this more fully below.

The relation between PEG solutions and germination is discussed by Brar et al. (1992) who found a negative correlation. They attributed the low germination not to osmotic stress but to reduced oxygen diffusion due to a thicker water film around the seed.

Lad (1986) also found that germination decreased as PEG 4000 increased in concentration. Smith et al. (1989) had similar results using PEG 6000 solutions. Ashraf et al. (1990) also showed that, under PEG solutions, germination percentage is reduced. Evans and Stickler (1961) worked with ifferent osmotic tensions from -0.05 to -0.6 MPa and concluded that germination decreased with increases in osmotic tensions.

Table 5.3. Osmotic potentials of 21% PEG solutions as prepared and at seed level after 2 days in ragdolls for germination under PEG treatment.

Year	Rep	Ragdoll	Solution	(Ragdoll -Solution)
		---------------------------MPa	-------------------------------	
1995	1	- 0.748	-0.398	0.350
	2	- 0.651	- 0.374	0.277
	3	- 0.665	- 0.422	0.243
	4	- 0.573	- 0.409	0.164
	Average	-0.591	-0.400	0.191
1996	1	- 0.690	- 0.365	0.325
	2	- 0.560	- 0.385	0.175
	3	- 0.623	- 0.365	0.258
	4	- 0.806	- 0.428	0.378
	Average	-0.669	-0.385	0.284

Table 5.3 shows the osmotic potentials of 21% PEG solutions in which the seeds were germinated. The differences in osmotic potential between ragdolls and the prepared solutions can likely be attributed to increasing concentrations of PEG inside of ragdolls due to the water adsorption by the toweling and the uptake by seedlings. Variations in final water potential among ragdoll replications were observed. They can likely be attributed to slight variations in water evaporation during the incubation.

The mean germination in PEG was 87.7% in 1995 and 88.4% in 1996. Standard germination using distilled water (Chapter 4) provided 88.3% and 87.2% in 1995 and 1995, respectively. The differences are not significant. This result does not fully agree with most of the preceding cited literature. However, Ashraf et al. (1990), who worked with two sorghum varieties and a range of 0 to - 0.9 MPa of osmotic potentials also did not find differences at -0.48 MPa for one cultivar after 48 hr. Similar results were found by Dart et al. (1992) using regression equations to show that germination was above 80% when water potential was -0.1 to -0.4 MPa. Brar et al. (1992), modeling sorghum establishment, discussed the effect of temperature and water content on seeds. They found a relationship between low temperature, high water potential, and low emergence; however, at 25.5°C the maximum emergence was unaffected by changes in osmotic potentials from -0.03 to -0.3 MPa. Gurmu and Naylor (1991) established that the minimum water potential which permits germination of sorghum seeds is between -0.73 and -1.15 MPa.

The “imbibition period” (24 hours in distilled water) used in our research likely affected germination rates, increasing the percentages as compared with seeds that would have been placed directly into PEG solutions. These imbibed seeds were not exposed to an initial water stress with PEG. (The rationale of this protocol was to try to mimic what might occur in the field when the soil moisture is initially high and consequently osmotic tensions are near 0 MPa). Under these test conditions, germination events in the seeds could have been well advanced before PEG exposure.

The relationship between field emergence and response to osmotic stress is discussed by Hadas (1977), who tried to estimate seed germination under field conditions using PEG 6000. He concluded that this is not an accurate method to predict field emergence, because it tests only the effect of adverse soil water conditions. However, he noted PEG-induced stress could be useful to define seed vigor or predict seed germination. Stout et al. (1980) agreed with Hadas and concluded that germination in osmotica can only predict the ability of a cultivar to germinate in low soil water content and that the osmotic stress was not due to decreased water uptake but to the inhibition of cell metabolism.

Radicle length of the seedlings

In both years, the hybrids used in our experiment showed differences in radicle length when the seeds were germinated in PEG (as they did in 1996 when germinated in distilled water).

This result agrees with Evans and Stickler (1961), who used four varieties and four osmotic tensions. They concluded that the radicle length differed with variety with a tendency toward reduction in length with increases in tension. Working with two cultivars, Gurmu and Naylor (1991) found that one had longer radicles and coleoptiles than the other under low water potentials. According to Ashraf (1990), root length decreased when osmotic potential decreased from 0 to -0.9 MPa, and varieties showed differential responses under this treatment. Lad (1986) found that radicle growth decreased from 4.4 to 0 cm with increases in PEG concentrations from 0 to 50 mM.

The effect of PEG treatment on radicle length can be measured when compared with the standard germination test (Table 5.4). PEG treatment reduced the radicle length by 50.5% in 1995 and 45.2% in 1996 (Table 5.4). This result agrees with the preceding cited researchers. Regressing the radicle lengths in PEG with field emergence revealed no significant linear relationship ($P = 0.47$ for 1995 and $P = 0.103$ for 1996), and the determination coefficients were very low ($r^2 = 0.054$ and 0.139 for 1995 and 1996, respectively). With this result, it can be concluded that radicle length in this PEG test was not a good predictor of field emergence.

Table 5.4. Comparison between the standard germination and PEG treatment on the radicle length of 3-day-old sorghum seedlings. Each value is the mean of 23 (1995) or 30 (1996) hybrids.

Year	**Medium**	**Radicle length**	**Difference**
		mm	%
1995	H_2O	49.3 $^{a *}$	
	PEG	24.3 ^{b}	-50.5
1996	H_2O	41.9 ^{a}	
	PEG	22.9 ^{b}	-45.2

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

Weight of the seedlings

In both years, the seedling fresh weights varied by hybrid. The overall effect of PEG on reducing seedling weight can be seen when comparisons are made with the standard germination test. The combined hybrids means for seedling weight for both distilled water and PEG are shown in Table 5.5. The osmoticum reduced fresh weight by about one-third in each year.

Table 5.5. Means of the fresh weight of 3-day-old seedlings from standard germination (H_2O) and from PEG treatments for 1995 and 1996. Each value is the mean of 23 (1995) or 30 (1996) hybrids.

Year	Treatment	Fresh weight	Difference
		mg	%
1995	H_2O	71.7[a*]	
	PEG	48.5[b]	-32.3
1996	H_2O	77.3[a]	
	PEG	51.4[b]	-33.7

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

When seedling weight in PEG was regressed with field emergence, the results did not show this test to be a good predictor of field emergence ($P<0.14$ in 1995 and $P<0.021$ in 1996). The percent of water uptake decreased markedly with decreases in osmotic potentials. Gurmu and Naylor (1991) observed a fresh weight reduction of almost 50% when they decreased osmotic potential from 0 to -1.15 MPa and the radicle length decreased from 63 to 38 mm. A second hybrid behaved similarly; however its fresh weight was more strongly reduced, and its radicle length was shortened even more, from 52 to 25 mm. They concluded that the first hybrid was more drought resistant.

Arguments similar to those of Gurmu and Naylor (1991) might be used in our study, by looking at Tables 5.1 and 5.2 and the rankings for radicle length and seedling fresh weight. The 1995 hybrids GW9089, SS115, SS1211, and FFR321 (with high combined rank averages for length and weight) might be more drought resistant than SS160, P8212, CAR630, and CAR837. In 1996 the hybrids SS1211, FFR321, SS1313, and SS115 had rankings that suggest that they might be more drought resistant than DK40, XS345, P8212, and CAR730. Some hybrids ranked well in both years (SS1211, FFR321, and SS115), while P8212 and XS345 appeared near the bottom twice.

Contrast across years of hybrids in both trial years

The 14 hybrids that were tested in both years were analyzed and contrasted one year against the other for the same hybrid. The hybrids XS345, CAR837, KS714, P8118 showed significant differences in percent germination under PEG. The hybrids KS714, P8118, P8212, and SS160 differed in radicle length, while, with the other ten hybrids, no differences between years were observed. The variable fresh weight of the seedlings was more sensitive for some hybrids; half of the hybrids tested showed significant differences. Differences from year to year can be attributed to one of the following factors: seedlots treated differently after harvest from one year to the other, seedlots experienced environmental differences during the preharvest period, or variation in laboratory testing procedures from one year to the next.

Table 5.6. F and P values of ANOVA contrasts between years for germination, fresh weight of seedlings, and radicle length of seedlings in PEG of the14 hybrids tested in both years.

Hybrid	Germination		Fresh weight		Radicle length	
	F value	P value	F value	P value	F value	P value
CAR775	0.01	0.9276	0.06	0.8023	2.02	0.1595
CAR837	9.68	0.0026	10.06	0.0022	1.47	0.2287
FFR321	1.33	0.2526	116.96	0.0001	3.39	0.0694
KS714	10.98	0.0014	14.49	0.0003	49.39	0.0001
P8118	5.31	0.0238	0.35	0.5578	4.53	0.0365
P8212	2.07	0.1537	0.01	0.9183	12.51	0.0007
P8305	10.62	0.0016	1.87	0.1755	0.09	0.7593
P8310	0.52	0.4735	3.38	0.0699	0.12	0.7316
P8446	0.52	0.4735	7.27	0.0086	0.04	0.8341
SS115	0.52	0.4735	1.30	0.2582	1.01	0.3179
SS1211	1.68	0.1986	1.37	0.2446	2.28	0.1350
SS1313	1.68	0.1986	4.76	0.0321	0.15	0.7028
SS160	0.52	0.4735	29.29	0.0001	13.39	0.0005
XS345	31.56	0.0001	8.28	0.0051	3.64	0.0601

Sensitivity of hybrids to the osmotic stress tests

The three variables (germination, radicle length, and fresh weight) were evaluated for the hybrids planted in 1995 and 1996. The formula used was:

Δ value = [(standard value - osmotic value) / (standard value)] x 100

Percent change in radicle length (Δ radicle length) and seedling fresh weight (Δ fresh weight) showed significant differences between hybrids in both years. Percent change in germination was not affected by osmotic stress in either year (Tables 5.7 and 5.8). The radicle length of 3-day-old seedlings was significantly reduced under PEG (compared to distilled water) in both years ($P<0.01$). Some hybrids were more sensitive to osmotic stress than others. The range of inibition was from 18 to 63% in the 1995 Trials (Table 5.7) and from 25 to 59% in the 1996 Trials (Table 5.8). However the correlation between Δ length and field emergence was not significant for either year, and the r^2 values were very low (data not shown).

Some hybrids used in 1995 were more drought sensitive than the others as seen by PEG reduction of radicle length. The hybrids FFR321 and SS1211 were shortened less than 21%, while the hybrids SS160, KS714, and CAR775 were reduced by more than 60% in radicle length. A similar result was found for the 1996 hybrids; P8446 and FFR321were less sensitive to PEG, with reduction of radicle length of less then 29%. By contrast, CAR730 and DK18 were shortened more than 59%.

The variable Δ fresh weight was also significantly different in both years, but only X604 with 23.2% reduction differed from FFR321, X19225, SS160, and CAR630 with more than 35% reduction in 1995. In 1996 the hybrid CAR775 was significantly reduced compared with DK18, and the reduction range was from 27.5% (CAR775) to 41.5 % (DK18).

Table 5.7. Sensitivity of germination, radicle length, and fresh weight of the sorghum hybrids to osmotic stress test [(standard-osmotic stress / standard) x 100] for the hybrids in 1995 Virginia Sorghum Trials.

Hybrid	Δ germination		Δ radicle length		Δ fresh weight	
	%	Rank	%	Rank	%	Rank
P8699	7.9 [a*]	1	52.1 [a b]	10	34.8 [a b]	5
CAR737	7.1 [a]	2	35.2 [a b]	20	34.5 [a b]	6
X604	5.7 [a]	3	40.5 [a b]	17	23.2 [b]	23
SS115	5.1 [a]	4	41.6 [a b]	16	31.9 [a b]	14
P8446	4.7 [a]	5	39.8 [a b]	18	31.8 [a b]	15
KS936	4.0 [a]	6	52.7 [a b]	9	28.0 [a b]	20
FFR321	3.6 [a]	7	20.3 [b]	22	36.7 [a]	1
GW1114	2.1 [a]	8	51.5 [a b]	12	31.9 [a b]	13
CAR577	1.9 [a]	9	42.0 [a b]	15	31.7 [a b]	16
CAR837	1.1 [a]	10	55.2 [a b]	5	33.8 [a b]	9
P8310	0.0 [a]	11	45.2 [a b]	13	33.2 [a b]	10
P8305	-0.2 [a]	12	38.3 [a b]	19	34.3 [a b]	8
CAR630	-0.9 [a]	13	53.1 [a b]	7	35.5 [a]	4
GW6089	-1.3 [a]	14	33.4 [a b]	21	32.7 [a b]	11
SS160	-1.3	15	63.3 [a]	1	35.9 [a]	3
P8212	-1.7 [a]	16	52.0 [a b]	11	34.3 [a b]	7
P8118	-2.6 [a]	17	54.6 [a b]	6	32.4 [a b]	12
CAR775	-2.6 [a]	18	61.4 [a]	3	30.6 [a b]	18
KS714	-3.5 [a]	19	61.9 [a]	2	27.1 [a b]	21
SS1211	-7.0 [a]	20	18.8 [b]	23	26.4 [a b]	22
XS345	-8.3 [a]	21	43.3 [a b]	14	30.1 [a b]	17
GW8046	-8.7 [a]	22	56.4 [a b]	4	30.1 [a b]	19
X19225	-10.7 [a]	23	53.0 [a b]	8	36.4 [a]	2
Average	-0.2	-	46.3	-	32.1	-

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

Table 5.8. Sensitivity of germination, radicle length, and fresh weight of the sorghum hybrids to osmotic stress test [(standard-osmotic stress / standard) x 100] for the hybrids in 1996 Virginia Sorghum Trials.

Hybrids	Δ germination		Δ radicle length		Δ fresh weight	
	%	Rank	%	Rank	%	Rank
XS345	7.8 a *	1	45.5 a b	13	27.8 a b	29
DK36	77 a	2	50.3 a b	7	30.7 a b	24
CAR647	6.7 a	3	48.0 a b	8	36.7 a b	5
CAR837	4.7 a	4	37.6 a b	25	31.9 a b	19
DK40	3.4 a	5	45.3 a b	16	28.7 a b	27
P8212	3.8 a	6	58.0 a	3	37.2 a b	3
SS160	1.3 a	7	53.8 a b	5	33.7 a b	13
X12027	0.6 a	8	35.7 a b	27	34.7 a b	11
SS1313	0.6 a	9	42.4 a b	20	30.8 a b	23
DK45	0.1 a	10	33.8 a b	28	31.5 a b	21
KS711	-0.3 a	11	38.2 a b	23	28.7 a b	26
DK55	-1.0 a	12	47.8 a b	10	36.7 a b	4
GW9089	-1.2 a	13	46.1 a b	11	35.2 a b	9
P8118	-1.3 a	14	47.8 a b	9	33.8 a b	12
CAR775	-1.5 a	15	51.9 a b	6	27.5 b	30
P8282	-1.6 a	16	45.5 a b	23	36.4 a b	7
P8414	-2.1 a	17	41.0 a b	21	35.0 a b	10
DK47	-2.5 a	18	54.0 a b	4	32.6 a b	16
SS1211	-2.6 a	19	38.2 a b	24	32.9 a b	15
CAR730	-3.8 a	20	59.0 a	1	38.8 a b	2
P8446	-3.8 a	21	28.6 b	29	71.2 a b c	5
DK18	-4.3 a	22	58.9 a	2	41.4 a	1
KS735	-4.6 a	23	43.9 a b	19	31.6 a b	20
KS714	-4.6 a	24	44.6 a b	17	30.8 a b	22
CAR627	-4.9 a	25	36.6 a b	26	32.0 a b	18
P8305	-5.2 a	26	44.4 a b	18	36.0 a b	8
SS115	-6.0 a	27	39.1 a b	22	32.1 a b	17
DK54	-6.0 a	28	45.3 a b	24	33.4 a b	14
P8310	-9.5 a	29	45.8 a b	22	30.5 a b	25
FFR321	-14.7 a	30	24.8 b	30	28.1 a b	28
Average	-1.5	-	44.4	-	33.1	-

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

The PEG concentration used in our experiment was effective in retarding radicle development. The general description of the radicles in PEG was shorter, weaker and thinner when compared with those in the standard germination test. Regressions with field emergence were not significant for this variable in either year.

Regressions of germination, radicle length, and seedling fresh weight with field emergence in Orange in 1996 were done to compare osmotic stress with field performance in a drought year (see Table 3.4). For all three variables, the data did not show significance correlations, with P values above 0.20 and r^2 values below 0.20 (data not shown).

In sum, we conclude that osmotic stress of sorghum germination and early seedling growth can be imposed using PEG. Such stress results in retarded growth and can detect differences between genotypes in sensitivity to stress. However such differences do not appear to be related to emergence potential under the field conditions we faced.