SEEDLING EVALUATION OF GRAIN QUALITY RESPONSES AND DROUGHT TOLERANCE IN MAIZE

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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GENERAL INTRODUCTION

Poor seed quality encompasses many things including microbial damage, insect damage, foreign material, lack or low levels of essential amino acids or vitamins etc. However, in breeding programs, seed certification and seed trade negotiations, corn seed users are more concerned about the quality of the seed in terms of germinability, stand establishment under field conditions, and the vigor of the seedlings. These seed characteristics depend on many factors such as the storage duration and conditions, moisture content of the seed at harvest, drying temperature, seed coat and structure damages during harvest, insects and disease damage, pericarp thickness or endosperm mutants.

Seed germination and seedling vigor tests have been used as indices of seed quality in seed control programs for several years. The germination test remains the principal and accepted criterion for seed viability. Test results, however are obtained under favorable germination conditions which are seldom encountered in the field. Therefore, germination results often overestimate field emergence (Association of official seed analysts,1981.n.32).

The concept of vigor and its definitions are as diverse as the people working on the problem. Seedling vigor is highly complex. At the biochemical level it involves energy and biosynthetic metabolism, coordination of

cellular activities, and transformation and utilization of reserve foods (Cardwell,1984).

In corn (Zea mays L), as well as any other cereal, the endosperm is the first source of energy for early growth. Then, new leaf material, by photosynthesis, becomes the source of energy after depletion of the endosperm (Cooper and Macdonald, 1970).

The significance of the endosperm, lies mainly in the fact that it plays a major role in the development and maintenance of a medium suitable for growth of the young embryo (Brink and Cooper, 1947). However, several mutations have been reported (Creech, 1968) which alter the type and quantity of carbohydrates, including starch in the kernels. For instance, it has been reported that the waxy mutant differs from normal dent corn in sugar and starch content. Also, the starch in waxy endosperm consists entirely of amylopectin instead of amylose. High amylose maize has more protein and oil and less endosperm than normal dent corn.

It appears then, that mutants influence the composition of the endosperm. The proportion of sugar, starch, and the type of starch (amylose or amylopectin) have a great influence on germination and seedling vigor.

In addition to normal dent corn, two other kinds of corn are used throughout the world, i.e., flint and floury corn. The floury kernel is opaque to light while the flint kernel is translucent, that is it transmits light. In the

flint and pop types of endosperm, the horny (corneous) layer extends over the top of the kernel so there is no denting due to shrinkage as in dent corn. Floury corn kernels have no horny endosperm and therefore also do not dent. Because of these differences, the objective of the first part of this study is to determine the influence of those three kernel endosperm types (flint, dent, and floury), on germination, seedling vigor and stand establishment.

The second part of this study deals with the selection of corn genotypes for drought tolerance under simulated drought conditions. It is well established that world crop production is limited by environmental stress. Statistics show that only 10% of the world's arable land may be classified as nonstressed. About 20% of the land is limited by mineral stress, 26% by drought stress, and 15% by freezing stress (Blum, 1985). Of all stresses, drought stress seems to be the most important. Its effect on crop production is of great importance in African countries, and other third-world countries as well.

In breeding programs, selection for drought tolerance is usually made under stress in the field. Such a procedure is slow and costly. An alternative method is to select in controlled environments (laboratory or greenhouse) under simulated stress conditions, using chemicals such as polyethylene glycol, mannitol or sodium (Williams et al, 1969. A.Blum et al, 1979. Kilen et al, 1969. Burlyn et al

1971.). Studies conducted on maize (Williams et al, 1967; Kilen et al, 1969.) showed a positive and high correlation between the results obtained in the laboratory and those obtained under field conditions.

The objective of the second part of this study, is to develop a relatively simple screening method for drought tolerance. Known and unknown drought tolerant and susceptible genotypes were grown to characterize their responses during the seedling stage under simulated drought conditions, using polyethylene glycol (PEG) 8000. With such a method, one should be able to predict the performance of corn genotypes in the field under drought stress.

PART I: EFFECTS OF DIFFERENT KERNEL ENDOSPERM TYPES ON GERMINATION, SEEDLING VIGOR, AND STAND ESTABLISHMENT IN MAIZE.

INTRODUCTION

Seed germination is the renewal of growth and development of a dormant embryo, and, in general, is caused by a change in environmental conditions. The ability of seed to undergo germination depends upon many factors, and the capability of seed lots to germinate is of great concern for seed users. Under natural conditions, seeds of a particular species may vary greatly in germinability. Throughout the world, where corn is commonly grown, three endosperm types of kernels are identified as flint, dent, and flour by their external appearance, and internally by the structure and distribution of starch grains in the endosperm. The objective of this part of the study was to determine the effects of flint, dent and floury endosperm on germination, seedling vigor and stand establishment in maize. The study was conducted in three environments; (1) the laboratory, (2) the greenhouse and (3) the field.

LITERATURE REVIEW

Seed Germination

Growth activity is quickly resumed by the embryo, when corn kernels are placed under moisture and temperature conditions favorable for germination. In official seed testing laboratories a period of five to six days is considered ample to determine viability at optimum temperature, 30 C (86F). Under field conditions, emergence of the seedling occurs in about seven to ten days, varying with soil temperature and moisture (Kiesselbach, 1949).

Germination is defined (Cardwell, 1984) as the sequence of events transforming a quiescent embryo into a metabolically active, synthesizing structure. Seed germination is a response to several environmental, physiological, and/or morphological factors. Choice of a germination test depends on the objectives and the resources available to the researcher.

Thus, some seed analysts or seed companies perform either a laboratory germination test, a field germination test or a combination of both. The association of official seed analysts (1983) defines laboratory germination as "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions".

From an agronomic view, germination begins when the seed is placed in a moist soil and ends when the seedling emerges above the soil surface and becomes autotrophic (Cardwell, 1984).

If properly matured, dried to a safe moisture content, and stored under favorable conditions, a corn seed may retain its viability for several years. The germination process consists of three stages, 1) imbibition (which occurs in viable and non-viable seeds), 2) initiation of enzymatic and meristematic activities and, 3) the growth of the radicle and plumule, and their elongation through the seed coat (Cardwell,1984).

The two main components of the seed are the embryo and the endosperm. They provide the basic potential of determining response during germination and initial growth (Wright, 1971). The endosperm provides nutrients for growth and development of the embryo (Stanley and Butler, 1961). The quantity of food reserves available during germination is related to seed size (Cooper and Mac Donald, 1970). In a given seed lot, heavier seeds usually possess greater growth potential than light seeds, since they are better able to furnish the energy needed for germination and initial growth of seedings (McDaniel, 1969). Within lines and temperature regimes, the larger, round kernels tended to perform better than the smaller, flat ones (Andrews,1982).

Cooper et al (1970) reported that root and shoot

growth in corn was highly correlated with endosperm weight loss through 12 days (r = .99 **). They found that the amount of energy for growth during the period of emergence of the first leaf until emergence of the third leaf, as measured by co_2 fixed, is negligible compared to the large amount of energy contributed by the endosperm. This emphasized the great importance of the endosperm during germination and early plant growth. They also found that for root and shoot growth, about 23.7 mg of endosperm was consumed per seedling and per day. When the endosperm was partially removed, root and shoot growth and leaf area were reduced. They concluded that the endosperm was important for growth of the seedling and for supplying adequate leaf area for a more rapid post-germination growth rate.

Kiesselbach (1949) reported that in field corn, the accumulation of starch continues until all the interior cells contain large quantities. The cells, however, are not filled equally in all regions of the endosperm. The starch grains in the "horny" region are angular and packed very closely together. The spaces between starch granules contain protein. The starch grains in the "starchy" region are rounded and less closely packed. The difference between dent and flint corn remains in the amount of horny starch in the upper part or crown of the kernel; in flint and pop types this horny layer extends over the top of the kernel so there is no denting due to shrinkage as in dent corn. Soft (flour)

corn has no horny endosperm and also does not dent. Waxy corn differs from other starchy corn in that the starch of the endosperm consists entirely of amylopectin and contains no amylose.

Amylose is a linear polymer of glucose, and amylopectin is a branched polymer of glucose. A number of genes are known to affect the carbohydrate composition of corn endosperm. Some genes are known to change the amylose-amylopectin ratio, seed germination and seedling vigor. In a review of mutants, Smith (1984) indicated that the ratio can be changed by the waxy (wx), sugary (su_1), sugary-2 (su_2), dull (du), opaque (O_2), or amylose extender (ae). Shrunken-2 (sh_2) and brittle (bt) also affect the carbohydrate composition of the endosperm. Genes may also interact to change the ratio. For instance, su_1 and du interact to increase the amylose content of endosperm starch to about 65%, compare to 25 % in the normal dent corn.

The effects of mutant genes on corn seed germination and seedling vigor have been investigated in several studies. Styer et al (1981) found that under stress conditions germination of sweet corn seeds with high-sugar shrunken-2 (sh_2), was reduced and produced seedlings less vigorous than sugary (su_1) seeds. Nass et al (1966) reported that normal endosperm seeds reached 100% germination 9 days after planting under greenhouse conditions, but none of the

seeds with endosperm mutants attained 100% germination. Su_1 and sh_1 were significantly lower in germination under greenhouse conditions, showing that these mutants were inferior to normal. A similar experiment conducted in a growth chamber at 15 C, showed that o_2, sh_1, and su_1, had fewer numbers of seeds germinated than normal. Mutants were found to produce changes in the components of the endosperm that were either favorable or unfavorable for germination and early seedling growth.

Seedling Vigor

The definition of seedling vigor is still controversial. Woodstock (1965,1969) defined vigor as "That condition of good health and natural robustness in seed which, upon planting, permits germination to proceed rapidly and to completion under a wide range of environmental conditions". It is assumed that "good health" means freedom from disease as well as purely physiological "well being".

Perry (1973) defined seedling vigor as "a physiological property determined by the genotype and modified by the environment, which governs the ability of a seed to produce a seedling rapidly in soil, and the extent to which the seed tolerates a range of environmental factors". From those definitions and other concepts of vigor stated by many other authors, a committee of the International Seed Testing Association (ISTA) proposed in

1977 a definition of seed vigor which was adopted by the ISTA congress. The definition follows: "Seed vigor is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence". Seeds which perform well are termed "High vigor seed", while those which perform poorly are called "Low vigor seed".

In general, the object of the vigor test is to identify seed lots which are capable of rapid and uniform seedling emergence in the field, and seed lots with high emergence ability under unfavorable environmental conditions.

Seedling vigor therefore results, from the interaction of genetic and environmental components during seedling emergence. It is used in seed production, seed quality control programs and breeding programs to provide the seed user with a better product.

The following tests are frequently used to assess vigor:

1. Accelerated aging of seeds at high relative humidity (near 100%) and high temperature (40-45 C) for a few weeks.

2. Cold testing of seeds is used to assess tolerance to soil pathogens in a cold, wet soil. Seeds are usually planted in nonsterilized soil at 70% of water-holding capacity. The cold test for corn is conducted at 10C for 7 days, and seeds are then transferred to 25 C and germination counts made 4 days later.

3. Conductivity test measures the loss of solute sugars, starches, protein, etc.

4. Cool germination testing is commonly used to estimate low temperature tolerance of cotton seeds (18 C).

5. Seedling growth rate is frequently used as an index of vigor to show differences in seed lots due to genotype, seed size, production location, and freeze damage. Germination is conducted on paper towels placed in a darkened germinator at 25 C for 7 days.

6. Seedling vigor classification is based on physical examination of seedlings for prompt development and freedom of defects of the root system, hypocotyl, and epicotyl.

7. Root elongation after 96 hours of germination has been used as an index of vigor for lettuce (Lactuca sativa L.).

8. Tetrazolium chloride (commonly 2,3,5-triphenyl tetrazolium chloride) tests the internal integrity of the embryo by staining those tissues exibiting dehydrogenase enzyme activity. Dead tissues do not stain.

The above tests, when used in conjunction with germination tests, give a rather broad spectrum of information on the quality of a seed lot.

Seed viability and seedling vigor are influenced by many factors. Among these are seed maturity and environmental stresses such as temperature, moisture, fertilizer, and

mechanical damage, which occur during maturation. Cardwell (1986) showed that corn germinated 87% after 5 years, 70% after 10 years, and 36% after 20 years, pointing out the effects of storage conditions and storage duration on seedling vigor. Seed size was positively correlated with seedling vigor, although for some cereal crops, that relationship was not consistent because of greater mechanical injury associated with large seeds during harvesting and processing.

Several mutations have been reported to alter the type and quantity of carbohydrate including starch (Creeck, 1868). It has been reported that waxy mutant was different from normal corn in sugar and starch content. Styer et al (1981) concluded that poor seedling vigor in corn was linked to low total carbohydrate content of sh_2 endosperm. Kernel vigor of sixteen combinations of four genes which alter reserve endosperm, i.e., amylose-extender (ae), dull (du), sugary (su), and waxy (wx), was measured to evaluate the potential of these genotypes for sweet corn (Rowe et al, 1987). Genotypes with the ae phenotype had poor vigor and exibited some reduced germination.

Stand Establishment

A good stand is essential for maximum yield of any crop. Poor stands with maize are most frequent when planting

is followed by a period of cold wet weather. This favors the development of pathogens which hinder germination and growth of seeds or young seedlings (Tatum et al, 1943). Weather is a prime factor in such poor stands, but it has been observed repeatedly that different genotypes of seed vary in performance under favorable conditions.

Stand establishment was reported to be related to some processing operations such as artificial drying which can cause some damage to the seed. Meyers (1924) found that broken pericarps resulted in reduced stands in the field when the soil was wet and cold.

Soil characteristics are among the most important factors influencing stand establishment under field conditions. Many soils, and particularly acid soils that are low in organic matter content, tend to form a dense crust under the beating of raindrops (Hillel, 1961). Soil crusting, either by mechanical obstruction or by limitation of aeration can hinder germination and seedling growth. On the other hand, seedbed compaction often adversely effects germination and subsequent growth (Parker and Taylor, 1965).

Stand establishment is also affected by seed size, soil temperature, and moisture availability. It has been shown that large wheat seeds germinate faster, have larger seedling dry weights and produce more vigorous seedlings than smaller seeds.

In a study of millet genotypes, seed size was

significantly and positively correlated with seedling vigor, ear length, yield, and size of seed produced (Chhina and Phul, 1982). Seedling emergence was 22% greater for large, dense millet than small, light seed.

However, seedling emergence is related to soil temperature since the meristem is at ground level and is the region of temperature perception. A study conducted by Singh and Dhaliwal (1972) showed that sorghum emergence was 97.5%, 92.5%, and 82.5% at 30, 35 and 60 C, respectively, with no germination at 45 C.

It has been established that rate of germination decreases with increasing soil water suction (Doneen and McGillivray, 1943). Final germination percentage similarly diminishes at low soil moisture contents. The ability of seed to germinate at low soil water contents depends on the plant species. Each species appear to have its own threshold suction value for germination (Hunter and Erickson, 1952). Values reported are 12.5 bars for corn, 7.9 bars for rice, 6.6 bars for soybean, and 3.5 bars for sugar beets. The importance of water during plant establishment is first seen during the germination process. In order for germination to start, a minimum soil moisture content is needed for the imbibition process. The presence of water in the seed tissue, induces a number of metabolical reactions. Lack of sufficient water after the imbibition process, will slow the process of cell division and embryo growth, resulting in

poor seedling vigor.

Hence, soil moisture conditions exert a dominant influence on stand establishment, not only at a cellular level, but also at the level of soil properties. Soil structure, soil water potential, and seed-soil surface contact determine the rate of moisture uptake by the seed in soil. Increasing seed surface contact with liquid water in the soil will decrease germination time and increase germination percentage. The number of emerging seedlings decreases rapidly as moisture tension exceeds 0.7 MPa, and as stress increases (Cardwell, 1984).

A number of factors besides soil moisture and other properties, have been shown to influence stand establishment in the field. Cardwell found that high concentrations of fertilizer and soluble salts in soil solutions restrict germination and field emergence due to osmotic (and in some cases ion toxicity) effects.

Moreover, seeding depth, date of planting, seedbed preparation, and planting equipment can influence stand establishment. Pathogens inhabiting the seed or soil, may reduce stand establishment whenever seedling emergence is delayed by low temperatures, high soil moisture, or other conditions more favorable for pathogens than for germination and emergence of seeds.

In short, seed germination, seedling vigor and stand establishment, in addition to being under genetic control,

depend on the environment and the interaction between genotype and environment.

MATERIALS AND METHODS.

Experiment I: Laboratory studies

A. Germination tests

During the summer of 1987, germination and seedling vigor tests were conducted in the laboratory at Kansas State University. Thirteen Kansas corn inbred lines were used. Four were of flint kernel type: K21, K22, K24, and K26. K24 and K26 had some floury parental sources in their pedigrees. Four lines were of normal dent kernel type: K41, K55, K724, K731. Five were of floury kernel type: K811, K812, K813, K814, and K815. Because of limited seed supplies, bulklots of seed of different age groups were used.

Germination tests were conducted according to the standard test (AOSA Handbook, 1981). Forty seeds were put on two well moistened standard paper towels, 12"x18". Seeds were soaked in bleach (sodium hypochlorite) for 2 to 3 minutes, washed to protect them from mold and arranged in two rows of 20 seeds each. Rows were 4" from the edge and about 4" away from each other, to provide enough room for radicle and shoot development. They were covered with a third moistened paper towel. The paper towels were rolled and placed in a plastic bucket (36cm x 30cm), and covered with a poly-bag to prevent the loss of moisture. The bucket was placed in a germinator at constant temperature of 25 C ±

2 for 7 days. Another container filled with water was put in the germinator to keep the humidity at about 100%. Germination tests were conducted in the dark. The first count was made after 4 days; seeds were then returned to the dark chamber and the second count was made after 7 days. The experiment was a randomized complete block design. seeds were considered to be germinated when the radicle broke the seed coat and emerged.

B. Seedling Vigor Tests

Two seedling vigor tests were used (AOSA,1983):

1. The seedling growth rate test, which measured the extent of dry matter accumulation in the seedling. The test was performed by assessing seedling root and shoot dry weight.

2. The promptness index test, which gave the speed of germination.

These two tests were performed in the same way as the standard germination test, using the same experimental conditions. For the promptness index, a germination count was made every day for 7 days. After the last count (7th day) normal, abnormal, and dead seedlings were separated.

Normal corn seedlings have a vigorous primary root, usually with adventitious roots present. If no primary root is present, there are at least two vigorous adventitious roots. The leaves must be green and vigorous. Abnormal

seedlings have no roots, or no primary root and only short, weak adventitious roots, and the leaves are not green.

Shoots and roots of normal seedlings were cut from the kernels, placed in different envelopes and dried in the oven at 80 C for 24 hours. Dry weights were then measured to determine dry-matter accumulation of the seedlings. Experimental design was a randomized complete block with 2 replications per block. Germinators were blocks.

Experiment II: Greenhouse study

In the fall of 1987, the same seed sources, except K24 and K815, were used for planting in the greenhouse. K24 and K815 were eliminated because of insufficient seeds. Planting was done in pots, at a depth of 2 inches in a completely randomized design with 3 replications. Twenty seeds were used per genotype. Adequate moisture was supplied during the 3-weeks growing period . No special treatment was applied to the soil or the seeds. Temperature in the greenhouse was about 84 F in daytime and 74 F at night. Germination percentage and number of plants/plot were recorded. Roots and shoots of seedlings were dried at 100 C for 72 hours, and weighed.

Experiment III: Field study

The field planting was made on May 6th 1988, at K.S.U Agronomy Farm. The objective was to evaluate germination (emergence) and stand establishment under field conditions. Experimental units were two row plots in a completely randomized design with 3 replications. Twenty seeds were planted per row in each plot. The number of plants that emerged (germination), and among these, those which gave a 2 or 3 leaf-seedling (stand establishment) were recorded after one month.

RESULTS AND DISCUSSION

Experiment I: Laboratory studies

A. Germination tests

The results of the germination tests, expressed in percent of germination for both counts, and the analyses of variance are presented in Table 1. The first count provided some information about the vigor of the seed and its ability to germinate rapidly under adverse conditions. Highly significant differences among inbreds were found in both counts, indicating that there was considerable variation among genotypes within the same type of endosperm, although on the average, some groups performed better than others (Table 2).

TABLE 1. Analyses of variance for two germination counts of thirteen corn inbred lines.

Source		mean squares	
	DF	1st count	2nd count
Rep	3	213.8 *	95.6 *
Inbred	12	553.0 **	439.5 **
Error	36	66.9	27.2

**, * significantly different at .01 and .05 level respectively.

Table 2 gives the mean separation for both counts for all kernel endosperm types. For flint sources, the highest means were obtained with K21 and K22. Likewise, K41, K724, K731, were the highest among dent sources. However, it is noteworthy that two flint sources with flour background K24

TABLE 2. Mean germination responses of thirteen inbred lines for two periods, 4 days and 7 days

INBRED	ENDOSPERM TYPE	1st COUNT	2nd COUNT
		(%)	(%)
K731	Dent	98.2 a	98.8 a
K724	Dent	97.5 ab	98.2 a
K41	Dent	97.5 ab	98.2 a
K21	Flint	96.3 ab	98.2 a
K22	Flint	93.2 abc	97.0 a
K812	Floury	88.8 abcd	93.8 abc
K813	Floury	86.9 abcd	90.7 bcd
K815	Floury	86.3 bcd	88.3 cd
K26	Flint	84.4 cd	87.5 cd
K24	Flint	83.3 cd	92.5 abcd
K811	Floury	78.8 d	86.3 d
K55	Dent	64.4 e	71.9 e
K814	Floury	62.5 e	65.0 e
LSD (.05)		11.8	7.5

Means within column with the same letter are not significantly different.

and K26 performed poorly in their group. Also, a dent source, K55, gave a very poor response probably due to the age of the seed and the storage condition that affected its viability. Floury kernel type inbreds performed poorly in general, although K812 and K813 gave satisfactory results for both counts.

Because of the variation in results within each group, the same analysis of variance was performed after grouping the inbreds within each kernel type endosperm. K55 was not taken into account in grouping dent inbreds because of its extremely low performance , Table 3 . Results showed that normal dent inbred lines performed better for both counts

although their differences were not statistically significant from flint sources. Floury sources gave the lowest germination percentage.

TABLE 3. Summary of germination means of three endosperm types.

GROUP	ENDOSPERM TYPE	1st COUNT	2nd COUNT
		(%)	(%)
1	Dent	97.7 a	98.3 a
2	Flint	94.7 a	97.5 a
3	Floury	87.3 b	90.8 b
LSD (.05)		6.05	4.4

Means within column with the same letter are not significantly different.

However, it should be noted that floury sources used in the study were opaque-2 sources. It has been shown by Smith (1984) that in addition to waxy , sugary, and dull mutant genes, opaque-2 genes can also change the ratio amylose-amylopectin in the endosperm. That ratio is 25% amylose to 75% amylopectin in normal dent endosperm. Also, Nass et al (1966) reported that normal endosperm was superior in greenhouse germination compared to endosperm mutants. Moreover, it has been established by previous workers that endosperm high in amino acid content performs poorly in germination. These findings seem to be consistent with our results where the floury o_2 sources high in lysine content, gave lower germination percentages.

Besides the genetic composition of the endosperm, the accumulation and structure of the starch grain may account for the differences in germination between flint, dent, and floury endosperm types. Indeed, the main difference between flint and dent was in the amounts of horny (corneous) starch in the upper part of the kernel, while flour corn has no hard starch. Normal dent corn, therefore, is physically closer to flint corn than it is to flour corn. That was confirmed by the low germination percentage obtained for K24 and K26, which were not pure flint sources. The presence of floury endosperm in the pedigrees of those lines was apparently sufficient to affect germination results.

B. Seedling Vigor tests

Results of the two standard tests used are summarized in table 4 . The promptness index test (PI) gives the speed of seed germination. The formula used was a modification from Maguire, 1962.

$$PI = \Sigma \frac{\text{number of seeds germinated}}{\text{days of count}}$$

The seedling growth rate test (SGR) gives the dry weight of a normal seedling (AOSA Handbook No 32).

$$SGR = \frac{\text{seedling dry weight}}{\text{total number of normal seedlings}}$$

The root to shoot dry weight ratio was computed to show the relative development of root over shoot, and to show how the development of either part influences seedling vigor. Highly significant differences were observed among inbreds for both tests and for the root to shoot ratio. The inbred x germinator interaction was not significant indicating that the results were consistent over germinators.

TABLE 4. Analyses of variance for promptness index (PI), seedling growth rate (SGR), and root to shoot ratio (R/S).

Source	DF	means squares PI	SGR	R/S
Rep	1	442.0 **	55	0.004
Germinator	1	92.0	254 *	0.020 **
Inbred	12	916.0 **	361 **	0.040 **
Inbred x germinator	12	36.3	76	0.002
Error	25	33.3	36	0.002

**, * significantly different at .01 and .05 level respectively.

Inbred lines were grouped by kernel type endosperm for ease of comparison. Normal dent inbreds germinated more rapidly as compared to flint and flour kernel inbreds, Table 5. Also, they appeared to accumulate more dry matter, although SGR means of dent and flint are not significantly different. Dent inbreds had the smallest root to shoot ratio, indicating that they accumulated more dry matter in the shoot than in the root. Floury type inbreds gave the poorest performance, although they had the highest root to shoot ratio. They showed less vigor, but accumulated more dry matter in root.

TABLE 5. Mean responses of three corn endosperm types for PI, SGR, and R/S ratio.

GROUP	ENDOSPERM TYPE	PI	SGR	R/S
			(mg)	
1	Dent	66.4 a	59.3 a	.55 b
2	Flint	52.7 a	58.7 a	.53 b
3	Floury	46.5 b	54.2 b	.70 a
LSD (.05)		7.1	4.1	.06

Means within column with the same letter are not significantly different.

In this study, dent inbreds appeared to be the most vigorous, and therefore, would be expected to be more capable of stand establishment under unfavorable field conditions. A vigorous seed lot is more likely to succeed under a wide range of environmental conditions.

Rate of germination (Fig. 1) also indicated the superiority of dent kernel type endosperm over flint and floury sources used. One might assume that dent endosperm is capable of completing the germination process more rapidly, inducing a rapid growth of the embryo, compared to flint and floury type endosperm. However, mutant genes, known to reduce germination, can also reduce seedling vigor. Styer et al., (1981) found that high sugar sweet corn with shrunken-2 (sh_2) seeds germinated poorly and had poor seedling vigor. They concluded that poor performance was linked to low total carbohydrate content of sh_2 endosperm. Rowe and

Garwood (1987) found that the amylose-extender (ae) gene, which gives high oil and protein endosperm instead of starch, conditioned poor vigor and reduced germination compared to normal allele.

Those findings may explain in part why floury endosperm performed so poorly. The floury kernel, because of the presence of opaque-2 (o_2) mutant gene and probably other mutant genes influencing the contents of some amino acids, contained less starch and consequently gave poor germination percentage, germination rate, and seedling vigor.

The reduction in germination and seedling vigor may reflect a close relationship between the embryo and a given endosperm type. Excision of the embryo and its growth in the absence of the endosperm could help explain that relationship.

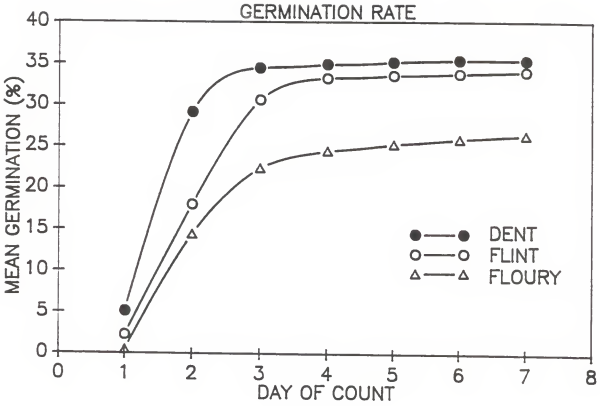

Fig.1: Germination rate of three endosperm types of maize over a seven day period.

Experiment II: Greenhouse study

This study was designed to determine the effects of the three types of endosperm on germination, seedling vigor and stand establishment in different environments. Analysis of variance results (table 6) showed that there were highly significant differences among inbreds for germination, seedling weight, and stand establishment.

TABLE 6. ANOVA of greenhouse study for germination (GERM), stand establishment (STD) and seedling weight (SWT).

		mean squares		
Source	DF	GERM	STD	SWT
Rep	2	332.6 *	275.7	5739
Inbred	10	2227.0 **	2032.0 **	9737 **
Error	20	90.1	97.4	2843

**, * significantly different at .01 and .05 level respectively

Tables 7 and 8 summarize greenhouse study results. Dent inbreds germinated better than other endosperm types in the greenhouse. The same pattern was observed earlier in the laboratory studies. Dent types had the highest percentage of stand and the highest seedling weights.

TABLE 7. Inbred line responses in the greenhouse study, 1987.

Inbred	Endosperm	std	germ	swt
		(%)	(%)	(mg)
K731	Dent	98.3 a	98.3 a	309 ab
K22	Flint	93.3 ab	95.0 a	255 abc
K41	Dent	86.7 abc	95.0 a	213 c
K812	Floury	81.7 abc	83.3 ab	188 c
K21	Flint	76.7 bcd	85.0 ab	213 c
K724	Dent	75.0 cde	75.0 bc	237 bc
K26	Flint	61.7 def	65.0 cd	210 c
K811	Floury	58.3 ef	60.0 cd	207 c
K813	Floury	56.7 f	58.3 d	189 c
K55	Dent	25.0 e	25.0 e	340 a
K814	Floury	18.3 e	18.3 e	334 ab
LSD (.05)		16.8	16.1	90.8

Means within column with the same letter are not significantly different.

TABLE 8. Summary of endosperm means in greenhouse study for germination, stand and seedling weight.

Endosperm	germ	std	swt
	(%)	(%)	(mg)
Dent	89.4 a	86.6 a	253
Flint	8I.6 ab	77.2 ab	226
Floury	67.2 b	65.5 b	195
LSD (.05)	18.6	18.8	70.5

Means within column with the same letter are not significantly different.

Experiment III: Field study

Analysis of variance results for field germination and stand establishment are given in table 9. Highly significant

differences among inbreds for both parameters were observed. The table 10 shows the same trend observed in the greenhouse and laboratory studies. Here, once again, K26 was the poorest among flint sources and K55 was the poorest among dent sources. Floury sources, as before, had the poorer performance compared to flint and dent sources. Germination and stand establishment responses are summarized in table 11. Again dent sources gave the highest means, followed by flint and floury sources.

TABLE 9. Analysis of variance for germination and stand establishment study under field conditions, 1988.

Source	DF	mean squares	
		Germ	Std
Rep	2	160.8	120.6
Inbreds	10	760.4 **	797.4 **
Error	20	110.8	104.8

** significantly different at .01 level.

TABLE 10. Responses of corn inbred lines for germination and stand establishment in field study.

Inbred	Endosperm	Germ	Std
		(%)	(%)
K731	Dent	94.1 a	93.3 a
K724	Dent	88.3 ab	85.8 ab
K21	Flint	85.8 abc	83.3 abc
K4I	Dent	78.3 abc	75.8 bc
K22	Flint	78.3 abc	76.6 abc
K26	Flint	76.6 abc	73.3 bc
K812	Floury	73.3 bc	70.0 bc
K813	Floury	70.0 cd	68.3 c
K814	Floury	52.5 de	50.8 d
K811	Floury	51.6 e	48.3 d
K55	Dent	45.8 e	42.5 d
LSD (.05)		17.9	17.4

Means within column with the same letter are not significantly different.

TABLE 11. Summary of inbreds responses for germination and stand in the field.

Endosperm	Germ	Std
	(%)	(%)
Dent	86.9 a	85.0 a
Flint	80.2 a	77.7 a
Floury	61.8 b	59.3 b
LSD (.05)	13.9	13.8

Means within column with the same letter are not significantly different.

Simple Correlation Coefficients

Simple correlation coefficients between laboratory, greenhouse and field results are given in table 12. Laboratory results for seed germination were highly and

positively correlated with greenhouse (.82**) and field (.74*) findings. Greenhouse germination results were also highly and positively correlated (.83**) with field results. Seedling growth rate (SGR) was not significantly but positively (.62) correlated with laboratory and greenhouse dry weight.

Promptness index (PI) was positively and highly correlated with greenhouse and field germination and stand establishment. Laboratory germination was highly correlated with stand establishment in the greenhouse and in the field.

TABLE 12. Simple correlation coefficients between laboratory germination (GERMI), promptness index (PI), seedling growth rate (SGR); greenhouse germination (germ2), stand establishment (STD2), seedling weight (SWT); and field germination (GERM3), stand establishment (STD3).

	GERM1	SGR	PI	GERM2	STD2	SWT	STD3	GERM3
GERM1		-.90**	.46	.87**	.86**	-.53	.74*	.74*
SGR			-.37	-.80**	-.78*	.62	-.74*	-.75*
PI				.74*	.75*	.30	.79*	.79*
GERM2					.99**	-.29	.83**	.83**
STD2						-.25	.84**	.84**
SWT							-.11	-.12
STD3								.99**
GERM3								

**, * significant at .01 and .05 level respectively.

SUMMARY

This study showed that corn seed germination and seedling vigor may be accounted for in part by the type of kernel endosperm. For the sources used, dent kernel type endosperm was the best performer in terms of percent, and rate of germination, dry matter accumulation and establishment of stand in the soil under greenhouse and field conditions. Flint endosperm, which was much closer to dent in starch composition and structure, was second after dent, and floury was the lowest. Results obtained with flint lines possessing floury background, confirmed the evidence that floury endosperm gave reduced germination, seedling vigor and stand establishment.

PART II: COMPARATIVE STUDY OF SEVEN MAIZE GENOTYPES AT SEEDLING STAGE FOR DROUGHT TOLERANCE.

INTRODUCTION

Drought may be defined as absence of rainfall for a period of time long enough to cause depletion of soil moisture and damage to plants. The reaction of plants to dehydration may be considered from two standpoints: survival and yield. Survival from severe dehydration is of great importance with respect to crops grown in areas where moisture shortage is common, especially at the early stages of plant growth. Cultivars capable of successful production in such regions must possess drought tolerance at the seedling stage as well as at later stages. The above situation occurs frequently in tropical regions where corn is grown.

The goal of this study is to determine if tolerance in seedling stage is correlated with responses of corn genotypes to water stress in later growth stages in the field; and to study the effectiveness of the seedling screening method used.

LITERATURE REVIEW

Successful crop production in tropical climates depends heavily on water supply of the environment. Drought stresses as meteorological events, may be either seasonal or temporal and unpredictable, but their effects on crop production depend on crop genotype, stage of the growth cycle, and severity and duration of stress. Corn breeders in tropical regions, therefore, must consider drought tolerance as one of their primary concerns.

Studies of drought response in corn, its effects on crop production, mechanisms of drought resistance or tolerance, and evaluation procedures have been investigated by several authors.

Splitter (1966) reported that the capacity of the corn plant to withstand drought is dependent in part upon the water gathering ability of the roots. A plant which can draw moisture from larger and wider areas, can resist drought better than those with less extensive root systems. Plants with small root system may be unable to penetrate the lower strata and surrounding areas to obtain moisture from the soil. The same conclusion was drawn by Misar (1954), who observed that drought resistant lines had greater numbers, more branching, and better development of roots.

Hague (1955) observed that a resistant strain of corn had a higher root-top ratio, and more branching of secondary

roots than less resistant strains. Harber (1938), compared the weights of roots of susceptible and resistant inbred lines of corn, but did not find significant differences that would account for differences in ability to withstand drought.

Kirkham et al (1984) measured canopy temperature of drought resistant and drought sensitive genotypes of maize to compare the temperature of known drought resistant and susceptible lines and hybrids. They found that drought resistant inbred lines had warmer leaves, and the canopy temperatures of hybrids were 5 C cooler than those of inbreds, although the temperatures of the hybrids with drought resistant parents was about the same as those of hybrids with drought-sensitive parents. The same conclusion was reached earlier in 1981 by Mtui et al., who observed that hybrids of maize had cooler canopy temperature than inbreds. Splitter (1966), found that varieties with greater numbers of leaves and greater leaf blade areas yielded more than varieties with fewer and smaller leaves. Water deficits however, result in decreased plant growth especially when tissues and organs are in stages of most rapid growth (Kirkham et al., 1972).

Various screening methods for water stress are being developed and used in controlled environments to overcome problems associated with direct selection under ambient field stress conditions. These methods involve laboratory

and greenhouse tests using several chemicals. In corn, results obtained in controlled environments, appeared to be well correlated with those of the field (Williams et al, 1967; Kilen and Andrew, 1969).Williams et al.,(1967) used a mannitol solution at 15 atm osmotic pressure as germination medium to screen corn genotypes for drought tolerance. Blum et al.,(1979) performed a series of experiments in wheat to evaluate the significance of seed germination and seedling growth, using polyethylene glycol-6000 (PEG) solutions as moisture-stress-inducing media. They concluded that tolerance to water stress in growing seedlings can be screened for by using PEG 6000 containing nutrient solutions, but cannot be predicted from germination tests in stress solutions.

Results of seedling studies of corn plant by Kilen and Andrew (1969) under water stress conditions in the greenhouse, were highly correlated with field responses. Leaf blade areas, and heights of resistant plants were less affected than those of susceptible lines. However, they found the average number of stomata per unit of leaf blade surface area to be approximately the same for resistant and susceptible lines.

Similar studies conducted on soybean by Sammons et al. (1970), showed that seedling stem elongation and leaf expansion can be used to characterize plant responses to drought stress.

Hence, more attention is being given to the use of morphological and physiological measurements for screening seedlings for drought and heat tolerance. Selection for seed yield under field conditions requires a full seasons' data, but may not be reliable due to the excessive variability encountered. Screening material under laboratory or greenhouse conditions maybe a useful alternative if laboratory results are highly correlated with field tests for a given crop. For instance, evidence has been presented that alfalfa, (Medicago sativa L.) accessions which emerge at -0.65 Mpa osmotic pressure in the laboratory emerge and survive better in the field under drought stress than accessions that do not emerge under stress in the laboratory (Rumbaugh and Johnson, 1981).

Several physiological characteristics in crops have been reported to be reliable indicators for selection of drought tolerant germplasm. These characteristics include seed germination and seedling growth in hydroponic solutions of low osmotic potential (Blum et al., 1979; Richard, 1978; Sammons et al., 1979; Stout et al.,1980; and Williams et al., 1967).

Success of the above approaches will require evidence that the drought tolerant cultivars tested in the laboratory reflect drought tolerance under field conditions. Previous studies showed that significant correlations exist between field and laboratory data in corn (Kilen and Andrew, 1969).

MATERIALS AND METHODS

These studies were conducted in spring, 1988 at Kansas State University. Seven maize genotypes, 5 inbred lines and 2 hybrids, were used. seed of genotypes was produced in Kansas except for one which came from Morocco. Kansas lines have been extensively studied in the field for their drought tolerance characteristics (Splitter, 1966; Kirkham et al, 1984). Genotypes and their drought stress responses are given in table 13.

Drought conditions were simulated in the laboratory using polyethylene glycol (PEG) 8000, obtained from Sigma Chemical Saint Louis, Missouri. The growth medium was made by dissolving PEG in nutrient solution. The necessary amount of PEG was determined by using the table developed by Burlyn et al (1973) which relates the concentration of PEG (g/kg H_2O) to temperature.

TABLE 13. Inbred lines and hybrids used in the study, and their previously observed response to drought stress.

GENOTYPES	Drought Reaction
K724	sensitive
K731	sensitive
B73	top fire in heat
H28	tolerant
ML	unknown
H28 x K55	tolerant x tolerant
K73I x K41	sensitive x tolerant

Three solutions of different water potential were made using 3 plastic buckets (24x25cm). The first concentration

of about -0.1 MPa, was made using a full-strength nutrient solution. That potential was estimated from the results of a study done by Kirkham (1969), which gave a water potential of about -0.05 MPa to a half-strength nutrient solution. The second medium had a water potential of -0.3 MPa and the third had -0.5 MPa. The experiment was conducted at a constant temperature of 23 C. These potentials were chosen, after a preliminary study that showed no growth of our seed sources in potentials less than -0.6 MPa. The PH of the solutions was about 5.70.

Seeds were germinated on paper towels under normal conditions, according to standard germination test procedures(Aosa Handbook, 1981) at 25 C ± 2. After 4 days of germination initial root and shoot lengths were recorded for 5 normal seedlings per genotype. Selected seedlings were transplanted into buckets containing 6 liters of solution. Plastic trays were cut to fit the buckets, with rows of holes about 2mm in diameter. The radicles were placed into the holes when the seedlings were transplanted and roots were then submerged in the solution. Kernels and shoots were surrounded with a cotton pad to keep seedlings upright. Light was provided by the laboratory ceiling lights and a Gro and Sho light of intensity 39.32 $\mu M/m^2/sec$, suspended at about 38cm above the buckets. An air supply device was designed to provide plants with oxygen.

Plants were grown for 10 days. Light and dark periods

of 16 and 8 hours respectively were provided. To assess seedling growth rate under stress conditions, heights of seedlings were measured to the tip of the longest leaves, each day during the growth period. A split plot experimental design with 3 replications was used. Water potentials were whole plots and genotypes were subplots.

Leaf area measurements were obtained after 10 days on the first and the second leaves of each plant using a portable area meter (LI-COR Model LI-3000). Final root lengths and seedling fresh weights were also recorded. Stomatal density (number of stomata/mm^2) was determined under microscope, on the first leaf where stomata were well differentiated. Observations were made at 10x10 power, after the leaf cuticle had been removed by applying clear nail polish as described by Kirkham (1972). Four observations were made on the adaxial and abaxial surface of the leaf. Leaf slices were preserved in 70° ethanol before observation. Stomatal density was determined on control plants only. All seedlings were dried in the oven at 90 C for 48 hrs and their dry weights were recorded.

RESULTS AND DISCUSSION

Root and Shoot Growth

Several researchers have reported that root system growth and development are of some importance for plants to withstand drought (Splitter 1966, Hague 1955, Misar 1954). Under severe drought conditions, plants which can draw moisture from larger and wider areas, can resist drought better. Drought-tolerant lines are expected to have greater numbers of roots with more branching and better development than drought susceptible lines.

Results showed that root elongation of seedlings grown in -.3 MPa and -.5 MPa water potential was inhibited. Differences among genotypes were highly significant (table 14). Greatest root development was obtained from the Moroccan line (ML) and the hybrid H28 x K55. Least root elongation was obtained with inbred H28 which was considered to be a tolerant line, table 15. Therefore, root development alone cannot be used to characterize seedling response to drought according to our data.

However, water deficit is known to decrease plant growth, especially when tissues and organs are in stages of most rapid growth (Kirkham et al., 1972). Results for shoot growth are summarized in tables 14 and 15. Differences among genotypes were highly significant. A comparison of means (table 15) showed that ML and Hybrid H28 x K55 had the highest means for shoot growth. Inbred lines B73 and H28

were intermediate and K724, K731 and K731 x K41 were lowest.

TABLE 14. Analyses of variance for dry matter accumulation (DM),root to shoot ratio (R/S), seedling growth (SG) and root elongation (RE) under water stress.

Source	DF	DM	R/S	SG	RE
		(mg)		(cm)	(cm)
Block	2	2203.0 **	0.01 *	278.4 **	62.0 **
Concentration	2	27229.0 **	0.10	1038.5 **	005.0 **
Error a	4	496.6	0.02	45.5	18.2
Genotype	6	958.0 *	0.01	82.6 **	62.0 **
G x C	12	240.4	0.01	21.4	41.0 **
Error b	36	392.9	0.01	19.6	8.0

**, * significantly different at .01 and .05 level respectively.

TABLE 15. Corn genotype responses for Dry Matter, Root/Shoot ratio, Seedling Growth, and Root Elongation.

GENOTYPES	DM	R/S	SG	RE
H28 x K55	256 a	.79 ab	16.7 a	11.1 a
ML	252 a	.73 b	16.0 a	12.0 a
B73	247 ab	.81 ab	14.7 ab	7.9 b
K724	239 abc	.82 a	10.5 bc	8.1 b
H28	239 abc	.78 ab	14.5 ab	4.6 c
K731 x K41	233 bc	.83 a	10.6 bc	7.5 b
K731	227 c	.83 a	9.1 c	6.1 bc
LSD (.05)	18.95	.08	4.2	2.7

Means within column with the same letter are not significantly different.

While root elongation under drought stress did not seem to distinguish clearly between drought tolerant and drought susceptible genotypes in this study, it appeared that shoot growth could be used to differentiate between them. It is important to mention that hybrids were superior

to their parents for root elongation and shoot growth. In table 15, for example, H28 x K55 performed better than H28 and K731 x K41 was superior to K731, although the differences were not statistically significant.

Fig. 2 showed that at -.3 MPa, inbred ML and the hybrid H28 x K55 were the best in terms of plant growth, and H28 and B73 were intermediate. However, at -.5 MPa, H28 appeared to adjust more to severe stress, followed by the hybrid H28 x K55 and the line ML. B73 again was intermediate. K724, K731 and K731 x K41 were consistently the lowest from -.1 MPa to -.5 MPa.

Dry Matter Accumulation and Root to Shoot Ratio

Dry matter accumulation under stress conditions is of great importance because it involves photosynthesis and thus influences the ability of a plant to produce high yield. Some studies have shown that drought resistant corn strains have higher root/top ratio than drought-susceptible ones (Hague 1955).

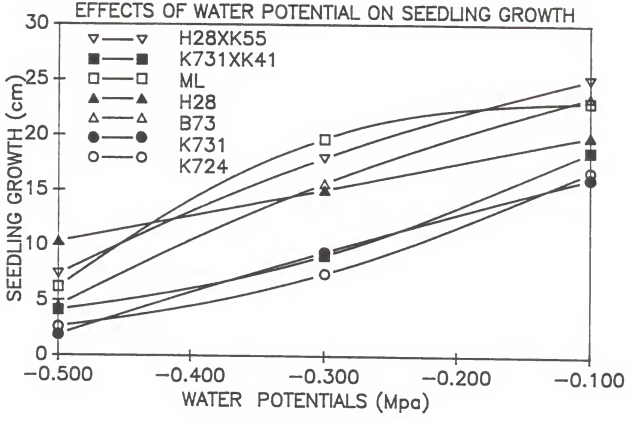

Fig.2: Plant growth as influenced by water potential.

Analyses of variances results for seedling dry weight and root/top ratio are given in table 14. Differences among genotypes were significant for dry matter accumulation, but not for the root/top ratio. The highest ratios were obtained from K724, K731 and K731 x K41. Highest dry matter was obtained with ML and H28 x K55. This result does not agree with Hague's conclusion. High ratios obtained with susceptible lines at seedling stage may be explained by a rapid decrease in susceptible seedlings' top growth under water stress, while they were partitioning more dry matter to the root.

Leaf Blade Area (cm^2) and Seedling Water Content (mg).

Seedling water contents were evaluated on a fresh weight basis as defined in the following formula:

$$\frac{\text{fresh weight - dry weight}}{\text{fresh weight}}$$

Several investigators have shown that some genotypes resist drought by producing less leaf area when stress occurs (Kearney and Shantz, 1911). Varieties with greater numbers of leaves and greater blade area were found to yield more under water stress conditions (Splitter, 1966).

Our results (table 16 and 17) showed no significant differences among genotypes, but leaf blade area declined with decreasing water potential from -0.1 MPa to -0.5 MPa.

Greatest leaf area however was obtained with ML and H28 x K55. Lowest leaf areas were obtained with K724, K731 and K731 x K41 while B73 and H28 were intermediate in the trait. Results showed that B73, H28, ML and H28 x K55 held more water in their tissues and organs thus avoiding desiccation longer. That may be accounted for by the ability of roots of these lines to gather water more efficiently, or by the stomatal opening mechanism to minimize water loss by transpiration. Leaf blade areas and the water-content results appeared to be associated with plant responses to water stress in the field.

TABLE 16. Analyses of variance for leaf area (LA), and seedling water content (WC).

SOURCE	DF	mean squares	
		LA	WC
		(mm^2)	(mg)
Block	1	31.6 *	1622.0 **
Concentration (C)	2	54.7 **	3371.4
Error a	2	0.5	474.1
Genotype (G)	6	7.2	407.3 *
G x C	12	2.7	149.7
Error b	18	4.2	1228.0

**, * significant at .01 and .05 level respectively.

TABLE 17. Mean responses of seven corn sources for leaf area and seedling water content

GENOTYPES	LA	WC
ML	5.4 a	52.2 ab
H28 x K55	5.0 ab	56.6 a
B73	4.5 ab	56.2 a
H28	4.0 ab	50.3 ab
K731	3.2 ab	42.5 bc
K731 x K41	3.0 ab	33.6 c
K724	2.4 b	45.3 abc
LSD (.05)	2.4	13.4

Means within column with the save letter are not significantly different.

Stomatal Density (stomata / mm^2)

The role of stomata in plant water and Co_2 regulation in photosynthesis and transpiration is well established. Early investigations on stomatal frequency were mainly concerned with their effects on transpiration or drought tolerance. Turner (1969) reported that there was no correlation between stomatal number and transpiration in corn canopies. In contrast, Dobrenz et al (1969) found stomatal frequency of six blue panicgrass clones highly and negatively correlated to drought tolerance. A study conducted by Liang et al (1975) in grain sorghum, showed that grain yield was negatively correlated with stomatal density, suggesting that high stomatal frequency may be undesirable for high grain production. Miskin et al (1972) studied barley lines which had either high or low stomatal frequencies and found that, under conditions that favored

high transpiration lines having low stomatal frequencies had higher stomatal resistances and transpired less water than lines with more stomata. Furthermore, high photosynthetic rate was reported for bean varieties with fewer stomata per unit leaf area (Izhar and Wallace, 1967).

We determined stomatal density on the adaxial and abaxial leaf surfaces. Our attention was focused on the number of stomata per unit area on the upper and lower surfaces. We tried to determine the relationship between the ratio of the density of stomata on the adaxial surface to that on the abaxial surface (A:A) and the known drought tolerances of genotypes tested. Results showed that the differences among genotypes were highly significant, table 18 and 19.

Table 19 presents the comparison of means for the genotypes studied and shows that the smallest ratios were obtained with H28, ML and H28 x K55. These genotypes have many more stomates on the abaxial surface compared to the adaxial surface. That characteristic, although related to the genotype, may represent an adaptation which minimizes the loss of water through transpiration. The measurement of stomatal resistance would, perhaps, be more conclusive. However, the stomatal density per se did not differentiate genotypes for their drought tolerance.

TABLE 18. Analyses of variance for stomatal density (stomata/mm²) on both adaxial (AD) and abaxial (AB) surface of the first leaf,and the ratio adaxial to abaxial (A:A).

SOURCE	DF	means squares AB	AD	A:A
Rep	2	32.3	26.7	0.001
Genotypes	6	103.9 **	139.6 **	0.034 **
Error	12	18.6	18.2	0.004

** significantly different at .01 level.

TABLE 19: Genotypic responses for stomata number on the abaxial (AB) and adaxial (AD) surface and the adaxial/abaxial ratio (A:A).

GENOTYPES	AB	AD	A:A
	(st/mm²)	(st/mm²)	
H28	63.9 a	34.4 bc	.54 d
H28 x K55	62.0 ab	39.0 b	.63 dc
K731 x K41	61.9 ab	50.7 a	.82 a
B73	55.2 bc	37.3 b	.67 bc
K724	53.6 c	37.8 b	.69 bc
ML	52.3 c	27.9 c	.53 d
K731	48.4 c	36.9 b	.69 bc
LSD (.05)	7.6	7.5	.10

Means within column with the same letter are not significantly different.

Stress indices

To characterize genotypes by the extent to which they respond to water stress, we computed water stress indices for root development, dry matter accumulation, leaf area and plant growth.

The following formulas were used:

- Root elongation stress index (RESI) =

$$\frac{\text{root length of stressed seedlings}}{\text{root length of control seedlings}} \times 100$$

- Dry matter stress index (DMSI) =

$$\frac{\text{dry matter of stressed seedlings}}{\text{dry matter of control seedlings}} \times 100$$

- Leaf area stress index (LASI) =

$$\frac{\text{leaf area of stressed seedlings}}{\text{leaf area of control seedlings}} \times 100$$

- Plant growth stress index (SGSI) =

$$\frac{\text{(final-initial shoot length) of stressed seedlings}}{\text{(final-initial shoot length) of control seedlings}} \times 100$$

Results, summarized in tables 20 and 21, showed that all the genotypes responded to water stress to about the same extent for root development, dry matter accumulation, and plant growth since differences among genotype were not significant. They did react differently concerning the decrease in leaf area. Even so, inbreds ML, H28 and B73 showed less decrease in root elongation under drought stress. ML had less stress level for dry matter accumulation. H28 x K55, ML, H28 and B73 had less decrease in leaf area under stress conditions, and they also showed

the least decrease in plant growth. The stress indices confirmed that H28, ML, H28 x K55 and B73 were less affected by water stress.

TABLE 20. Analyses of variance for root elongation stress index (RESI), dry matter stress index (DMSI), leaf area stress index (LASI), and seedling growth stress index (SGSI).

SOURCE	DF	means squares			
		RESI	DMSI	LASI	SGSI
Block	2	131.0	197.2	650.8	555.5
Concentration	1	272.6	293.4	2508.0	6969.0
Error a	2	169.8	17.2	160.9	1395.0
Genotypes	6	96.5	110.4	1939.0 *	933.0
G x C	6	98.5 **	16.6	374.6	885.0
Error b	24	95.3	81.6	403.5	554.0

**, * significant at .01 and .05 level respectively.

TABLE 21. Genotypic responses for stress indices.

GENOTYPES	RESI	DMSI	LASI	SGSI
H28	29.5	73.8 b	71 a	71 a
ML	30.5	86.3 a	64 a	60 ab
H28 x k55	22.5	76.3 ab	64 a	52 ab
B73	28.1	78.6 ab	40 ab	44 ab
K724	19.6	79.6 ab	25 b	43 b
K731 x k41	24.3	82.3 ab	25 b	40 b
K731	23.5	75.6 ab	19 b	36 b
LSD (.05)	11.6	10.7	30.9	28.1

Means within column with the same letter are not significantly different.

Simple Correlation Coefficients

Simple correlation coefficients between parameters are given in table 22. Results showed that most of the parameters measured can be used to evaluate seedlings for their drought tolerance. Seedling growth was positively correlated with leaf blade area (.91**), seedling water content (.83**), dry matter accumulation (.91**) and leaf area stress index (.89**), but negatively correlated with the stomata density ratio (-.78*) and the root to shoot ratio (-.76*). Also, these parameters tended to be well correlated among themselves, indicating that drought tolerant genotypes were less affected by water stress in terms of growth, leaf blade areas, water contents and dry matter accumulations. They also developed more shoot, and distributed more stomata on the abaxial surface of the leaf than the adaxial surface.

TABLE 22. Simple correlation coefficients between seedling growth (SG), leaf area (LA), seedling water content (WC), stomata density ratio (A:A), dry matter accumulation (DM), root elongation (RE), root to shoot ratio (R:S), root elongation stress index (RESI), dry matter stress index (DMSI), leaf area stress index (LASI), and seedling growth stress index (SGSI).

	SG	LA	WC	A:A	DM	RE	R:S	RESI	DMSI	LASI	SGSI
SG	.9I**	.83*	-.78*	.91**	.55	-.76*	.54	.09	.89**	.69	
LA		.76*	-.69	.79*	.61	-.79*	.65	.20	.79*	.54	
WC			-.72	.83*	.41	-.55	.33	-.18	.68	.46	
A:A				-.65-	.28	.88**	-.61	-.01	-.89**	-.89**	
DM					.76*	-.66	.25	.23	.70	.44	
RE						-.55	.0I	.64	.30	.03	
R/S							-.68	-.40	-.82*	-.77*	
RESI								.21	.60	.64	
DMSI									-.06	-.09	
LASI										.92**	

**, * significant at .01 and .05 level respectively.

SUMMARY

These studies showed that the responses of the genotypes studied in field conditions, were well correlated to the laboratory results. Genotypes such as H28, and H28 x K55 which were drought tolerant in field studies also showed drought tolerance in the laboratory. Likewise, K731 and K724 were drought susceptible in both environments. The Moroccan line which showed drought tolerance in these studies is

expected to be drought tolerant in the field. Also, it is well established that heat susceptible genotypes are drought susceptible as well. In that regard, B73, known to top fire in heat, was classified in the intermediate group in this study.

It is noteworthy that the hybrid of drought tolerant parents was drought tolerant in this study. In addition, if one of the parents was susceptible, the hybrid was susceptible or less tolerant. The screening method used was simple and rapid. It does not require specialized training, and can be used on corn to predict approximate field responses of several genotypes.

Concerning the parameters measured, the combination of plant growth, leaf area, dry matter accumulation, and the stomatal density ratio can be used to screen for drought tolerance. Polyethylene glycol (PEG) 8000 satisfactorily induced water stress.

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SEEDLING EVALUATION OF GRAIN QUALITY RESPONSES AND DROUGHT TOLERANCE IN MAIZE

by

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ABSTRACT

Seed quality is of great concern to farmers and other seed users. Good quality seed sources are likely to do well under field conditions in terms of germinability, plant vigor and establishment than poor quality sources. Many factors are known to affect seed and seedling characteristics. In regions where moisture stress is common, however, cultivars capable of successful production must possess drought tolerance at the seedling stage as well as at later stages. The objectives of this study were: (1) to determine the effects of three kernel endosperm types i.e. flint, dent and floury on germination, seedling vigor and stand establishment; and (2) to compare seven maize genotypes at the seedling stage for drought tolerance. Part 1 of the study was conducted in the laboratory, greenhouse and the field. Results showed that dent sources were the best performers in all three environments.

The second part of the study was done in the laboratory using polyethylene glycol (PEG 8000) as a moisture stress inducing agent to screen corn genotypes. Results correlated well with those obtained in earlier field studies. Parameters such as leaf area, plant growth, dry-matter accumulation, and stomatal density ratio can be measured to screen for drought tolerance.