CORN SEED GERMINATION AND SEEDLING ELONGATION AS INFLUENCED BY AN AMINO ACID

by

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INTRODUCTION

Increasing essential amino acids such as lysine and tryptophan in the endosperm of corn has been a goal of corn breeders. Breeding high lysine and tryptophan corn has made rapid progress since discovery of the opaque-2 gene. One of the obstacles has been maintaining yield ability as high as normal hybrids without loss of quality. Lysine and tryptophan levels as influenced by the opaque-2 and floury-2 genes are quantatively inherited, therefore, identifying individual plants which possess the favorable genes is necessary to increase frequencies of these genes in the selected populations. Previous studies at Kansas State University have shown that genetically different lines of corn responded differentially to added lysine during germination. This suggested that germination responses might be used as a screening tool to select for certain seed characteristics.

The present study was undertaken to investigate genotypic responses of corn seed germination and subsequent seedling elongation in L-lysine monohydrochloride solution. Relationships between germination responses and levels of lysine and tryptophan in the endosperm were also investigated.

LITERATURE REVIEW

Effects of several amino acids on red clover seed germination have been studied to determine amino acid specificity of a cultivar type for identification purposes (16). It was concluded that the magnitude of inhibition by amino acids was not sufficient to screen cultivar types.

Miflin (21) reported isolated barley embryos showed differential responses in fresh and dry weight accumulation when fifteen amino acids were supplied singly or in combinations under sterile conditions in the culture medium with nitrate. L-lysine significantly reduced the growth of embryos after seven days at 20 C. Arginine, tyrosine, proline, threonine, homoserine, methionine, leucine, valine, and isoleucine also showed significant (P = .05) inhibition of fresh and/or dry weight accumulation. The inhibitory effects of those amino acids were also apparent on root elongation. Similar results were obtained with isolated oats embryos (10).

In oats (10) and barley embryo cultures (21), L-lysine inhibition on seedling growth was partially alleviated by the presence of arginine in the medium. Such metabolic antagonism has been found with other amino acids. Canavanine, a structural analogue of arginine, showed a metabolic antagonism with arginine in immature maize embryo culture (53).

The discovery of the relationship of opaque-2 and floury-2 corn endosperm mutants with high lysine levels in endosperm has brought about extensive studies on the nutritional value and agronomic characters (17, 23, 24, 25, 38, 49). Nass and Crane (24) investigated germination responses of the mutant endosperm genes under near isogenic backgrounds. The opaque-2 gene tended to reduce rates of germination and seedling growth in comparison with normal endosperm type. On the other hand, floury-2 germinated faster than the normal. Sugary-1 and shrunken-1 genes significantly reduced the rates of germination and seedling growth.

According to Mertz, Bates and Nelson (20) amino acid composition of opaque-2 endosperm has been modified in three respects which are amount of acid soluble fraction, amount of zein, and the ratio of zein to

glutelin. Lysine and arginine contents in the opaque-2 endosperm of corn were increased by the changes of protein composition.

Oaks (29) reported that a mixture of amino acids added to the culture medium temporarily supported corn seedling growth and delayed nitrogen loss from the endosperm of germinating seeds. The amino acids supplied in the germinating medium were shown by Oaks and Beevers (27) to be released and transported from endosperm to embryo axis. Miflin (21) and Harris (10) in separate studies indicated that due to the antagonism between lysine and arginine, availability of these amino acids to isolated barley and oats embryos seemed to regulate seedling growth in the culture media. Wright and SRB (53) observed that added lysine relieved differently the inhibition of immature corn embryo growth of different hybrids in the presence of canavanine in the culture medium.

MATERIALS AND METHODS

Open-pollinated varieties from Kansas corn breeding stocks were the main sources of materials for germination screening tests in this study. Twenty seeds from each source ear were treated with Spergon and germinated at 18-20 C on filter paper with germ side up. Petri dishes were placed in a cabinet in a dark room. Five mililiters of L-lysine monohydrochloride solution (7.5 mg/ml in distilled water) were added at the beginning of the test with subsequent additions of 2 ml on the third and sixth day. After seven days individual seeds with developing radicle and plumule were classified as fast germinators, while seeds with no development or slight radicle emergence were designated as slow.

Two plants derived from fast germinating seeds and two from slow germinating seeds of each ear source were planted in breeding plots in 1971. One plant of each selected group was self-pollinated, and the other was used for crossing; crosses were made within and among selected groups of the different sources.

Germination responses of S_1 and F_1 seeds were measured in the spring of 1972. Procedures were the same as with the parental sources except petri dishes were placed inside a humid plastic container to prevent dehydration of seeds during germination. Daily progress of seedling emergence and elongation in lysine solution was noted on twenty seeds of each ear source for eight days. Radicle and plumule emergence were considered to be indicators of germination, while elongation increments of 1 cm were used as indices of seedling growth for line evaluations in the progeny tests. Two replicated progeny tests in lysine solution and distilled water were conducted at 18-19 C in a dark room.

S_4 progenies from the crosses of some Kansas inbred lines and an opaque-2 source were the materials used to study the relationship between lysine and tryptophan levels in the endosperm and germination rates, and rates of seedling elongation in lysine solution. The experimental procedure was similar to that used previously for the progeny tests. Simple correlation coefficients were calculated between daily total rates of seedling emergence and elongation of the progeny lines in the lysine solution and laboratory measurements of lysine and tryptophan content in the endosperm.

The study of the effect of lysine concentrations upon germination and early seedling elongation involved application of 2.5 mg/ml, 5.0 mg/ml,

and 7.5 mg/ml of lysine in distilled water to seeds of inbred lines used. An additional combination of Spergon treated seeds and a lysine concentration of 7.5 mg/ml was also included for each line tested. Germination and seedling elongation responses of endosperm mutant genes, opaque-2 and floury-2, in lysine solution were compared to each normal counterpart in isogenic backgrounds. The experimental procedure was the same as previously used. Three replications for the study of effect of lysine concentrations and two replications for the study of the mutant gene effect at 18-19 C in a dark growth chamber were used for statistical analyses.

The laboratory measurement of tryptophan level in endosperm employed a method described by Hernandez and Bates (1969). Lysine levels were determined by a colorimetric method in which protein is hydrolyzed by papain and a colored complex is formed between lysine and 2-chloro, 3,5-dinitropyridine (private communication, Villegas, A.).

RESULTS AND DISCUSSION

Correlation Studies

Results: Simple correlation coefficients were calculated in Table 1 and Table 2 between levels of lysine and tryptophan in the endosperm and each daily total rate of germination and seedling elongation, and products of all combinations of the four variables. Experiment 1 was conducted in a dark room at about 18-19 C. Experiment 2 and 3 were conducted in a dark growth chamber at 18 C. Experiment 2 was accidentally subjected to a high temperature of 26 C during the period from 24 to 36 hours after

the start of the germination test. Experiment 4 utilized the combined data from experiment 1 and 3.

It appeared that relationships between levels of lysine and tryptophan and responses of germination and seedling elongation were not consistent in all experiments as indicated by the significance tests, Table 1. The rate of plumule emergence on the fifth day and that of plumule elongation on the eighth day were correlated significantly with tryptophan level in experiments 1 and 4. Significant correlations ($P < .01$) were obtained between rate of germination and tryptophan level in experiment 2. Coefficients of variability for lysine and tryptophan levels were lowest in experiment 3. Significant correlations ($P < .01$) between levels of lysine and tryptophan were obtained.

Higher correlation coefficients were obtained between levels of lysine and tryptophan and products of certain combinations of germination and seedling elongation rates, Table 2. Seven combinations of higher rates of germination and/or seedling elongation considered were significantly related to higher level of tryptophan in the endosperm in experiments 1 and 4. Experiment 2 also showed a high relationship of tryptophan level with germination and seedling elongation rates. Lysine level was related significantly ($P < .01$) to high rates of seedling emergence only on the fourth and fifth days in experiment 1.

Discussion: Seed lots which produced (germinated and elongated) their seedling faster tended to have high tryptophan level in the endosperm. Based on the correlation obtained between tryptophan and lysine levels, it appeared that the same seed lots or seeds with high tryptophan level

Table 1. Simple correlation coefficients between daily total percentage rates of germination and seedling elongation and levels of lysine and tryptophan in the endosperm.

Experiment		Daily germination and seedling elongation indices											L/T	C.V.
		4RGM	5RGM	5RGR	5PGM	6RGM	6RGR	6PGM	7RGR	7PGM	8PGM	8PGR		
1	lysine	.394*	.192	.015	.346	.061	.219	.245	.154	.037	.029	.157	.616**	26.97
	trypto.	.389	.379	.215	.559*	.248	.321	.332	.279	.039	.179	.545*		21.84
2	lysine	.243	.147	.203	.320	.082	.138	.105	.336	.121	.129	.134	.572**	21.38
	trypto.	.439*	.597*	.510*	.287	.588*	.489*	.328	.484*	.403	.275	.257		22.63
3	lysine	.152	.019	-.106	-.134	.026	.020	-.117	.112	-.040	-.076	-.108	.590**	19.30
	trypto.	.484*	.221	.298	.135	.058	.304	.107	.213	-.050	-.096	.265		18.19
4	lysine	.301	.154	-.086	.136	.084	.176	.192	.180	.091	.090	.070	.611**	25.12
	trypto.	.355	.293	.225	.429*	.215	.306	.340	.286	.129	.176	.422		20.47

*Significant ($P < .05$).
**Significant ($P < .01$).

Note: RGM refers to radicle emergence, RGR for radical elongation. PGM refers to plumule emergence, PGR for plumule elongation. Numerical prefix indicates duration of germination by days. L/T is for correlation coefficients between lysine and tryptophan levels. C.V. is for lysine and tryptophan levels in each experiment.

Table 2. Correlation coefficients between levels of lysine and tryptophan in the endosperm and products of percentage rates of germination and seedling elongation in all combinations of 4RGM, 5PGM, 6RGR, and 8PGR.

Experiment		Combinations										
		4-5	4-6	4-8	5-6	5-8	6-8	4-5-6	4-5-8	4-6-8	5-6-8	4-5-6-8
1	lysine	.511**	.349	.316	.407	.349	.192	.484*	.431*	.307	.356	.404
	trypto.	.695**	.373	.567**	.639**	.672**	.539*	.658**	.692**	.531*	.679**	.649**
2	lysine	.366	.205	.277	.244	.345	.119	.278	.365	.212	.234	.267
	trypto.	.427*	.519*	.379	.451*	.400	.425*	.454*	.440*	.435*	.460*	.439*
3	lysine	.009	.087	.023	-.138	-.178	-.127	-.051	-.054	-.052	-.209	-.128
	trypto.	.366	.421	.427	.226	.236	.342	.333	.371	.393	.255	.326
4	lysine	.240	.259	.184	.136	.067	.070	.212	.127	.162	.046	.098
	trypto.	.519*	.325	.471*	.444*	.388	.429*	.482*	.418*	.434*	.361	.377

*Significant ($P < .05$).
**Significant ($P < .01$).

might also have higher lysine content in the endosperm. Low coefficients of variability of levels of tryptophan and lysine may explain the poor relationship between levels of amino acids and rates of germination and seedling elongation in experiment 3.

Ingle, Beevers and Hageman (15) reported that amino acids liberated in the corn endosperm in germination were transported immediately to the embryo axis. Oaks and Beevers (27) observed that soluble neutral and basic amino acids were reduced faster from isolated corn embryos cultured in a glucose and inorganic nitrogen medium, than from embryos of the intact seeds. They postulated that synthesizing those amino acids in the isolated corn embryo from inorganic nitrogen sources did not keep up with consumption in protein synthesis for embryo growth. The data obtained herein suggested that endosperm lysine and tryptophan levels had some bearing on embryo growth and subsequently high rates of seedling emergence and elongation.

Selection Studies

Results: Lysine and tryptophan contents of endosperm and rates of seedling emergence of progenies of selected lines from open-pollinated varieties are presented in Table 3 and 4.

Mean endosperm tryptophan levels of fast germinating selfs and crosses (13 combinations of fast x slow, 4 of slow x fast) of selfed progenies from 17 ear sources were significantly higher than those of the slow germinating group according to the t-tests shown in Table 3. Reciprocal crosses were identified as A or B. The A's were relatively high and B's

Table 3. Endosperm lysine and tryptophan contents and the rates of seedling emergence responses at 18-19 C of 4RGM, and 5PGM to lysine solution in the S_1 and F_1 progenies.

O.P source	Fast				Slow				Cross			
	T2/ ug/ml1/	L3/ ug/2ml1/	4RGM (%)	5PGM (%)	T	L	4RGM	5PGM	T	L	4RGM	5PGM
43-1	8.5	230	0.0	0.0	6.5	215	42.5	2.5	8.5	212	17.5	2.5
43-2	6.2	150	17.5	5.0	6.8	145	2.5	0.0	8.6	217	12.5	0.0
43-3	9.1	245	25.0	0.0	8.1	260	15.0	0.0	9.5	270	0.0	0.0
43-4	6.2	190	5.0	0.0	6.8	185	17.5	0.0	7.8	200	2.5	0.0
43-5	9.1	240	0.0	0.0	9.4	230	7.5	0.0	7.7	215	10.0	0.0
43-6	7.2	217	10.0	0.0	6.1	212	2.5	0.0	7.3	225	0.0	2.5
43-7	7.8	238	0.0	2.5	6.8	195	0.0	0.0	5.8	208	0.0	0.0
43-8	6.4	230	0.0	0.0	5.5	190	0.0	0.0	7.0	225	2.5	0.0
42-1	7.8	255	0.0	0.0	5.5	230	5.0	0.0	6.4	243	2.5	0.0
42-2	9.5	226	2.5	0.0	7.7	255	0.0	0.0	7.8	226	0.0	0.0
57-1	7.8	210	22.5	0.0	6.9	226	0.0	0.0	7.3	217	0.0	0.0
57-2	10.5	242	0.0	0.0	9.2	217	0.0	0.0	7.8	208	10.0	0.0
80-1	6.2	226	22.5	0.0	6.2	208	2.5	0.0	7.7	240	7.5	0.0
95-1	7.1	208	70.0	42.5	6.7	185	97.5	52.5	10.2	282	30.0	2.5
38-1	7.3	230	0.0	0.0	6.2	195	77.5	20.0	6.9	230	9.5	0.0
36-1	8.5	255	25.0	0.0	6.4	217	2.5	0.0	8.0	217	32.5	0.0
54-1	---	---	0.0	0.0	---	---	2.5	2.5	---	---	2.5	0.0
$\bar{X}$ =	7.82 ±.69	224 ±14.0	11.76	2.94	6.92 ±.60	210 ±15.0	15.14	4.70	7.76 ±.60	227 ±12.2	9.11	0.44

t-tests

between	lysine	tryptophan
fast and slow	t_c = 1.468^{ns}	t_c = 2.080*
cross and slow	= 1.841	= 2.095*
fast and cross	= 0.305^{ns}	= 0.132^{ns}

* ($P < .05$).

1/Hydrolyzate of papain solution.
2/Lysine level.
3/Tryptophan level.

Table 4. Endosperm lysine and tryptophan contents and the rates (%) of seedling emergence responces of 4RGM, and 5PGM to lysine solution in reciprocal progenies.

	O.P source	T ug/ml	L ug/2ml	4RGM (%)	5PGM (%)
Slow					
A.	30-1 x 43-10	6.5	208	82.5	15.0
B.	43-10 x 30-1	5.5	210	5.0	5.0
A.	38-2 x 43-11	8.5	200	15.0	0.0
B.	43-11 x 38-2	8.0	208	0.0	0.0
A.	43-22 x 43-13	6.1	195	17.5	0.0
B.	43-13 x 43-12	7.7	244	0.0	0.0
A.	43-14 x 54-2	6.7	207	60.0	0.0
B.	54-2 x 43-14	6.0	180	0.0	0.0
A.	01-1 x 43-15	8.4	227	77.5	50.0
B.	43-15 x 01-1	8.2	255	50.0	2.5
A.	115-1 x 93-1	6.9	212	7.5	0.0
B.	93-1 x 115-1	8.3	230	0.0	0.0
Fast					
A.	57-3 x 30-2	7.0	227	55.0	0.0
B.	30-2 x 57-3	6.3	183	25.0	2.5
A.	82-1 x 108-1	7.8	217	27.5	2.5
B.	108-1 x 82-1	8.4	183	20.0	0.0
A.	57-4 x 6-1	7.9	240	42.5	10.0
B.	6-1 x 57-4	8.2	243	32.5	0.0
A.	108-2 x 30-3	8.2	244	67.5	10.0
B.	30-3 x 108-2	6.7	184	12.5	0.0

A. $\bar{X} = 7.4 \pm .614 = 217.7 \pm 11.74 = 45.25 = 9.75$
B. $\bar{X} = 7.33 \pm .85 = 212.0 \pm 20.86 = 14.50 = 1.00$

t-tests
$t_c = 1.591^{ns} \quad = 0.538^{ns}$

were low in rate of radicle emergence on the fourth day. Ten combinations of reciprocal crosses made between slows, or fasts did not show mean differences in endosperm lysine and tryptophan levels, suggesting that no appreciable differences were found in endosperm lysine and tryptophan content among originally selected groups.

The rates of seedling emergence in lysine solution differed among strains and progenies of selected groups within each ear source, Table 3. Generally slower seedling emergence rates on the fourth and fifth days, Tables 3 and 4 indicated lower levels of tryptophan and lysine in the endosperm, based on the relationship obtained in the previous section. A few exceptions were noted, for example, selection O.P 95-1 and 01-1 x 43-15 gave fast germination responses, but were not high in endosperm lysine and tryptophan.

Discussion: In general, germination of the S_1 and F_1 progenies of selected groups was slow at 18-19 C in lysine solution and distilled water (Table 11 in appendix). Using a lysine germinating medium and relative rates of germination and seedling elongation as selection criteria, greater selection effectiveness was obtained with respect to tryptophan than lysine content in the endosperm. The observed mean differences obtained in tryptophan level from the different germination response groups indicated such a relationship in the correlation studies. Pinnell (1949) reported that maternal influence in a cold test of corn seed germination was greater than paternal influence in reciprocal crosses. Mean endosperm lysine and tryptophan levels of both classified groups based on rate of radicle emergence in ten combinations of reciprocal crosses were not different in

spite of apparently different responses in germination. Fast germination of some lines could not be explained on the basis of the amino acid levels in the endosperm. It was noted, however, that responses of seedling emergence rates in lysine solution were different and might be associated with seed characteristics other than lysine and tryptophan levels in the endosperm.

Effects of lysine on rates of germination and seedling elongation

Results: Lysine Concentration Mean germination responses of inbred lines to all treatments of lysine solutions and distilled water, and mean effects of each treatment to all inbred lines tested are presented in Table 5. Significantly higher germination rates in the responses of K-801 and K-812 were found. K-802, K-201G, and K-41 showed significantly slower rates of germination and seedling elongation. Endosperm lysine and tryptophan levels of K-812, K-802, K-201G, and K-41 seemed to correspond to their responses of germination and seedling elongation in lysine solution. However, the high rates of germination and seedling elongation of K-801 was not reflected in its levels of these amino acids.

Added lysine appeared to inhibit rates of germination and seedling elongation, Table 6. There was no significant effect of the use of higher lysine concentrations upon the rate of radicle emergence on the fourth day. On the other hand, plumule emergence and elongation rates on the fifth and eighth days, respectively, were decreased as concentrations of added lysine increased. The degree of inhibition which resulted from

gradual increase of lysine concentration in the germinating medium increased linearly when treatments were equally spaced, Tables 7, 8, and 9.

Results: Responses of Genotypes Three isogenic lines with floury-2 or opaque-2 endosperm mutant gene were tested for germination response in lysine solution and in distilled water, Table 10. In lysine solution, a significant difference ($P < .05$) was observed only between slower germination rate of floury-2 endosperm type and that of normal in the genetic background of Oh 07. Floury-2 genotype with Oh 43 background germinated faster than normal in distilled water, but it was slower than normal in that of Oh 07. The line (B 37) with opaque-2 genotype appeared to delay plumule emergence as compared with the normal counterpart in both lysine and water germinating media. Since different genetic backgrounds seemed to alter responses of the mutant genotype in germination, it was difficult to conclude a specific effect of the mutant endosperm genes on rates of germination.

Discussion

Differential responses of inbred lines germinated in lysine solution at 18-19 C were observed. Higher rates of germination did not always indicate corresponding high levels of tryptophan and lysine in the endosperm. The data indicated that corn seed germination might not be strongly related to lysine and tryptophan levels in the endosperm. The magnitude of inhibiting corn seed germination by lysine increased linearly as concentrations increased. Nass and Crane (24) studied endosperm mutant gene effects on germination in nearly the same genetic backgrounds. The present study revealed that a specific effect of endosperm mutant gene on

Table 5. Mean percentage of seedling emergence and elongation of inbred lines to all treatments of lysine solution.

Lines	4RGM	5PGM	8PGR	T (ug/ml)*	L (ug/2ml)*
K-801	89.33	80.33	77.66	7.8	243
K-812	90.33	62.44	60.99	15.5	344
K-802	30.33	16.66	32.99	7.9	243
K-201G	15.33	14.33	27.33	6.1	245
K-41	38.33	15.00	37.33	6.3	245
LSD .01	19.39	16.69	21.50		

*Hydrolyzate of papain solution.

Table 6. Mean percentage of seedling emergence and elongation of all inbred lines tested in response to each treatment of lysine monohydrochloride solution.

Treatments	4RGM	5PGM	8PGR
H_2O	56.66	50.33	65.99
2.5 mg/ml*	57.66	44.33	52.66
5.0 mg/ml	54.66	36.99	49.66
7.5 mg/ml	50.99	30.33	37.33
7.5 mg/ml + Spergon	43.44	26.66	30.66
LSD .01	19.39	16.69	21.50

*In distilled water.

Table 7. Analysis of variance on rate of radicle emergence on the fourth day.

Source	D.F	SS	MS	F
Inbred lines	4	73143.328	18285.808	47.952**
Lysine concent.	4	2023.333	505.833	1.326
linear	1	1666.688	1666.688	4.370
residual	3	356.645	118.819	0.311
Inbreds x lysine concent.	16	2633.315	164.582	0.432
Error	50	19066.808	381.335	
Total	74	96866.781		

**Significant (P < .01).

Table 8. Analysis of variance on rate of plumule emergence on the fifth day.

Source	D.F	SS	MS	F
Inbred lines	4	58921.251	14730.312	52.111**
Lysine concent.	4	5701.322	1425.330	5.042**
linear	1	5642.666	5642.666	19.042**
residual	3	58.656	19.552	0.069
Inbreds x lysine concent.	16	2508.664	156.791	0.555
Error	50	14133.468	282.669	
Total		81264.705		

**Significant (P < .01).

Table 9. Analysis of variance on rate of plumule elongation on the eighth day.

Source	D.F	SS	MS	F
Inbred lines	4	27184.616	6796.152	14.501**
Lysine concent.	4	11401.308	2850.325	6.082**
linear	1	11093.985	11093.984	23.671**
residual	3	307.319	102.439	0.218
Inbreds x lysine concent.	16	2795.328	174.708	0.373
Error	50	23433.398	468.667	
Total	74	64814.646		

**Significant (P < .01).

Table 10. Mean percentage germination responses of endosperm mutants and normal types in isogenic backgrounds.

Lines	Genotype 1/	Lysine (7.5 mg/ml)		Distilled water		T	L
		4RGM	5PGM	4RGM	5PGM	ug/ml	ug/2ml
Oh 43	n	12.5	15.0	12.5 *	20.0 *	6.1	194
Oh 43	fl_2	20.0	7.5	50.0	60.0	7.8	228
Oh 07	n	90.0 *	92.5 *	80.0	92.5 *	8.9	220
Oh 07	fl_2	50.0	22.5	60.0	50.0	11.5	370
B 37	n	62.5	37.5	97.5	80.0 *	8.7	262
B 37	o_2	87.5	12.5	90.0	45.0	15.2	328

*Significant (P < .05).
1/n = normal endosperm.
fl_2 = floury-2
o_2 = opaque-2

germination in lysine solution could not be detected so that differences in genetic backgrounds were assumed to influence or confound the genotypic expression on germination.

CONCLUSIONS

Differential magnitudes of the inhibitory effect of L-lysine monohydrochloride on corn seed germination at 18-19 C were observed among genetically different seed lots. These differences were analyzed to investigate relationship of lysine and tryptophan levels determined in the endosperm including pericarp to the degrees of inhibition of germination and seedling elongation in lysine solution.

Significant correlation between rate of seedling emergence and level of tryptophan in the endosperm was obtained in S_4 progenies of crosses between opaque-2 source and Kansas inbred lines. Lysine and tryptophan levels were highly correlated also. Progenies of open-pollinated varieties and a few inbred lines tested showed some seeds could germinate fast regardless of low levels of lysine and tryptophan in the endosperm.

Seedling selection from open-pollinated varieties based on relative germination and seedling elongation rate in lysine solution at a concentration of 7.5 mg/ml in distilled water at 18-19 C was effective in increasing mean endosperm tryptophan levels of S_1 progeny of fast germinators and F_1 crosses including 13 combinations of fast x slow, and 4 of slow x fast as compared to the mean of S_1 progeny of slower germinators. Some reciprocal progenies appeared to be different in rate of seedling emergence in lysine solution at 18-19 C.

The magnitude of inhibition of corn seed germination and seedling elongation by lysine solutions increased linearly as concentrations increased (2.5 mg/ml, 5.0 mg/ml and 7.5 mg/ml in distilled water). The degree of inhibitory effect of lysine in a germinating medium on several inbred lines tested at 18-19 C differed significantly, depending on their genetic compositions. Effects of endosperm mutant genes, floury-2 and opaque-2, on rate of seedling emergence were not clear, so it appeared that these gene effects were apparently influenced by or confounded with different genetic backgrounds.

It was concluded that the germination test in lysine solution at 18-19 C as used in this study was not effective for selecting high lysine and tryptophan corn seeds.

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APPENDIX

Table 11. Mean percentage radicle emergence (on the fourth day) of S_1 and F_1 progenies of selected lines in distilled water at 18-19 C.

O.P source	Fast	Slow	Cross
43-1	2.5	50.0	42.5
43-2	5.0	22.5	25.0
43-3	7.5	25.0	0.0
43-4	12.5	5.0	27.5
43-5	10.0	10.0	30.0
43-6	12.5	5.0	0.0
43-7	15.0	2.5	0.0
43-8	2.5	0.0	0.0
42-1	2.5	37.5	20.0
42-2	7.5	0.0	0.0
57-1	45.0	20.0	2.5
57-2	0.0	0.0	25.0
80-1	30.0	10.0	35.0
95-1	90.0	97.5	50.0
38-1	2.5	80.0	17.5
36-1	50.0	2.5	57.5
54-1	10.0	0.0	15.0

Table 12. Analysis of variance on the germination responses of endosperm genotypes in isogenic lines at 18-19 C.

Source	D.F.	SS	M.S.	F.
4RGM in lysine solution (7.5 mg/ml)				
Lines	2	8487.500	4243.750	5.580*
Genotypes	3	2281.250	760.4161	6.186*
Error	6	737.500	122.916	
4RGM in distilled water				
Lines	2	7962.500	3981.250	6.412*
Genotypes	3	1862.500	620.833	8.764*
Error	6	425.000	70.833	
5PGM in lysine solution (7.5 mg/ml)				
Lines	2	4512.500	2256.250	1.212
Genotypes	3	5581.250	1860.416	12.942**
Error	6	862.500	143.750	
5PGM in distilled water				
Lines	2	2079.169	1039.584	0.673
Genotypes	3	4631.250	1543.750	25.551**
Error	6	362.500	60.416	

*Significant ($P < .05$).
**Significant ($P < .01$).

CORN SEED GERMINATION AND SEEDLING ELONGATION AS INFLUENCED BY AN AMINO ACID

by

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This study was undertaken to investigate genotypic responses of corn seed germination and subsequent seedling elongation in L-lysine monohydrochloride solution. Relationships between germination responses and levels of lysine and tryptophan in the endosperm were also investigated.

Open-pollinated varieties from Kansas corn breeding stocks were the main sources of materials for germination screening tests in this study. Some Kansas inbred lines, crosses of an opaque-2 source by Kansas inbred lines, and other lines isogenic for opaque-2 were also tested. Twenty seeds from each source ear were treated with Spergon and germinated in the dark at 18-20 C on filter paper with germ side up in petri dishes. Five milliliters of L-lysine monohydrochloride solution (7.5 mg/ml in distilled water) were added at the beginning of the test with subsequent additions of 2 ml on the third and sixth day. After seven days seeds with developing radicle and plumule were classified as fast germinators, while seeds with no development or slight radicle emergence were designated as slow.

Tryptophan levels and lysine levels were determined by a colorimetric method in which protein is hydrolyzed by papain.

Differential magnitudes of the inhibitory effect of L-lysine monohydrochloride on corn seed germination at 18-19 C were observed among genetically different seed lots. These differences were analyzed to investigate relationship of lysine and tryptophan levels determined in the endosperm including pericarp to the degrees of inhibition of germination and seedling elongation in lysine solution.

Significant correlation between rate of seedling emergence and level of tryptophan in the endosperm was obtained in S_4 progenies of crosses between opaque-2 source and Kansas inbred lines. Lysine and tryptophan levels were highly correlated also. Progenies of open-pollinated varieties and a few inbred lines tested showed some seeds could germinate fast regardless of low levels of lysine and tryptophan in the endosperm.

Seedling selection from open-pollinated varieties based on relative germination and seedling elongation rate in lysine solution at a concentration of 7.5 mg/ml in distilled water at 18-19 C was effective in increasing meat endosperm tryptophan levels of S_1 progeny of fast germinators and F_1 crosses including 13 combinations of fast x slow, and 4 of slow x fast as compared to the mean of S_1 progeny of slower germinators. Some reciprocal progenies appeared to be different in rate of seedling emergence in lysine solution at 18-19 C.

The magnitude of inhibition of corn seed germination and seedling elongation by lysine solutions increased linearly as concentrations increased (2.5 mg/ml, 5.0 mg/ml and 7.5 mg/ml in distilled water). The degree of inhibitory effect of lysine in a germinating medium on several inbred lines tested at 18-19 C differed significantly, depending on their genetic compositions. Effects of endosperm mutant genes, floury-2 and opaque-2, on rate of seedling emergence were not clear, so it appeared that these gene effects were apparently influenced by or confounded with different genetic backgrounds.

It was concluded that the germination test in lysine solution at 18-19 C as used in this study was not effective for selecting high lysine and tryptophan corn seeds.