# **Environmental Influences on Wheat Lysine Content**

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The lysine content of wheat flour protein, as well as the protein content itself, was unaffected by the addition of 12.5 or 25 cm of added irrigation water, or by 30 or 47% shade during the growing season. Comparisons were conducted on Thatcher and Idaed 59 spring wheat varieties grown at Lind and Pullman, Wash., respectively, in two separate years. Furthermore, the lysine content of the spring wheat varieties NapHal and CI 6127 was unaffected in growth chamber experiments by increased air temperatures during the growing period as compared with a simulated normal schedule of temperatures. The protein content of CI 6127 grown at the higher temperatures was significantly greater.

Extensive studies have been made in recent years in an effort to identify wheat varieties sufficiently high in lysine to be useful for breeding more nutritious wheat (Johnson and Mattern, 1972). Although some progress has been made, high protein wheats are more easily found than those whose protein is unusually high in lysine (Johnson and Lay, 1974). Furthermore, varieties that appeared to be high in lysine in the initial studies usually were no longer high when regrown in different locations or years (Johnson and Mattern, 1972, pp 64, 67). Clearly, it would be of value to wheat breeders to know what environmental factors during the growing cycle of the plant are responsible for variability in lysine content. Conceivably, it might also be possible deliberately to influence the lysine level through agronomic practices.

It is well known that the protein level of wheat can be raised by nitrogen fertilization (Sosulski et al., 1963), and many studies (Price, 1950, was one of the earliest) have shown that the lysine content of the protein generally decreases as the protein level increases. However, no specific studies have been made to determine the influence of any other environmental factors on wheat lysine levels. This report describes studies on the effects of soil moisture, shading, and air temperature conducted under controlled conditions.

#### MATERIALS AND METHODS

Field Studies. Experimental plots were set up at Lind, Wash., in 1969 and 1970, and in Pullman, Wash., in 1969 and 1971 to measure the possible effect of shading and of added water on the lysine content of wheat. An area approximately  $35 \times 25$  m was selected at each location having very uniform soil structure and slope. Within each area, 15 sub-plots  $2.5 \times 2.5$  m (6 rows) were laid out. Three each of these were assigned, by random methods, for treatment with 30% shading, 47% shading, 12.5 cm of added water, 25 cm of added water, and as control sub-plots.

For the shading, plastic screening material with the specified percentage of shading was stretched on a wooden framework about 1.5 m above ground level to cover an area  $3.6 \times 4.2$  m. This was put in place when the wheat was from 6 to 10 cm tall. At least half the sub-plot in the east-west direction was shaded for 6 to 6.5 hr in late May and mid-June, and from north to south all but about 0.4 m on the south side. Water was applied from sprinkler heads placed on 1.2 m standpipes and directed upward. This was done on windless days, when coverage was quite even over an area with a radius of at least 2 m. Soil cores

	Lind 1969 <sup>b</sup> 14 April	Lind 1970 <sup>b</sup> 3 April	Pull- man 1969 <sup>b</sup> 9 May	Pullman 1971 <sup>b</sup> 22 April
First Water Treatment,	12.5 ci	n Applie	ed to 6	Sub-plots
Days after planting	44	58	40	76
Cumulative precip- itation (cm) from previous September	26.6	22.9	57	54
Maximum soil moisture, <sup>a</sup> %	11.7	15.1	24.1	
Second Water Trea	atment,	12.5 cm	Additi	ional
Applied to 3	3 of Ab	ove Sub-	plots	
Days after planting	66	76	67	104
Cumulative precip- itation (cm) from previous September	26.9	23.4	58.3	55.8
Maximum soil moisture $a$ %	9.4	14.7		17.0

<sup>a</sup> The maximum soil moisture was at about 1.2 m depth at the time of the first watering, and at about 1.5 m depth at the second watering. <sup>b</sup> Experiment and planting date.

to a depth of 1.6 to 2 m were taken with a King tube before each watering, and composited samples at 0.3-m depth intervals were later analyzed for moisture content.

Pullman (and Lind, even more so) is in an area of low precipitation and high insolation in summer, depending largely on stored soil moisture for wheat production. The hard red spring wheat variety, Thatcher, was used for the experiments in Lind. The total precipitation for 1969 (Sept 1968 to Sept 1969) was 27.2 cm, while that for the corresponding 1970 period was 24.2 cm. The soft white spring wheat variety, Idaed 59, was used for the Pullman experiments. The total comparable 1969 precipitation for Pullman was 58.5 cm, and for 1971 it was 59 cm. Table I gives the watering schedules, including cumulative precipitation data (from the previous September), and soil moisture conditions.

Wheat was harvested by hand from approximately the center half of the two or three center rows of each sub-plot. It was threshed and cleaned in experimental machinery and the cleaning was completed by hand. Test weights were determined as the weight of wheat to fill a beaker of known volume, after the procedure was checked against a number of samples of known test weight. The wheat was milled in a Brabender Quadrumat Junior mill. The material passing an 80-mesh screen was taken as flour. Nitrogen was determined by the Kjeldahl method, and lysine on a Technicon TSM amino acid analyzer.

Growth Chamber Studies. Wheat was grown in four steel boxes each containing Palouse silt loam soil with a

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Table II.	Schedule	of	Growing	Conditions	in	Growth	Chambers
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		Air tempe	erature, °C				
Time at which	D	ay	Ni	ght	Soil	Dav	
made, days Norm	Normal	High	Normal	High	temp, °C	length, hr	
Initial	16.2	± 0.7	11.2	± 0.2	11	14.5	
30			100 01		13	15	
60	$18.8 \pm 0.5$	$23.1 \pm 1.3$	$13.0 \pm 0.1$	$15.2 \pm 2.0$	16	16	
92	$27.8 \pm 0.6$	$31.2 \pm 0.9$	$15.2 \pm 3.7$	$22.7 \pm 2.4$	23	15.5	
130	Harvest of hig	h temperature whea	it				
138	Harvest of nor	mal temperature wi	heat				

Table III. Protein and Lysine Contents of Wheat Flours from Shaded and Watered Plots<sup>a</sup>

	Control plots	12.5 cm added water	25 cm added water	30% shade	47% shade	Std dev
Lind 1969						
% protein <sup>b</sup>	16.1	15.7	16.6	16.0	14.8	0.52
g of lysine/100 g of protein	2.09	2.20	2.16	2.05	2.22	0.13
Test weight, lb/bushel	58.1	58.1	58.6	58.8	58.2	0.63
Lind 1970						
% protein <sup>b</sup>	16.1	16.0	16.1	16.2	16.5	0.62
g of lysine/100 g of protein	2.22	2.27	2.32	2.17	2.13	0.11
Test weight, lb/bushel	55.4	54.6	54.8	55.5	55.8	1.21
Pullman 1969						
% protein <sup>b</sup>	11.1	10.8	10.4	12.2	11.2	0.71
g of lysine/100 g of protein	2.48	2.54	2.56	2.35	2.42	0.13
Test weight, lb/bushel	61.4	60.9	61.2	62.0	62.0	0.94
Pullman 1971						
% protein <sup>b</sup>	11.4	11.4	11.4	11.3	12.0	0.76
g of lysine/100 g of protein	2.38	2.32	2.31	2.33	2.28	0.22
Test weight, lb/bushel	58.8	58.0	58.0	58.6	59.1	0.37

<sup>a</sup> Mean values from triplicate plots. Duplicates for 47% shade in Pullman 1969, since one of the sub-plots was accidentally partially flooded on one occasion. <sup>b</sup> N  $\times$  5.7; 14% moisture basis.

surface  $75 \times 52$  cm and a soil depth of 80 cm. Partridge and Shaykewich (1972) caution against using small soil volumes. Water was circulated through the walls of the boxes to maintain controlled temperatures. The space above the boxes was enclosed in clear polyethylene film sealed to the boxes, and supported on a metal framework. Controlled temperature air was circulated through this space. Air temperature was measured continuously by a sensor hung in the middle of the space, and soil temperature by a sensor buried about 10 cm below the surface. The boxes were lighted by a bank of 16 Gro-lux 215-W WS/VHO fluorescent lights about 1.3 m above the soil surfaces, isolated by a sheet of mylar, as well as the polyethylene enclosure, and by six 60-W incandescent lamps above the enclosure and about 1 m above the surface. The illuminance at the soil surface fell from about 14000 to 10000 lx as the lamps aged during the course of the experiment.

Deionized water was added periodically through the bottom of the boxes by a gravity feeding system. A layer of sand about 7 cm deep in the bottom of the boxes aided in horizontal distribution of the water.

In two of the boxes, conditions of air and soil temperatures and day lengths were arranged to be representative of those in the field. In the other two boxes, air temperatures were several degrees higher during the latter part of the growing season. Table II gives the schedules for the growing conditions. Figures for standard deviations are included to show that while temperature controls usually functioned efficiently, some problems occurred during certain periods.

The spring wheats, NapHal (PI 176217) and CI 6127, were planted in three rows each per box. Twelve days later each row was thinned to approximately 14 plants. NapHal has shown promise for both high protein and high lysine qualities in the Nebraska studies (Johnson and Mattern, 1975), while the lysine level of CI 6127 appeared to be strongly influenced by environment (Johnson and Mattern,

Table IV. Protein and Lysine Contents of Wheats Grown at Different Temperatures<sup>a</sup>

	NapHal		CI 6127	
	Whole wheat	Flour	Whole wheat	Flour
% protein <sup>b,c</sup>				
Normal temperatures	17.7	15.3	13.7	12.9
Higher temperatures % lysine in protein <sup>d</sup>	18.0	16.0	16.4	14.8
Normal temperatures Higher temperatures	$3.4 \\ 3.3$	2.6 2.6	3.2 3.2	$\begin{array}{c} 2.22 \\ 2.54 \end{array}$

<sup>a</sup> Mean values from duplicate experiments. <sup>b</sup> N  $\times$  5.7; 14% moisture basis. <sup>c</sup> Standard deviation = 1.06.

<sup>d</sup> Standard deviation = 0.15.

1972, p 67). Based on a soil analysis, calculated amounts of ammonium nitrate and 16-20-0 fertilizers were added along each row 17 days after planting, and additional ammonium nitrate was supplied at the midpoint of the growing season.

The wheat was harvested and threshed by hand. Test weights were measured, and the flour was obtained as described above.

## RESULTS

**Field Studies.** Table III gives the results for the field studies of the effects of shading and supplemental watering. Analyses of variance were carried out for each year and location separately and then combined. No significant differences (5% level) in protein or lysine levels of the flour protein or in test weights of the wheat occurred for any of the treatments. Since no differences in protein levels were caused by any of the treatments, it was not necessary to make allowances for the effect of protein content on the lysine levels.

Growth Chamber Studies. The effects of an altered temperature regimen on the composition are shown in Table IV. In this experiment, analyses were done on both whole wheat and flour. Higher growing temperatures did

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result in increasing the protein content of the wheat, though the analysis of variance showed that the increase for NapHal was not statistically significant at the 5% level. This effect of temperature has been noted before (Sosulski et al., 1963). However, there was no significant effect of temperature on the lysine content of the protein in flour or whole wheat of either variety. The difference in the case of flour from CI 6127 approaches statistical significance at the 5% level, but it is unlikely to be real since there was no difference in the whole wheat levels of lysine in protein.

The growth and maturation of the wheat appeared to be fairly normal, although normal for NapHal is not very good. The rate of air circulation necessary to control air temperature evenly caused some lodging.

## DISCUSSION

It is well established that many wheat varieties which produce high lysine grain when grown in one location do not do so in another. The results reported here indicate that this is not due simply to moisture supply, insolation, or air temperature alone. The variations in these factors explored in this work are fairly representative of those occurring in wheat growing areas, so that it is unlikely, though possible, that more extreme variations might have had an effect. The lysine differences in different locations hence remain unexplained. Soil differences could be the explanation, but the difficulties of setting up simulations of natural soil profiles under controlled conditions are intimidating. Furthermore, it may well be that a combination of conditions is the controlling factor.

The experience of the Nebraska workers seems to show that some varieties are more responsive than others to environmental effects on their lysine levels. The varieties used here may not have been such susceptible ones, although CI 6127 was chosen with this in mind. The results for NapHal, incidentally, bore out its reputation as a high-protein, high-lysine variety, as well as one with poor agronomic characteristics.

For wheat irradiated at 2.5 and 5.0 cal  $cm^{-2} hr^{-1}$  in growth chamber experiments, Partridge and Shaykewich (1972) reported higher yields and lower protein levels at the higher light intensity. They found no effect from variation in water supply. Natural sunlight, even when shaded, apparently supplies enough light that protein production is not limited.

The effect of growing temperatures on the protein levels in the growth chamber experiment might lead one to expect lower lysine to protein ratios at the higher temperatures. However, the protein levels were well above the point at which there is no longer a predictable correlation of protein content with the amount of lysine in the protein (Lawrence et al., 1958).

## ACKNOWLEDGMENT

The author wishes to express his appreciation to R. E. Davis and Hedwig E. Herrick for technical assistance; to C. Oldenburg and D. R. Coahran for aid in setting up the experimental facilities; to A. R. Halvorson for a soil analysis; to R. E. Allan, C. F. Konzak, W. L. Nelson, and M. Nagamitsu for help with the field studies; to F. E. Koehler and W. H. Gardner for advice on methods of conducting the growth chamber experiment; and to V. A. Johnson for providing seed of the NapHal and CI 6127 wheat varieties.

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Received for review August 4, 1975. Accepted October 20, 1975. Scientific Paper No. 4484, Projects 1910 and 0185 from College of Agriculture Research Center.