Gene for Improved Nutritional Value in Barley Seed Protein

Abstract. *Genetically dependent 20 to 30 percent increase in lysine per 16 grams of nitrogen results in improved nutritional values in feeding trials with mice and rats. The recessive gene was selected from the World Barley Collection. Other amino acids are also influenced by the gene. Protein content segregates independently of the changed amino acid pattern. The gene putatively influences the matrix proteins, which characteristically adhere to the starch grains in meal preparations. The morphological character permits rapid microscopic screening of single seeds without affecting viability. Low yield is considerably improved by crossing and selection.*

Cereals have a low content of amino acids such as lysine, methionine, threonine, and valine that are essential for monogastric animals. A high-protein, high-lysine (Hiproly) barley cultivar, CI 3947, was selected (*1*) from the World Barley Collection (*2*) with the dye-binding-capacity (DBC) screening technique as an estimate for basic amino acids, among them lysine. The acid dye Acilane Orange G (monosodium salt of 1-phenylazo-2-naphthol-6-sulfonic acid) combines at *p*H 2.6 with basic groups from these amino acids in the cereal proteins in suspension. The method is not completely selective for basic amino acids (*3*).

Lysine, threonine, valine, methionine, isoleucine, alanine, glycine, and aspartic acid were higher in Hiproly barley, whereas cystein, glutamic acid, proline, and NH_3 (after hydrolysis) were lower when compared with other varieties with comparable protein contents (16 to 18 percent dry matter) (*1, 4*). These differences were recorded in material from different localities in the United States and Europe (*1*). The question arises to what extent the changed amino acid composition of Hiproly could be used to produce high-yielding, nutritious barley varieties.

Hiproly is of Ethiopian origin (*5*). It is an erectoid type with naked, slightly shriveled seeds (1000 kernel weight about 40 g) and a long-day photoperiod. The endosperm is hard in Hiproly. The average number of seeds per spike is low (about 12), partly owing to sterility. Thus, Hiproly does not satisfy the requirements of modern agriculture at the present time—particularly with respect to yield.

A sister line, CI 4362, to Hiproly was isolated from the same original seed batch (*5*). This line has a similar habit, but it has smooth seeds that are heavier (1000 kernel weight about 50 g). The protein content is comparable to Hiproly, but it has a normal amino acid composition (*5*). Both lines, which were grown in the United States (*5*), were analyzed. The protein content was 19.6 percent and the lysine content was 4.1 percent for Hiproly as compared with 18.7 and 3.2 percent, respectively, for CI 4362. Normal, low-lysine cultivars and Hiproly, grown in Sweden, were also included in the investigation that included histological examination, fertilizer experiments, animal feeding trials, and genetic analyses.

The aleurone layer, endosperm cells, matrix proteins, and protein bodies were studied with the light microscope in 10-μm sections and in meal preparations from a hammer mill. Acilane Orange G at *p*H 2.6 was used for dyeing. Starch-free tissues, strongly positive with Acilane Orange G, were observed near the aleurone layer in sections of Hiproly and to a slightly lesser degree in sections of the sister line. Reference cultivars Foma, Domen, and especially CI 5195 had a greatly reduced development of this sublayer. In barley there are two distinct classes of starch granules: small, slightly oval ones with a distinct peripheral region that is birefringent as seen in meal preparations in the phase microscope, and large, slightly irregular, rounded granules (Fig. 1A). Hiproly is almost devoid of small starch granules, and the large ones are often crumbled. The most striking feature, however, is that the starch grains are firmly bound to the matrix proteins (Fig. 1B); for this reason it is difficult to obtain intact free granules from meal preparations. The sister line is com-

Table 1. Animal nutritional studies of a high-lysine barley (Hiproly) compared with a low-lysine reference barley with a comparable amount of crude protein (nitrogen × 6.25) in the seed.

	Protein (%)		Lysine (grams per 16 g of nitrogen)	14 days male CBA × Swiss mice growth test				9 days male rat nitrogen-balance test				
	Diet	Sample		Animals (No.)	Gain (g)	Protein consumed (g)	Protein efficiency ratio*	Animals (No.)	Nitrogen true digestibility (%)	Biological value (%)	Net protein utilization (%)	Lysine true digestibility (%)
Hiproly	9.40	16.81	4.08	6	8.9 ± 0.7	5.2 ± 0.4	1.72 ± 0.05	5	85.2 ± 2.5	76.0 ± 1.8	64.8 ± 2.7	78.7 ± 2.5
Reference	9.33	17.25	3.13	7	7.8 ± 0.5	5.0 ± 0.2	1.54 ± 0.09	4	82.0 ± 1.5	71.1 ± 2.1	59.2 ± 3.5	69.5 ± 3.2

* Grams of weight gained per gram of protein consumed.

Table 2. Cross between a high-yield, low-lysine (Sv 64625 male) and a low-yield, high-lysine (Hiproly female) barley. The P, F_1, and F_2 plants are analyzed for agronomic characteristics, crude protein, and dye-binding capacity (DBC) as an estimate of lysine.

	Plants (No.)	Straw length (cm)	Shoots per plant (No.)	1000 kernel weight (g)	Seeds per plant (No.)	Yield (grams per plant)	Crude protein (%) sample	DBC* per 60 mg of protein
Hiproly	14	65.2 ± 11	9.0 ± 6	39 ± 5	68 ± 62	2.7	19.6 ± 1.4	66.4 ± 1.2
Sv 64625	6	62.5 ± 9	11.7 ± 4	47 ± 7	175 ± 112	8.3	14.9 ± 0.9	64.1 ± 1.4
F_1	20	82.5 ± 7	10.6 ± 3	61 ± 5	145 ± 52	8.9	15.1 ± 1.0	64.2 ± 1.4
F_2, high lysine (classified by DBC)	19	75.8 ± 11	10.1 ± 4	45 ± 7	142 ± 81	6.3	17.2 ± 1.2	68.4 ± 1.0
F_2, low lysine (classified by DBC)	105	75.4 ± 11	10.5 ± 3	54 ± 8	144 ± 76	7.7	16.1 ± 1.8	63.4 ± 2.2
F_2, total	124	75.4 ± 11	10.4 ± 3	52 ± 7	144 ± 77	7.5	16.3 ± 1.6	64.2 ± 1.0

* DBC, micromoles of Acilane Orange G bound.

Table 3. Amino acid composition of seed yields from parental and F_2 plants in the cross Sv 64625 × Hiproly (see Table 2).

	Low-lysine parent Sv 64625	F_2 Lowly selection	Hiproly parent CI	F_2 Hily selection	Change
Plants	2	5	3	5	
Nitrogen (% of sample)	2.23	2.46	2.94	2.50	
DBC* per 60 mg of protein	63.1	64.0	66.1	68.5	
Relative amount of amino acids in protein					
Lys	100	96	120	120	+
His	100	96	99	104	
NH_3	100	100	87	88	−
Arg	100	94	103	103	
Asp	100	107	128	123	+
Tre	100	103	111	111	+
Ser	100	103	105	104	
Glu	100	104	92	92	−
Pro	100	104	91	93	−
Gly	100	101	111	110	+
Ala	100	99	117	119	+
Val	100	100	109	114	+
Met†	100	113	174	134	+
Ileu	100	102	110	119	+
Leu	100	98	102	106	
Tyr	100	101	105	104	
Phe	100	105	105	104	

* Dye-binding capacity, micromoles of Acilane Orange G bound. † Measured in nonoxidized hydrolyzates as methionine. Magnitude of difference is therefore unsafe.

parable to reference cultivars (Fig. 1A). A histological screening technique was developed that permitted single seeds to be classified by light microscopy without altering their viability. A small transverse incision was made in the seed and material was removed and scratched onto a microslide with a dissecting needle. The sample was mixed with a drop of Acilane Orange G (*p*H 2.6), and a cover glass was affixed.

No difference was found in the form or Acilane-Orange-G staining of the protein bodies between Hiproly and the low-lysine sister line. In corn (*6*), protein bodies contain zein and are reduced in the mutant opaque-2 (*7*). The amino acid composition of the corresponding prolamines in barley with low lysine content is slightly different from that of Hiproly (*1*), which supports the observation of morphologically unaltered protein bodies. On the other hand, the crude fractions of albumins, globulins, glutelins, and insoluble residual proteins from Hiproly and low-lysine varieties, compared in pairs, differ in amino acid composition (*1*). Lysine is higher in these fractions in Hiproly barley (*1*, *4*). Available data indicate a change at least in the composition of the matrix proteins of Hiproly.

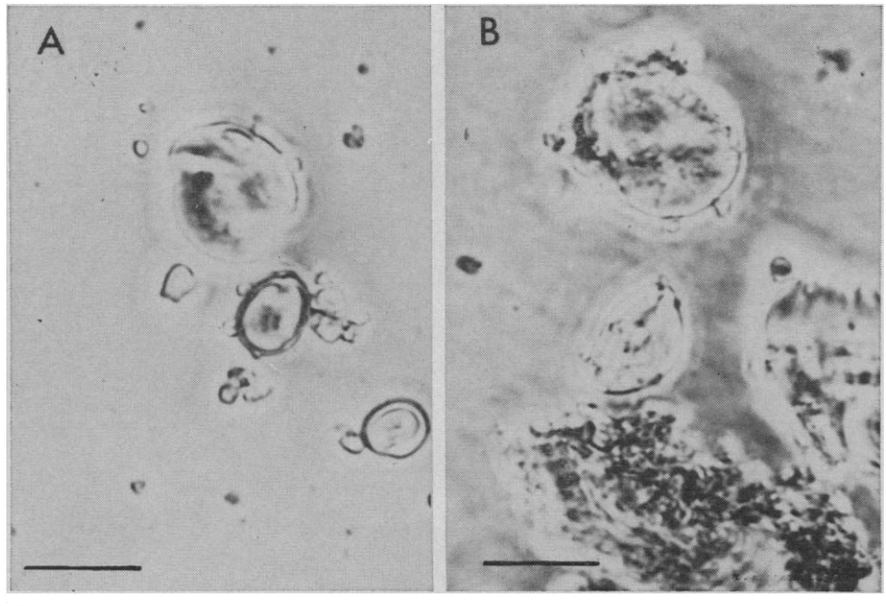

Fig. 1. Starch grains from meal preparations of single seeds (Acilane Orange G stain, phase contrast microscopy, line indicates 25 μm). (A) High-protein, low-lysine barley (Hiproly sister line CI 4362); small and large starch granules. (B) High-protein, high-lysine barley CI 3947 (Hiproly); large starch grain with attached Acilane-Orange-G-positive protein; deformed, large starch grain; and starch protein conglomerate.

In an experiment in which Hiproly and Foma barleys were grown at different concentrations of a complete nutrient solution, high protein contents tended to decrease the amount of lysine per 16 g of nitrogen. Hiproly varied 10.3 to 22.7 percent in protein corresponding to 4.86 to 4.02 percent lysine, whereas Foma, on the two extreme dressings, had 9.5 to 15.9 percent protein and 3.74 to 3.41 percent lysine per 16 g of nitrogen.

Feeding trials were made with limited amounts of Hiproly grown in Sweden and a reference mixture of four naked, six-row lines with 9.4 percent protein. Mice (*8*) were fed individually ad libitum, and rats were fed individually restrictively (*9*) (Table 1). In mice the protein efficiency ratio was increased with the Hiproly diet; and in rats true digestibility, the biological value of protein, and the net protein utilization (NPU) were likewise improved. True digestibility for lysine in rats, as measured with a fecal analytic method (*9*), was significantly increased on the Hiproly diet. The NPU of diets from the commercial varieties Birgitta and Kristina, not given here, were similar to those of reference barley feed material.

Four crosses with high-yielding, disease-resistant varieties were made in the field with Hiproly as the maternal parent. The F_1 and F_2 plants were grown in pots together with the parents outdoors in the summer. Seeds from 86 parental, 51 F_1, and 524 F_2 plants were analyzed for DBC at a constant weight of 60 mg of crude protein (nitrogen × 6.25) and agronomic measurements, and 93 amino-acid analyses were made. The results are exemplified by one of the crosses in Tables 2 and 3.

1) The F_1 has a low DBC compared with Hiproly. The histological classification of 100 seeds from one F_1 plant (F_2 seeds) was made as a blind experiment with 20 seeds from each of the parental cultivars intermixed. All parental seeds were correctly classified. The variation among the F_2 seeds was high, which indicates segregation. A ratio of 1 : 3.2 was found, which is not significantly different from an expected 1 : 3 ratio. Thirty-two of these seeds were analyzed for amino acids, of which nine had been histologically determined as high-lysine barleys. The amino acid

analysis in single determinations was different from the morphological classification in one case, which was probably due to an analytical error.

2) The F_2 plants segregated into two categories—one high in DBC and lysine (Hily) and one low (Lowly), both being distributed over the entire protein range from 11 to 22 percent (Table 2). The DBC per 60 mg of protein is negatively correlated with crude protein content equally in both categories.

3) In two crosses the ratio between plants high and low in DBC was not significantly different from 1 : 3, and in another two the same ratio differed significantly from 1 : 3.

4) DBC segregated independently of protein content and the naked and the erectoid characters.

5) From a comparison of the Hily and Lowly plants in the F_2, no regular differences with respect to the variation of time of ripening, straw length, number of tillers, and the number of seeds set could be ascertained. The Hily plants are considerably superior to Hiproly with respect to the last character (Table 2). The seed is smaller in the Hily than in the Lowly plants, but variation in both groups is considerable. There is no strongly expressed tendency for a negative correlation between high DBC (lysine) and yield in the F_2 material.

6) The DBC measurements were verified by amino acid analyses. Of 46 F_2 plants analyzed, two were erroneously classified by DBC determination, which is likely to be due to experimental error. Hily plants in the F_2 generation have an amino acid composition very close to Hiproly (Table 3), whereas the Lowly plants are comparable to the high-yielding, low-lysine paternal parent.

7) Six hundred F_3 seeds from F_2 plants that had amino acids analyzed were studied further with the histological screening technique in a blind test with a reference material. All Hily and Lowly lines were correctly classified. A wide range of morphological variation was recorded among the Hily and Lowly material. The adhesion of the starch grains to the matrix proteins is the critical Hily character. This character displayed a 1 : 3 segregation in the heterozygous F_2 plants in all crosses. The crosses with the ratio deviating from 1 : 3 in the DBC test of yields from F_2 plants (point 3 above) were hence not verified in the F_3 seeds. Hily lines were found that had a much larger proportion of small to large starch grains. Both starch grain types were undeformed.

Thus, the Hily character is due to a recessive gene or linked gene complex. In addition, a number of genes modify seed structure without interfering with the expression of the Hily gene as reflected by the amino acid composition.

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Elevation of Aortic Proline Hydroxylase: A Biochemical Defect in Experimental Arteriosclerosis

Abstract. *The relation of collagen synthesis to experimentally induced arteriosclerosis was studied by measuring proline hydroxylase activity. Gross aortic plaques were produced in rabbits by daily injection of epinephrine (intravenous) and thyroxine (intraperitoneal) for 4, 9, or 14 days. Activity of proline hydroxylase was significantly increased after 4 days of treatment and reached a peak, five- to sixfold increase, after 14 days of treatment. The increase in enzyme activity was correlated with the severity of observed arteriosclerosis. Increase in proline hydroxylase activity may be a possible biochemical defect in the aortas of rabbits with arteriosclerosis induced by injury.*

There is little information on the role of the arterial wall in atherogenesis. The connective tissue of the aortic wall was formerly regarded as metabolically inactive, but it has recently been shown to be an active tissue which responds to external and internal influences (*1*). Collagenous material, which is abundant in both medial and intimal regions of blood vessels, is a dominant component of human atherosclerosis (*1*). Tissue repair mechanisms have been implicated in various model systems of experimental atherosclerosis (*2*). Because accelerated collagen synthesis is a characteristic of healing wounds (*3*), we initiated studies on the collagen synthetic pathway in diseased aortic tissue. Proline hydroxylase activity was measured as a parameter of

Table 1. Proline hydroxylase activity in rabbit aortas after injection of thyroxine (Thy) (0.050 mg/kg for 14 days, intraperitoneally) or epinephrine (Epi) (0.025 mg/kg for the first 5 days and 0.050 mg/kg thereafter, intravenously), or both (Epi-Thy). The rabbits were killed at 4, 9, or 14 days after the beginning of the treatment. Enzyme activity is expressed as the mean (± S.E.) of the amount (dpm) of $[^3H]H_2O$ formed from [3,4-^{3}H]proline per milligram of protein per 30 minutes. Student's *t*-test was used to test for differences between activity in control and treated mice. Numbers in parentheses are the number of determinations.

Treatment	Proline hydroxylase activity		Number of rabbits with lesions graded
	Thoracic aorta	Abdominal aorta	0-1-2-3-4
	4 days		
Control	3896 ± 686 (7)	1422 ± 284 (7)	5-3-0-0-0
Epi-Thy	7064 ± 894 (7)*	2109 ± 273 (8)†	2-4-2-0-0
	9 days		
Control	3722 ± 490 (8)	1553 ± 274 (8)	5-3-0-0-0
Epi-Thy	8943 ± 1526 (9)*	4835 ± 1218 (9)‡	3-2-1-2-1
	14 days		
Control	2750 ± 325 (8)	1637 ± 130 (7)	6-2-0-0-0
Epi-Thy	14179 ± 770 (6)§	5661 ± 637 (6)§	1-2-0-1-2
Epi	4670 ± 1240 (6)†	2026 ± 363 (6)†	3-3-0-0-0
Thy	5659 ± 1050 (8)‡	2678 ± 316 (7)*	2-5-1-0-0

* $P < .01$. † $P > .05$. ‡ $P < .05$. § $P < .001$.