Amino Acid Composition of Isogenic Lines in Barley

Kernel weight, protein content, and amino acid composition were determined in 49 pairs of isogenic barley lines representing 13 spike or kernel characteristics. Hulled kernels differed from naked kernels in kernel weight, protein content, and concentrations of three amino acids; two-rowed and six-rowed kernels differed in kernel weight, protein content, and 15 amino acids; and there were differences in the concentrations of three amino acids in the proteins of blue and white aleurone barleys.

One of the basic questions in plant breeding concerns the contribution of genotypes to phenotypes. The answer to that question can be obtained by several methods of isogenic analysis which measure the difference between two alternate states of the gene AA and aa in the same background genotype. Isogenic lines are assumed to differ by a single gene (or a small block of genes). There are several methods of establishing isogenic lines as a basis for isogenic analysis. One of the most useful approaches is to evaluate mutants that have occurred in the past and have survived through many generations of natural selection (Wiebe, 1968).

Pairs of isogenic lines have been used to study differences in agronomic and malt quality factors between six-rowed and two-rowed spikes, differences in α-amylase activities between barleys with orange and normal-colored lemmas, and differences in agronomic and malt characters between blue and white aleurone barleys (Wiebe, 1968). Additional agronomic tests with isogenic and near isogenic lines of barley were described by Suneson et al. (1952) and by Suneson and Stevens (1957). Fasoulas and Allard (1962) and Qualset (1974) studied the importance of additive, dominant, and epistatic gene action of two small chromosomal segments for quantitative characters in barley.

Additional areas of potential usefulness of isogenic lines are in determining losses caused by diseases and insects, studying the optimum plant type for a particular environment, and determining the fitness of a gene in natural selection.

Recent advances in our understanding of protein synthesis have opened the door to studies at the molecular level. Those studies have been made almost entirely with bacteria and viruses. It is now, theoretically, possible to make such studies with higher plants. Isogenic lines are ideally suited for such studies because any biochemical or physiological difference between the members of a pair of lines has a good chance of being related to the gene under study (Wiebe, 1968).

The purpose of this communication is to report differences in protein contents and amino acid composition of isogenic lines and to call attention to the potential usefulness of studying isogenic lines in barley to provide basic information for the efficient manipulation of germ plasm as part of a system to improve nutritive value of cereal proteins.

We have reported previously on amino acid composition of two-rowed and six-rowed inbred-derived isogenic barley lines (Pomeranz et al., 1973). We now report results of studies on kernel weight, protein content, and amino acid composition of 49 pairs of isogenic barley lines; the 49 pairs were selected as they varied for 13 main and important spike or kernel characteristics.

MATERIALS AND METHODS

We used 49 pairs of inbred-derived barley isogenic lines developed by Dr. G. A. Wiebe. The selfed generation in which the two homozygous lines were selected ranged from F_{28} to F_{31}. The pairs were grown under irrigation at Aberdeen, Idaho in 1968, and are described in Table I; there are 55 because six of the pairs involved two spike or kernel characteristics.

Kjeldahl nitrogen and amino acids in acid hydrolysates (Beckman 121 automatic amino acid analyzer) were determined by published procedures (Robbins et al., 1971). Crude protein, estimated by multiplying the nitrogen

Table I. Description of Isogenic Pairs

Spike or kernel character	No. of isogenic pairs
Rough awned vs. smooth awned	6
Long-haired rachilla vs. short-haired rachilla	5
Hulled vs. naked	3
Yellow-green spike vs. blue-green spike	4
Black kernel vs. white kernel	2
Long-awned glume vs. short-awned glume	3
Lax spike vs. dense spike	2
Two-rowed spike vs. six-rowed spike	12
Deficiens spike vs. six-rowed spike	2
Blue aleurone vs. white aleurone	8
Awnless spike vs. short-full awn spike	4
Rough-awned tip vs. smooth-awned tip	2
Small lateral kernel vs. large lateral kernel	2
Total	55

Table II. Test of Significance of Sample Means with Pair Observations[a]

Parameter	Spike or kernel character: Hulled vs. naked	Two-rowed vs. six-rowed	Blue aleurone vs. white aleurone
Kernel weight	Δ	xx	
Protein	Δ	xx	
Lysine		xx	
Histidine		xx	
Ammonia		xx	
Arginine	Δ	xx	
Aspartic acid		xx	
Threonine		xx	
Serine		Δ	
Glutamic acid		xx	
Proline		xx	
Cystine	xx		
Glycine		xx	
Alanine		xx	
Valine		xx	x
Methionine		xx	x
Isoleucine		xx	
Leucine		xx	
Tyrosine	x		x
Phenylalanine		xx	

[a] Δ, x, and xx denote significance at the 10, 5, and 1% levels, respectively.

Table III. Average Kernel Weight, Protein Content, and Amino Acid Composition[a] of Isogenic Barleys

Parameter	Overall av	Two-rowed spike	Six-rowed spike	Deficiens spike	Six-rowed spike
Kernel weight, mg	39.35	50.05a	34.47c	43.90ab	35.50bc
Protein, %	13.42	14.89a	11.11b	16.15a	13.30ab
Lysine	3.61	3.53b	4.18a	3.45b	3.70b
Histidine	2.15	2.16b	2.33a	2.05b	2.10b
Ammonia	3.19	3.42a	3.06b	3.40a	3.20ab
Arginine	4.70	4.63b	5.25a	4.25b	4.65ab
Aspartic acid	6.54	6.11b	7.21a	5.75b	6.10b
Threonine	3.26	3.12b	3.45a	2.95b	3.20b
Serine	3.66	3.51a	3.63a	3.20a	3.30a
Glutamic acid	26.43	27.68a	24.80c	27.50ab	26.20bc
Proline	11.43	11.18a	9.45b	12.80a	12.10a
Half-cystine	1.12	1.12a	1.03a	1.25a	1.20a
Glycine	3.98	3.78b	4.40a	3.55b	3.75b
Alanine	4.20	4.02b	4.58a	3.70b	4.00b
Valine	5.10	5.01b	5.40a	4.90b	5.10b
Methionine	2.62	2.59b	2.87a	2.85ab	2.85ab
Isoleucine	3.66	3.66a	3.78a	3.60a	3.65a
Leucine	6.71	6.76b	7.02a	6.60b	6.80ab
Tyrosine	2.44	2.43a	2.50a	2.60a	2.70a
Phenylalanine	5.27	5.42a	5.12b	5.60a	5.40a

[a] Grams of amino acid per 100 g recovered from the column. Letters following the numbers denote results of Duncan multiple test at the 5% level of significance.

content by 6.25, is reported on a moisture-free basis. Amino acids are reported as grams per 100 g of amino acid recovered. Average recovery was 88.1%.

RESULTS AND DISCUSSION

The *t*-test was used to determine the significance of differences between means for pairs of isogenic lines for each barley character. Differences were not significant, at the 10% level, for the isogenic pairs of black vs. white kernels, long vs. short awned glumes, lax vs. dense spikes, and rough vs. smooth awned tips. Differences were significant at the 10% level, for the pairs rough vs. smooth awned (for kernel weight and aspartic acid), long vs. short haired rachilla (for percent protein), yellow-green vs. blue-green spikes (for percent protein and ammonia), deficiens vs. six-rowed spike (for percent protein), and awnless vs. short-full awn spike (for kernel weight and serine). For the pair small vs. large lateral kernels, the small lateral kernels contained significantly more (at the 5% level) protein than the large lateral kernels. The greatest numbers of significant differences were between the isogenic pairs two-rowed vs. six-rowed spikes, hulled vs. naked kernels, and blue- vs. white-aleurone kernels (Table II). Undoubtedly, increasing the number of isogenic pairs increased the probability of finding statistically significant differences. Kernels from the hulled vs. naked isogenic lines are characterized by more profound structural and gross compositional differences than kernels of any of the other sets of isogenic pairs. The hulled kernels differed from the naked kernels in kernel weight and protein content. Surprisingly, however, these were only minor differences in amino acid composition of proteins of the naked and hulled barleys. Two-rowed and six-rowed kernels differed, in kernel weight, protein content, and 15 amino acids; and there were differences in concentrations of three amino acids in the proteins of blue and white aluerone barleys.

The kernels from the two pairs of isogenic lines, two-rowed vs. six-rowed, and deficiens (two-rowed) vs. six-rowed were considered also as a group of four classification variables. Table III lists the average parameters for all samples that were studied (98) and the averages of the four classes. Kernels from the deficiens spikes did not differ in weight, protein content, or amino acid composition from kernels of corresponding six-rowed spikes. Kernels from the two-rowed spikes were larger and contained more protein than kernels of the corresponding six-rowed spikes. There were also large differences in amino acid composition of proteins in kernels from two-rowed and six-rowed barleys. The proteins of the two-rowed barleys contained more glutamic acid and proline, the two main amino acids of storage proteins, and phenylalanine and less of 11 amino acids; no significant differences were determined for serine, cystine, and isoleucine.

Mutations are of interest (a) in studies of mutagenesis, (b) as a source of information on gene action in physiological and biochemical processes, (c) in analyses of specific genetic problems and pathways of evolution and phylogeny, and most important (d) for the genetic improvement of cultivated plants by breeding (Scholtz, 1971).

The nutritional value of cereals is mainly limited by their protein content, amino acid composition, or both. It has been demonstrated that genetic improvement in protein quantity or protein quality is possible, that single-gene substitutions can result in marked increases in the contents of the limiting essential amino acids for nutrition of humans or monogastric animals, and that genes with significant effect on protein, lysine, and tryptophan contents can be used to improve the nutritive value of corn and barley (Johnson and Lay, 1974). Attempts of geneticists to elucidate the biochemical basis for mutant phenotypes and attempts of plant physiologists to utilize mutant genes as experimental tools in investigations of biosynthetic pathways were reviewed recently by Nelson and Burr (1973). According to those authors the most important needs and potential advances in biochemical genetics of higher plants lie in the development of supportive techniques. The availability of pairs of isogenic lines could enhance progress in those areas both in terms of understanding the underlying principles and of practical breeding for nutritionally improved cereals.

ACKNOWLEDGMENT

We thank F. S. Lai for the statistical analyses, G. A. Wiebe and D. M. Wesenberg for the barley samples, and J. T. Gilbertson for technical help with the analyses.

LITERATURE CITED

Fasoulas, A. C., Allard, R. W., *Genetics* **47**, 889 (1962).
Johnson, V. A., Lay, C. L., *J. Agric. Food Chem.* **22**, 558 (1974).
Nelson, O. E., Burr, B., *Annu. Rev. Plant Physiol.* **24**, 493 (1973).
Pomeranz, Y., Robbins, G. S., Wesenberg, D. M., Hockett, E. A., Gilbertson, J. T., *J. Agric. Food Chem.* **21**, 218 (1973).
Qualset, C. D., American Society of Agronomists, Proceedings of the Annual Meeting, Abstract No. 77, 1974.
Robbins, G. S., Pomeranz, Y., Briggle, L. W., *J. Agric. Food Chem.* **19**, 536 (1971).
Scholtz, F., *Barley Genet. 2, Proc. Int. Symp.*, 94–105 (1971).
Suneson, C. A., Schaller, C. W., Everson, E. H., *Agron. J.* **44**, 584 (1952).
Suneson, C. A., Stevens, H., *Agron. J.* 49, 50 (1957).
Wiebe, G. A., "Barley: Origin, Botany, Culture, Winterhardiness, Genetics, Utilization, Pests", Agriculture Handbook No. 338, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C., 1968.

Yeshajahu Pomeranz*[1]
George S. Robbins

Barley and Malt Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Madison, Wisconsin 53705
[1]Present address: U.S. Grain
Marketing Research Center
Manhattan, Kansas 66502

Received for review May 7, 1975. Accepted August 1, 1975. Cooperative investigation of the Agricultural Research Service, U.S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station, Madison. Mention of a trademark or proprietary product does not imply approval or recommendation by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Toxicity to White Mice of Corn Naturally Contaminated with Aflatoxin

Male and female mice of three different ages were fed continuously on balanced corn diets containing naturally occurring aflatoxin. The corn contained 286 ppb of aflatoxin and was fed in diets containing 5, 20, and 40% (14, 57, and 114 ppb) contaminated grain for 6 months. Animals were regularly observed for weight changes, general health, or toxicity symptoms, and at the end of the experiment their livers were examined for total lipid content. Weight gains of mice fed the highest aflatoxin levels were approximately the same as those on rations containing lower levels. No tumors were observed, but lipid accumulation in the liver was significant in animals ingesting the highest level of toxin-contaminated feed.

Aflatoxin has been fed in diets to a number of different animals and a variety of effects have been observed (Wogan, 1972). Carcinogenic activity of aflatoxin has been demonstrated in ducks, rainbow trout, ferrets, rats, mice, and monkeys (Louria et al., 1974; Wogan, 1965, 1972). Apparently, in animals poisoned by aflatoxin, the liver is the main organ affected. Toxin-induced lesions and high lipid accumulations in the organ have been described (Bourgeois et al., 1971; Butler, 1969; Wogan, 1973). Fatty degeneration of the liver has been observed in a number of animal species ingesting aflatoxin (Butler, 1969; Tung et al., 1972). In addition to liver symptoms, growth rates were also affected in ducklings and chickens ingesting aflatoxin (Lynd and Lynd, 1970; Smith and Hamilton, 1970). In feeding experiments with chickens, smaller amounts of the toxin were required to induce lipid accumulation in the liver than were required to cause growth repression (Tung et al., 1972).

Although numerous accounts describe symptoms in animals fed diets artificially contaminated with aflatoxin, few report effects of a diet of grain containing naturally occurring aflatoxin. In our research, we fed mice continuously on a balanced diet containing corn naturally contaminated with aflatoxin. We regularly observed the animals for weight changes, general health, or toxicity symptoms, and at the end of the experiment we examined their livers for size, color, and total lipid content.

EXPERIMENTAL SECTION

Experimental Animals. White mice (CD-1 outbred albino strain) from the Charles River Breeding Laboratories (Wilmington, Mass.) were 3, 5, and 8 weeks old. Half of each age group was male and half female. The animals were divided according to age and sex into groups of 10, and housed in a constant temperature (25°C), constant humidity (60%) room. Water and feed were supplied ad libitum. Mice were observed daily and weighed every other week for 6 months.

Special Diets. The contaminated grain was received from Missouri as whole, shelled white corn. FDA inspectors had found it to contain aflatoxin at levels above acceptable limits ("F.D.A. Recalls Corn Meal, Bread Mix Allegedly Tainted by Toxin", 1971). The particular lot of corn used in the feeding trials contained 286 ppb of aflatoxin B_1 and no aflatoxin G, as determined by thin-layer chromatography (TLC) analysis. B_1 isolated from the corn was confirmed by cochromatography with a sample of the pure compound.

The aflatoxin-containing corn was fed in special diet mixtures of 5, 20, and 40% of the total ration. Total corn content in the various mixtures was maintained at a constant level of 47.1% by addition of ground corn containing no aflatoxin. The corn, blended in a PK Blender to ensure homogeneity, was ground to 40-mesh grade in a Raymond laboratory hammer mill. A protein level of 20% (Mills and Murray, 1960) was achieved through addition of nonfat dry milk (Valley Lea Super Instant, Midwest Producer's Creameries, Inc., South Bend, Ind.). Commercial mixtures of minerals (Mineral Premix, CSM, Mallinckrodt Chemical Works, St. Louis, Mo.) and vitamins (Vitamin Premix, Roche Chemical Division, Hoffman-LaRoche, Inc., Nutley, N.J.) were added for dietary balance (Mills and Murray, 1960). Ingredients were blended, pelletized, and fed to mice in self-feeders. As negative controls, laboratory animal feed ration (Allied Mills, Inc., Chicago, Ill.) was fed to one group of mice in each category, and a diet containing aflatoxin-free corn to another group in each category. Compositions of the special diets are shown in Table I.

Liver Lipid Analysis. At the termination of the feeding trials, the mice were sacrificed for necropsy. Livers were removed, examined visually, pooled according to diet