Survey for a High Lysine Variety in the World Collection of Safflower

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Safflower meal is a valuable animal feed and shows promise of being useful as human food. Although rich in protein, the meal is deficient in the essential amino acid, lysine. In a survey of the world collection of safflower seeds, several foreign varieties were found to contain 15% more lysine, through two generations, than the average domestic commercial variety and 26% more lysine than the average foreign variety. In addition, plot tests on pure domestic varieties revealed that environmental effects on lysine content are highly significant.

Interest in safflower as a source of industrial oil, food oil, and feed supplement has stimulated agronomic and genetic research on this widely cultivated crop. The discovery and cultivation of mutant varieties for unusual oil composition (Knowles and Mutwakil, 1963; Applewhite, 1966) and for low hull content (Rubis, 1963) are opening up new potentials. The added dividend of a protein-rich meal gives great promise toward relieving the world food shortage (Kohler, 1966).

To date, breeding and agronomic programs have been directed toward improving disease resistance, seed yield, and oil content; reducing hull content; and modifying fatty acid composition. With the pressing need for a source of protein with high biological value, some research is now being directed toward meal improvement. The composition of the oil-free meal has been investigated recently (Guggolz *et al.*, 1968), and feeding tests on chicks have been performed (Halloran, 1961; Kohler *et al.* 1966; Kuzmicky and Kohler, 1968a, b; Kratzer and Williams, 1951; Valadez *et al.*, 1965). Completely decorticated safflower meal contains 65% protein. However, its lysine content, 2.7 grams per 16 grams N, is too low for optimum growth and feed efficiency when the meal is used as the primary source of protein in poultry rations. The other essential amino acids are well balanced in relation to poultry requirements. In the hope of finding breeding stock with substantially more than the average lysine content, all samples of safflower seeds in the world collection were analyzed for lysine by a rapid screening method. Promising samples were analyzed by high precision column chromatography. Genetic and environmental effects were demonstrated.

EXPERIMENTAL

Samples. The samples in the world collection of safflower seeds represent wild and cultivated plants from Europe, the Middle East, Africa, Asia, and Canada. The foreign seeds, after passing import quarantine, were planted in this country and their progeny were analyzed in the survey. The first 900 samples of safflower seeds were grown at the University of California, Davis, in 1963; the second group of 250 samples were grown in the same location in 1964; the last 800 samples were grown at the U. S. Department of Agriculture's Plant Introduction Station, Pullman, Wash., in 1966. Samples of domestic commercial varieties grown in California regional tests and experimentally crossed varieties were included in the survey. A total of 2197 samples was originally screened. After the survey was completed, several samples from the world collection were chosen for replanting at the University of California, Davis, in 1967. Seeds from individual plants were then assayed.

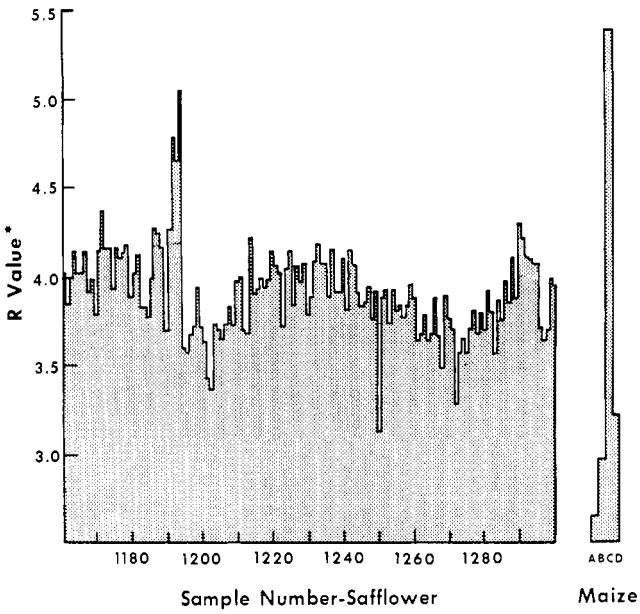

Figure 1. Screening graph. Maize: A, Mexican; B, Mexican; C, Opaque-2; D, Commercial hybrid

*Gram lysine/16 grams nitrogen = R value × 0.660

Methods. For the initial screening one gram of each whole seed sample was hydrolyzed by a survey hydrolysis procedure (Palter and Kohler, 1969). Lysine was determined enzymatically on an aliquot of each hydrolysate by a Technicon Auto-Analyzer. The color reaction of another aliquot with ninhydrin was used to determine total amino acid content (White and Gauger, 1967). The ratio of the lysine value to the ninhydrin value (R value) was calculated for each sample. A factor (0.660, std. dev. 0.027) for converting R value to grams lysine per 16 grams N was derived by determining Kjeldahl nitrogen values as well as lysine and total amino acids on 10 randomly selected samples. Maize samples of widely different lysine content were included in the survey as method check samples. The survey was completed by hand dissecting the samples that proved to be of interest and oil extracting and analyzing the kernel fractions for lysine by high precision amino acid procedures (Kohler and Palter, 1967).

RESULTS AND DISCUSSION

A convenient method of handling the great number of R values obtained in the screening was charting each sample as it was analyzed. Any deviation from the normal became apparent immediately. Figure 1 represents a portion of the

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sample screening graph. During this portion of the screening, the foreign sample containing the highest amount of lysine showed up. For comparison purposes, the results on maize samples are shown separately on the chart.

A high degree of correlation was found between calculated lysine values as determined from screening ratios and by the high precision lysine assay of the defatted kernels. Thirty-seven survey samples, chosen to cover the range of R values, were tested. Figure 2 is the regression line equation derived by a least squares fit from single survey and single high precision analyses. The correlation coefficient is 0.859. The significance of regression is at the 0.1% level [F (1,35) = 99.12].

The average R value of the samples in the world collection of foreign seeds was 3.80 (= 2.51 grams lysine per 16 grams N). The averages of the samples grown at the University of California in 1963 and 1964 and at Pullman in 1966 did not vary more than ± 4%. Forty-seven samples in the foreign collection had R values higher than 4.34 (two standard deviations above the mean). These samples were rescreened and nine samples showing the highest lysine content were chosen for decortication and analysis by high precision procedures. Kernel analysis of eight of these samples gave lysine values 12 to 19% higher than the calculated average of the foreign collection; the remaining sample, No. 1193, was 28% higher in lysine (3.22 grams lysine per 16 grams N).

The average R value of samples from the domestic and experimental varieties was 4.16 (= 2.75 grams lysine per 16 grams N). Samples with R values greater than two standard deviations above the mean were reanalyzed by screening procedures, and those with highest values were decorticated and analyzed by conventional procedures.

Included in the domestic and experimental collection were samples from a five-county regional test of pure varieties. The results for these samples are presented in Table I. An analysis of variance showed a significant varietal difference only at the 5% level. However, differences among counties were significant at the 1% level. Colusa County samples, verified by Duncan's Multiple Range Test, were unusually high. Samples from five of the Colusa County varieties were decorticated; kernel analysis checked quite well with the predicted survey calculated value. The fact that this county produced safflower seeds 12 to 17% higher in lysine than in average domestic commercial and experimental varieties presents the possibility of lysine improvement through changes in environmental conditions, presently not identified.

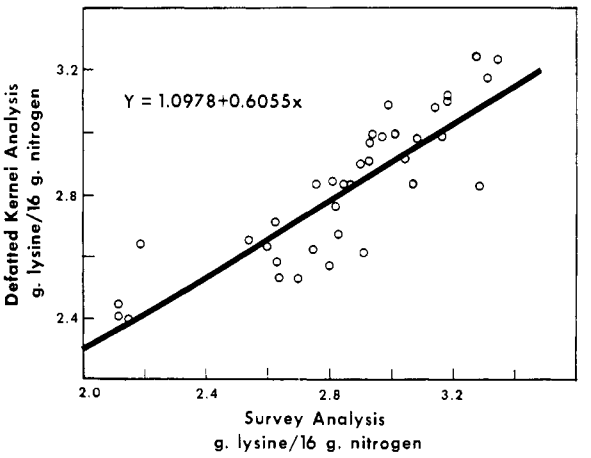

Figure 2. Regression line

Table I. Regional Test—1964. Calculated Grams Lysine/16 Grams N (Unequal Number of Replicates)

	County				
Variety	Colusa	Sacramento	San Joaquin	Kings	Sutter[a]
US-10	3.36	2.51	2.59	2.91	2.73 (2.71)
Frio	3.30	2.66	2.60	2.81	2.70 (2.72)
Gila	2.98	2.53	2.67	2.71	2.57 (2.69)
RR-63	2.68	2.39	2.59	2.56	2.55 ...
U-5	3.24	2.49	2.68	2.60	2.68 (2.64)
Ute	3.30	2.53	2.60	...	3.04 ...
Mean	3.14	2.52	2.62	2.72	2.71

[a] Figures in parentheses are for year 1965 and are not included in means.

Since the original survey was made on the collected seeds from several plants of the same variety, those from the non-commercial varieties having the highest lysine content were replanted, and seeds from individual plants were analyzed. Table II shows the results from replanting as well as for check row plants of US-10 variety. Although the lysine values of the second generation foreign seeds were generally not so high as the lysine values of the first generation, they were significantly higher than the mean value for original survey samples. The best individual plant seeds of the progeny were about equal in lysine content to the grouped paren

Table II. Comparison of Progeny with Parent Seeds

		Parent, Pooled plant seeds	Progeny, Seeds from individual plants				
Sample no.	Country of origin	Defatted kernel analysis (Grams lysine/16 Grams N)	No. of plants	Significant[a] variation among plants	Average[b] lysine content (Grams lysine/16 Grams N)	Std. dev.	Highest[b] individual plant lysine content (Grams lysine/16 Grams N)
Foreign collection		2.51[c]					
US-10 Check		2.63	36	...	2.63	0.07	2.79
1099	Iraq	2.99	18	No	2.80	0.09	2.98
1128	Pakistan	2.98	8	No	2.69	0.13	2.95
1191	Ethiopia	2.98	17	No	2.60	0.10	2.74
1192	Ethiopia	2.97	20	No	2.67	0.10	2.89
1193	Israel	3.22	20	Yes	2.85	0.12	3.17
1324	India	2.98	11	Yes	2.91	0.15	3.18

[a] Compared to variance of US-10 check plants.
[b] Derived from R values.
[c] Derived from R values of 1948 samples.

plant seeds. Evidence thus far indicates a genetic variation in lysine content. Two (No. 1193 and No. 1324) of the six varieties replanted showed considerable variability. Seeds from individual plants of the two varieties had lysine contents 26% higher than the average of those of the foreign collection and 15% higher than those of the average of domestic and experimental varieties. In the original plantings from the world collection, it seems unlikely that environmental conditions played a significant part in lysine variability. In the second planting, the check rows showed there was no environmental effect.

Although the work just concluded did not reveal any startling new safflower mutants, it did afford two new approaches for improving the lysine content of the meal. The domestic regional test showed that certain environmental conditions, still unknown, can appreciably affect the lysine content of safflower meal. More important, it was found that there is genetic variability in lysine content.

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