COMMUNICATION TO THE EDITOR

Rapid Ninhydrin Color Test for Screening High-Lysine Mutants of Maize, Sorghum, Barley, and Other Cereal Grains

TO THE EDITOR:

At a symposium in 1971 (1), we reported that the endosperm of *opaque-2* maize had a higher level of water-soluble free amino acids than the normal counterpart. Sodek and Wilson (2) also reported increased levels of free amino acids in *opaque-2* maize. In searching for a rapid color test to detect high-lysine mutants, the idea occurred to us that the excess free amino acids in such mutants could be measured with ninhydrin.

The ninhydrin reagent rapidly detects the difference in total free amino acids between the normal and its mutant counterpart. The differences observed are shown in Fig. 1. Five kernels of maize, 15 kernels of sorghum, and 10 kernels of barley (from which the glumes had been removed) were split lengthwise with a sharp knife and placed in 0.75 X 6 in. Pyrex test tubes. The kernels were covered with 10 ml. water, and 300 ± 50 mg. of dry ninhydrin-buffer (pH 5) mixture (16% ninhydrin, 58% sodium citrate, and 26% citric acid) was added. The contents were heated just to boiling and the tubes allowed to stand 5 min. In Fig. 1 it can be seen that the intensity of ninhydrin color is much greater in the tubes containing the high-lysine mutants of maize, sorghum, and barley than in the tubes containing the normal grains. To identify single kernels, a section of the crown containing endosperm and pericarp can be removed and the cut surface stained with ninhydrin solution[1] and compared with the same fragment of a normal control kernel. The remainder of the seed can then be planted. The test should be performed only on freshly cut seeds. In normal sorghum, bisected seeds stored for 1 year gave a strong test for free amino acids, suggesting proteolysis on the cut surfaces.

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[1] Ninhydrin reagent: Dissolve 400 mg. of reagent grade $SnCl_2 \cdot 2H_2O$ in 250 ml. of sodium citrate buffer. 0.2M, pH 5. Mix with 250 ml. methyl cellosolve containing 10 g. of ninhydrin (practical).

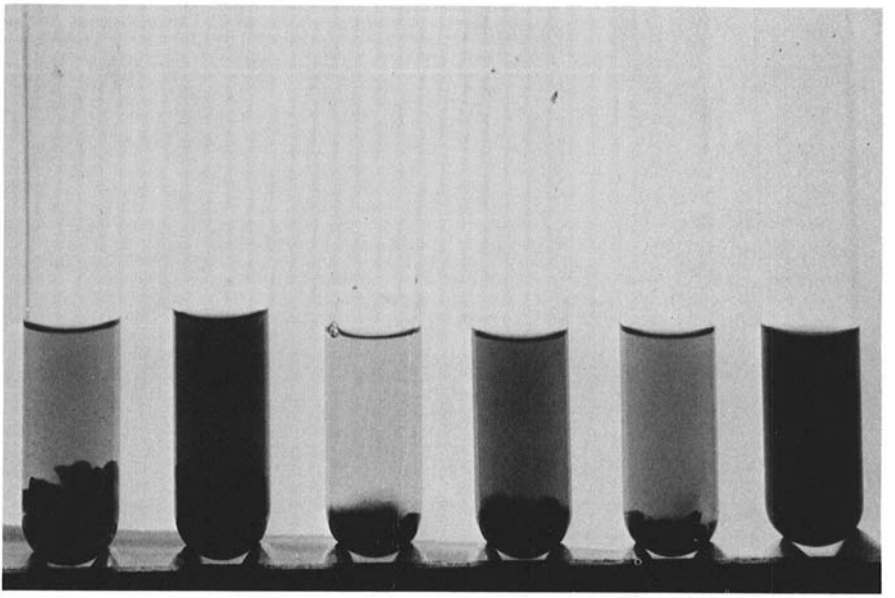

Fig. 1. Ninhydrin color test. From left to right: tube 1, P-A-G SX52 (normal hybrid maize); tube 2, P-A-G 50,001 (near isogenic *opaque-2* counterpart of P-A-G SX52); tubes 3 to 6, normal sorghum, high-lysine sorghum, normal barley, and high-lysine barley, respectively, as described in column 1, Table I.

In order to make a quantitative comparison, leucine equivalents per 100 mg. of protein were calculated for the various cereal grains of economic importance and for the high-lysine mutants of maize, sorghum, and barley described in this paper. One hundred to 500 mg. of defatted ground samples of whole kernels of the grains were suspended in 100 times their weight of distilled water and shaken at room temperature for 20 min. Samples were centrifuged and 0.15, 0.20, and 0.25 ml. of supernate placed in separate 0.75 X 6 in. Pyrex test tubes, the volume made up to 0.5 ml., and 1.5 ml. of ninhydrin reagent[1] was added. The tubes were heated in a boiling water bath for 20 min., cooled to room temperature, and 8 ml. of 50% *n*-propanol was added. The tubes were briefly mixed on a Vortex mixer and were read in a Spectronic 20 colorimeter at 570 nm. Increments of standard L-leucine solution were treated in a similar fashion and the micromoles of leucine plotted against absorbance to obtain a standard curve. The color obtained with the samples was then expressed in terms of leucine equivalents.

The free amino acids as measured by the ninhydrin reaction, the total protein, and the lysine content of whole grain samples of seven cereal species are listed in Table I. These data show that the high-lysine mutants of maize, sorghum, and barley contain 3 to 4 times more water-soluble, ninhydrin-reacting molecules per 100 mg. of protein than their normal counterparts. Rice, millet, and oats have leucine values in the range of those found for normal maize, sorghum, and barley. Wheat has slightly higher values. It is interesting to note that high-protein rice has about one-half the leucine value of low-protein rice.

TABLE 1. FREE AMINO ACIDS IN CEREAL GRAINS EXPRESSED AS LEUCINE EQUIVALENTS

Cereal Grain	Total Free Amino Acids μM. leucine/100 mg. protein	Protein %	Lysine g./100 g. protein
Maise 4854[a]	14.3	11.0	2.6
Maize 4858[a]	17.6	10.5	2.7
Maize 4855[a]	46.0	10.9	4.5
Maize 4856[a]	50.8	10.7	4.4
Maize 4857[a]	69.3	10.1	4.7
Sorghum, normal[b]	10.7	13.1	1.8
Sorghum, high-lysine[b]	49.5	18.5	3.3
Barley, Bomi[c]	19.5	19.8	3.1
Barley, high-lysine[c]	68.0	19.4	5.3
Rice BPI761[d]	8.9	15.4	3.4
Rice BB[d]	16.8	6.9	3.7
Wheat, Purdue 4930[e]	20.2	17.3	2.9
Wheat, Arthur[e]	23.1	15.4	2.7
Wheat, Genesee[e]	27.7	12.1	3.0
Millet[f]	9.8	14.5	1.9
Oats, Noble[g]	15.9	18.9	3.8
Oats, Dal[g]	16.1	20.3	3.9

[a]4854 and 4858: CIMMYT normal, yellow synthetics; 4855: CIMMYT yellow, hard-endosperm, *opaque-2* synthetic; 4856 and 4857: CIMMYT yellow, soft-endosperm, *opaque-2* synthetics. CIMMYT: International Maize and Wheat Improvement Center, Mexico City.

[b]Normal sorghum kernels; high-lysine *(hl hl hl)* kernels from F_2 segregating heads derived from crosses between "normal" (low-lysine) plants and the high-lysine sorghum line IS11758 (3).

[c]Bomi: Parent barley variety (4); high-lysine mutant: mutant 1508 from RISφ, Denmark (4).

[d]BPI761: high-protein rice, IRRI (5); BB: Blue Bonnet rice (5).

[e]Purdue 4930: A-6-28-2-1 Indiana high-protein; Arthur: variety of Indiana soft wheat; Genesee: variety of New York soft wheat.

[f]Finger millet; variety from Uganda supplied by S. A. Eberhart, Iowa State University, Ames.

[g]Noble: Wisconsin variety; Dal: Purdue University variety.

These determinations were made on mature seeds. Data which will be published elsewhere show that the free amino acid levels of both normal and *opaque-2* maize are higher in immature grain; thus, the differences observed might not be found if an immature normal grain was compared with a mature high-lysine mutant. Also, some variation in leucine values has been observed between normal maize genotypes. It is therefore recommended that any screening for high-lysine mutants be done using normal controls with comparable genetic backgrounds.

Hard vitreous endosperm types of *opaque-2* maize indistinguishable from normal maize have been developed recently (maize 4855, Table I, is an example). The simple test described here should quickly identify such kernels in the field or in the marketplace[2].

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