COMMUNICATION TO THE EDITOR

A Simple and Inexpensive Visual Method for Distinguishing Between High-Lysine and Normal-Lysine Barley Kernels

To the Editor:

Many high-lysine barley mutants have about 1.5 times the lysine content of corresponding nonmutant barleys. The difference might be sufficient to allow differences to be observed visually on the cut surface of a kernel with a specific staining method. A fluorescence staining technique specific for lysine might make the high-lysine kernels appear different. An attempt was made to develop a fluorimetric method with which one could classify the kernels into high- and low-lysine groups, or into three or four classes. This nondestructive method could be useful in barley breeding because it leaves the kernel end containing the embryo undamaged.

Fluorimetric methods for free lysine or lysyl residues of proteins have been devised (Blake 1981, Brand and Shaltiel 1964, Goodno et al 1981, Roth 1971, Roth and Jeanneret 1972). Brand and Shaltiel (1963, 1964) studied the fluorescence of peptides and amino acids induced with *N*-bromosuccinimide (NBS). The described method, based on the fluorescence caused by NBS, was developed during a pilot study that enabled the differentiation between kernels of Hiproly CI 3947 and Hiproly Normal CI 4362, as well as between kernels of Bomi Risø mutant 1508 and Bomi. Seeds produced during various years were used. Hiproly Normal and Hiproly are normal-lysine and high-lysine barley, respectively. Both have high protein content. Bomi and Risø 1508 are normal- and high-lysine barley, respectively, and both have normal protein content. The high lysine content of Hiproly and Risø 1508 is caused by different genes (Tallberg 1981).

Lysine was determined in meal with the trinitrobenzenesulfonic acid method described previously (Ahokas 1982). The values shown in Figs. 1 and 2 are means of two determinations.

Dry, mature kernels were cut transversely with a sharp scalpel at two thirds of the length from the embryo end, so that no whitish artifacts due to air layers occurred below the cut surface. Any loosely attached husks at the incised point were removed. Forceps were used to imbed the small pieces (without the embryo), with the cut edge about 1 mm above the surface, in a 7-mm layer of Blue Tack (Bostik AB, Sweden) supported on nonfluorescent brown cardboard. The embryo ends were kept on a thin layer of Blue Tack in the same order for identification. When the desired number of kernels was ready, the pieces were pressed deeper so that the cut surfaces sat horizontally about 0.5 mm above the surface of the Blue Tack. Reverse emptying was used to apply 5 μl of 30% H_2O_2 (Analytical Perhydrol, Merck) to each of the cut surfaces with a 5–50 μl Finnpipette (Labsystems Ky, Finland). For large samples, a Hamilton PB600 microdispenser attached to a 1725 syringe with a short blunt-point needle (Hamilton Bonaduz, Switzerland) was used. After 1 hr under room conditions, an additional 5 μl of H_2O_2 was added. The samples were left in the dark under room conditions for at least four days, and usually for one week.

N-bromosuccinimide (Sigma) was mixed at 0.1 molar concentration in 0.1*M* Na_2HPO_4-KH_2PO_4 buffer, pH 8. After standing in the dark for about 12 hr, the solution was filtered through a Whatman 41 filter. The filtrate was used for one day. Five microliters of the NBS solution was dispensed onto each of the cut, H_2O_2-treated kernel ends, followed by an additional 5 μl of NBS 1 hr later. After the treated surfaces had dried, the material was observed under ultraviolet (UV) light in the dark (Analysenlampe PL 320 Labor-Modell, Hanau, FRG, or Mineralight UVG-54, Ultra-Violet Products, Inc., CA). Under these conditions, the normal-lysine kernels fluoresce with a bright blue light, whereas the high-lysine kernels appear greenish and fairly dull (Fig. 1). Less than 10% of the Hiproly kernels and less than 5% of the Risø 13, 18, 19, and 1508 kernels failed to show the dull, greenish fluorescence. Less than 3% of the kernels of Hiproly Normal and Bomi might have been misclassed as high-lysine. These misclassifications are at least partly due to the variation in the lysine content between kernels. The fluorescence of the kernels can be observed for several months. Although the effect of indoor light had not been tested, the kernels were stored in the dark. The fluorescence difference tends to increase upon storage.

A surface area of about 6 × 16 mm was prepared from several kernels of each of the four genotypes on Blue Tack. The sides of the cut kernels were pared with a scalpel or sanded with sandpaper to fit side by side in two tightly adjoining rows. Kernel surfaces were chemically treated, or dry kernels that had been treated earlier were used. The surfaces of each genotype were exposed to a Zeiss KM 3 chromatogram spectrophotometer (Zeiss, FRG). The mercury (Hg) line excitation between wavelengths 325 and 440 nm was applied to record the intensity of fluorescence being transmitted through the barrier filter FL46 (principally transmitting wavelengths over 460 nm). The result with the Risø 1508 - Bomi pair is depicted in Fig. 2. The fluorescence intensities are consistent with the visual observations under mercury light sources in that the fluorescence intensities of the normal-lysine kernels are higher. The range of excitation wavelengths used is evidently important for the visible fluorescence because a polystyrene layer absorbing below 320 nm lowers the fluorescence only slightly, and because a UV photographic filter absorbing below 380 nm eliminates the fluorescence.

The effect of proteins containing lysine on the fluorescence of normal-lysine, blue-fluorescing kernels of Bomi was tested.

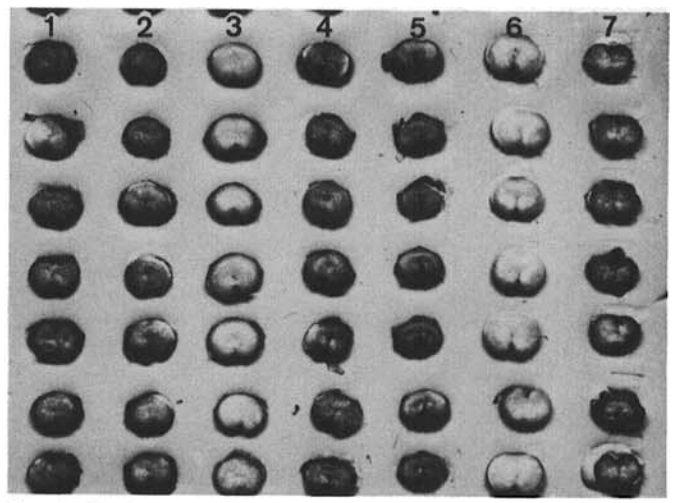

Fig. 1. Cut kernels stained by the *N*-bromosuccinimide procedure, illuminated with ultraviolet light, and recorded with panchromatic Kodak Tri-X pan film. Row 1 = Risø 13, 2 = Risø 18, 3 = Bomi, 4 = Risø 19, 5 = Risø 1508, 6 = Hiproly Normal, and 7 = Hiproly. The mutants Risø 18 and 19 are allelic with Risø 1508 (Jensen 1979a). Risø 13 is a high-lysine mutant of cultivar Bomi (Doll 1976) not allelic with Risø 1508 (Jensen 1979b). The normal-lysine barleys Bomi (3) and Hiproly Normal (6) emit the brightest fluorescence. Lysine contents of the meals: 1, 0.55%; 2, 0.57%; 3, 0.35%; 4, 0.59%; 5, 0.53%; 6, 0.45%; and 7, 0.69%.

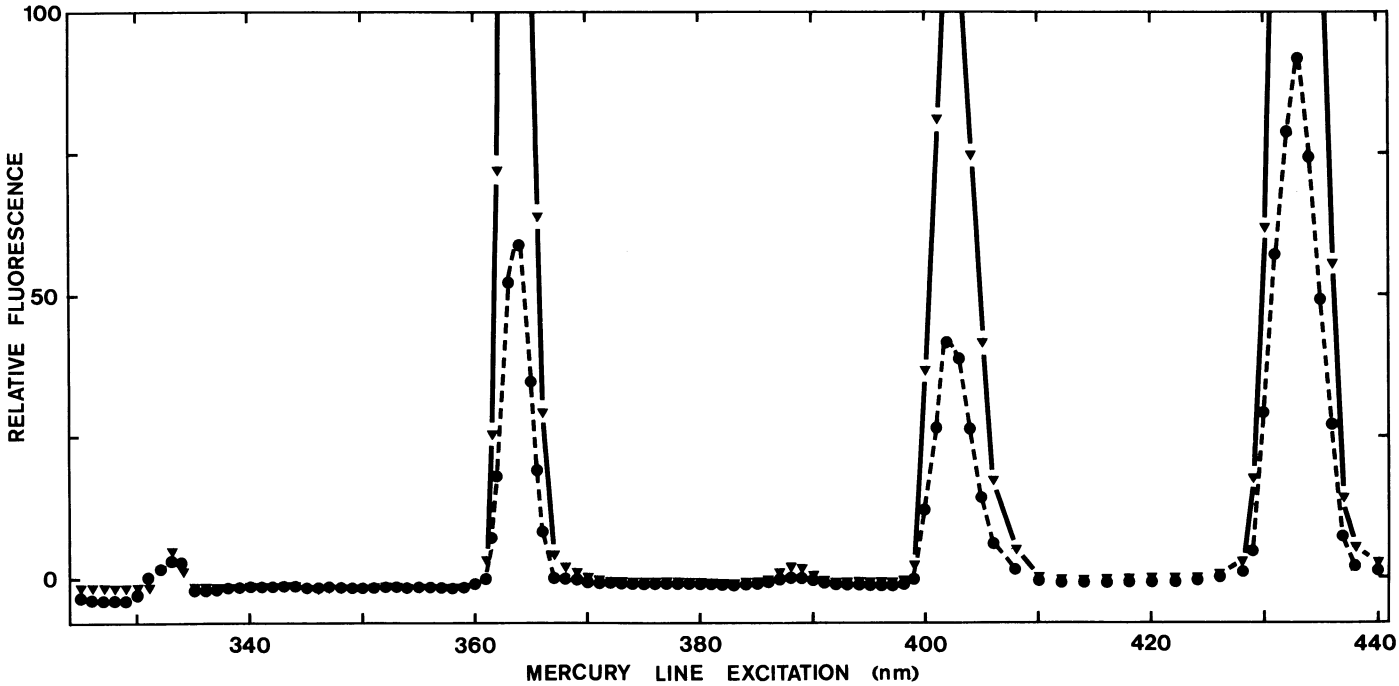

Fig. 2. Fluorescence recorded with a Zeiss KM3 chromatogram spectrophotometer from the cut *N*-bromosuccinimide-treated surfaces of kernels. Excitation with a mercury lamp, emission over 460 nm. Lysine content of Bomi (▼——▼) meal = 0.34%. Lysine content of Risø 1508 (●-----●) meal = 0.65%. Material from the 1978 season was raised under the same conditions. The intensities of cultivar Bomi at the mercury lines are two to three times higher.

Dissolved in the H_2O_2 solution, about 17 and 36 nmol of either bovine ribonuclease (Sigma type XI-A) or equine heart cytochrome C (Sigma type III), which contain 10 and 19 lysyl residues per molecule, respectively (Margoliash et al 1961, Smyth et al 1963), were applied to the cut surface of the kernels. After the NBS staining, these proteins changed the blue fluorescence of the Bomi control to greenish, the kernels with ribonuclease to dull greenish, and the kernels with cytochrome to bright greenish. The addition of L-glycine or L-lysine in H_2O_2 (up to 380 nmol per kernel) did not give rise to any visible change in the fluorescence of cultivar Bomi kernels. These observations support the concept that the greenish fluorescence is caused primarily by proteins, not by free amino acids or other cell components.

The purpose of the H_2O_2 treatment is to disorganize the cell contents at the cut surface. Hydrogen peroxide damages the lysyl residues of proteins (Sjöberg and Boström 1977) and may affect proteins via lipids (Gardner 1979).

The reactions with NBS and phosphate on kernels treated with H_2O_2 are obscure. The many cellular compounds particularly complicate the situation. Under certain conditions, NBS cleaves peptide bonds on tryptophyl and tyrosyl residues (Fontana et al 1981, Ohnishi et al 1980, Ramachandran and Witkop 1959). In acidic media, the basic amino acids consume NBS relatively rapidly (Schmir and Cohen 1961). The loss of ϵ-NH_2 groups of lysine in lysozyme under large amounts of NBS was previously described (Ramachandran and Witkop 1959).

This method differentiates the high-lysine kernels of low protein content (Bomi series, with 9–12% of N × 6.25) and of high protein content (Hiproly - Hiproly Normal pair with 15–17%). The method can differentiate other high-lysine variants of barley, as well as high-lysine segregants of the crosses with Risø 1508 and possibly with other mutants. The method may be extended to other small grain cereals having kernels that are easily cut.

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