Note on the Use of Sodium Hydroxide to Distinguish Red Wheats from White Common, Club, and Durum Cultivars[1]

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ABSTRACT

A color test employing sodium hydroxide was investigated as a method for distinguishing red wheats from white common, white club, and amber durum cultivars. A modified procedure was developed which required only 5 min and could be performed on a single kernel. Using the modified procedure, an untrained observer could correctly classify as red or nonred all 87 samples examined, which included 60 American wheat cultivars representing all classes. Yellow kernels (yellow berries) from samples of red wheats reacted the same way as did dark, hard, and vitreous kernels.

Objective methods are needed to permit the assignment of class for an unknown wheat seed sample, and a procedure that would quickly distinguish a red wheat from a nonred one would be useful. This distinction is usually made subjectively by visual inspection, but observers do not always agree. However, a simple objective test has been used by geneticists to classify a wheat sample as red or nonred after color development in sodium or potassium hydroxide.

The use of sodium or potassium hydroxide to distinguish red from nonred kernels is found in the literature as early as 1938 (Chmelar and Mostovoj) in a review of laboratory methods for testing the genuineness of variety. Quartley and Wellington (1962) tested the accuracy of the method on 19 red and white United Kingdom wheat varieties that had been treated with a seed dressing, and Kimber (1971) relied on the test in studying the inheritance of red grain color in wheat. In Kimber's procedure, wheat grains were soaked in 5% sodium hyroxide for 60–90 min. More recently, the test was described (Coles and Wrigley 1976) in a discussion of laboratory methods for identifying New Zealand wheat cultivars. We undertook to modify the test so that it could be run quickly and to evaluate the reliability of the procedure for wheat cultivars grown in the United States.

MATERIALS AND METHODS

Wheat samples were obtained from various growing locations and represented hard red winter, soft red winter, hard red spring, white common, white club, and amber durum wheats grown in the United States. Most samples were from the 1977 and 1978 crops, although a few were from the 1971–1976 crops. "Baker analyzed" sodium hydroxide used for color development was obtained from J. T. Baker Chemical Co.

Color was developed by exposing wheat kernels to 5.0% (w/v) sodium hydroxide at room temperature for 60 min. Samples consisting of 1–10 kernels were placed with 1.5 ml of base in the wells of a white porcelain spot plate, 118 × 90 × 13 mm, having cavities 21 mm in diameter by 7.5 mm deep. After 60 min, samples were classified as red or nonred on the basis of the color developed.

In a rapid version of the test, kernels were exposed to 1.5 ml of 5.0% (w/v) sodium hydroxide for 5.0 min at 55°C. Temperature was controlled by means of the apparatus shown in Fig. 1, a Tecam DB-3 block heater having an aluminum block specially machined to hold a spot plate like the one used in the 60-min test. Temperature of the block could be controlled to within ± 0.1°C over the range of 30–100°C. To maintain the sodium hydroxide solution at the desired temperature, insulating characteristics of the spot plate required setting the block temperature at a higher value. A thermocouple placed in the wells of the spot plate was used to

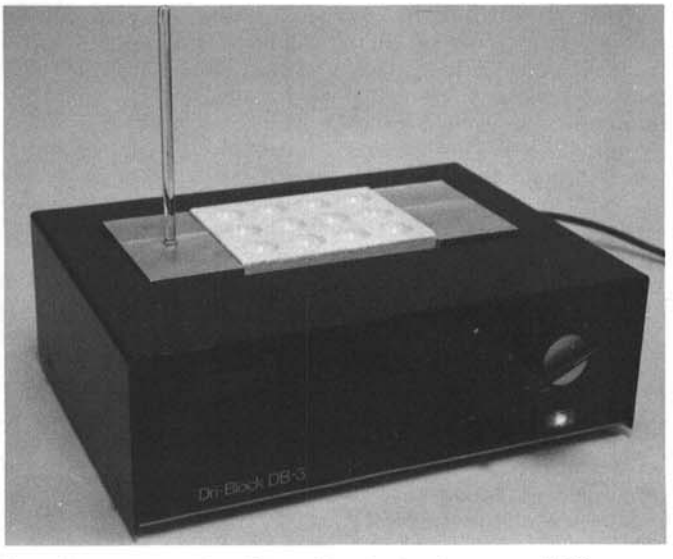

Fig. 1. Apparatus employed for rapid color development at 55°C.

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TABLE I
Wheat Cultivars Tested with Sodium Hydroxide[a]

Hard Red Winter	Soft Red Winter	Hard Red Spring	White Common	White Club	Durum
Buckskin (2)	Abe (3)	Angus	Daws	Barbee	Calvin
Centurk (2)	Arthur	Butte	Genesee	Moro	Cando (2)
Eagle (2)	Arthur 71 (3)	Chris (2)	Hyslop (2)	Omar (2)	Edmore
Gage (2)	Double Crop	Coteau	Ionia	Paha (3)	Rolette
Homestead (2)	Hart (3)	Era	Luke (2)		Rugby
Lancota (2)	Logan	Eureka	McDermid		Ward
Parker (2)	Monon	Kitt	Nugaines (2)		
Parker 76	Oasis	Monitor	Raider		
Sage (3)	Pioneer S-76	Olaf	Sprague		
Scout (2)	Roland	Red River 68	Tecumseh		
Sturdy	Ruler	Thatcher	Urquie		
Tam W101		Waldron (2)	Yamhill (2)		
Triumph 64 (2)		World Seeds 25			
Vona					

[a]If more than one sample of a cultivar was tested, the number of samples is indicated in parenthesis.

determine the block temperature needed to maintain the sodium hydroxide at 55°C, and this block temperature was used in subsequent experiments.

RESULTS AND DISCUSSION

Standard Procedure (Kimber 1971)

When placed in 5% sodium hydroxide at room temperature, white common, white club, and amber durum wheats become straw yellow in appearance, whereas red-grained cultivars develop a brown color. The yellow appears almost immediately, but the brown develops slowly over a period of more than an hour. We found the test to be quite reliable if 60 min was allowed for color formation. After color development, one could easily distinguish the red wheats from the nonred cultivars. Kernels that originally had been different shades of brown were either distinctly brown or yellow. Age of a sample did not have a noticeable effect on color development.

Five-Minute Modified Procedure

As the time allowed for color development at room temperature is reduced below 60 min, reliability of the procedure decreases rapidly because of the slow rate of formation of the brown color that identifies the red wheats. Time required to make a test is thus a major disadvantage of the standard method. Neither more concentrated sodium hydroxide nor a different base appreciably reduced the time required for color development. Color development was, however, much more rapid at elevated temperatures. At 55°C the test could be completed in 5.0 min, with results comparable to those obtained using the longer reaction time. Temperatures above 55°C, which gave even more rapid color development, were considered to be less satisfactory because of the increased evaporation rate of the sodium hydroxide solution. The test may be conducted in a test tube, petri dish, or any convenient container, but we preferred to use a white porcelain spot plate because the background made the colors more distinct.

Color Formation

The reason for color production when wheat seed is placed in strong base is not well understood. Researchers have known for some time, however, that at least one compound in wheat, possibly a flavone (Simpson 1935), gives a relatively strong yellow color when treated with base. In the absence of any interfering color, a wheat kernel placed in base would thus be expected to become straw yellow, as do the kernels of white common, white club, and amber durum wheats. In red wheats, on the other hand, a compound or group of compounds in the bran, probably the red pigment, becomes brown upon exposure to base, and, because it is in the bran, the brown color predominates. The fact that the brown color is indeed in the bran is easily demonstrated by slicing open a kernel after color development.

Yellow Berry

In red wheats a condition called yellow berry is characterized by kernels having a lighter appearance than usual, and we thought that these yellow kernels, or yellow berries, might affect the test adversely. The yellow berries, however, reacted the same way as did dark, hard, and vitreous kernels on exposure to sodium hydroxide. Color development was normal in both the 60-min procedure and the 5-min modification.

Reliability

To test the reliability of the two procedures, a total of 87 wheat seed samples, representing 60 cultivars grown in the United States (Table I), were examined. An attempt was made to obtain representative samples of each class of wheat grown. Included were five of the 10 American hard red winter wheats most extensively cultivated in 1974, the last year for which complete statistics are available (Reitz and Hamlin 1978). Also included were five of the top soft red winter wheats, three of the leading hard red spring wheats, four of the top white common wheats, the three principal white club cultivars, and two of the leading durum wheats, as well as cultivars that have become important since 1974.

The test was found to be completely reliable. An untrained observer could correctly classify all samples included in the study on the first attempt. This was true for the 60-min test and also for the 5-min modification. The modified procedure is therefore both reliable and rapid. Either version can be performed on a single kernel or on many, as required.

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