NOTE

A Rapid Method for the Detection of Sprouting in Populations of Wheat Kernels

Sv. A. JENSEN[1] and D. P. LAW[2]

In recent years, researchers have devoted much effort to developing rapid tests for detecting field germination or sprouting in wheat. The demand for such a test arises from the need to ensure that wheat deliveries containing sprouted kernels are not binned with those containing sound grains; the enzymes produced on germination, particularly α-amylase, have a deleterious effect on the processing quality of the milled flour.

A number of methods for detection of sprouting have been developed that utilize the determination of α-amylase either directly or indirectly. The falling number and amylograph procedures, which measure the viscosity of flour paste heated under controlled conditions, are commonly used in milling and baking laboratories (Kruger and Tipples 1980). These and other available methods, ie, nephelometric (Campbell 1980) and fluorometric methods (Marchylo and Kruger 1978), are, however, not generally suitable for use at wheat receival depots. In a recent paper (Kruger and Tipples 1980) it was reported that only one highly sprouted kernel in 90 g can have a substantial effect, especially on the falling number and amylograph values. As a consequence, few highly sprouted kernels in a wheat sample can result in the rejection of the whole sample, although it may have good milling and baking properties. Visual examination of the embryo of wheat kernels is, therefore, the basis of many classification systems.

This note outlines a simple fluorescence technique for determining sprouting in populations of wheat kernels. The method allows the visualization of hydrolase activity in the endosperm of germinated wheat kernels by the use of a lipase-sensitive fluorochrome (Jensen and Heltved 1982).

MATERIALS AND METHODS

Wheat

A series of 36 Australian wheats harvested in 1981 was evaluated, except for one sample. The sample set included six genotypes grown at six locations. The samples had a range in falling number of 68–684 sec (Table I).

Falling Number Determination

The standard ICC method (ICC 1967) was followed, and a 7-g sample of ground wheat was used. Results are reported in seconds.

Visualization of Sprouted Wheat Kernels

Fifty individual wheat kernels from a sample were mounted in one working operation on a cernit plate, using the Seed Fixation System described by Heltved et al (1982). Four plates were obtained from each wheat sample, thus providing assessments based upon 200 individual wheat kernels. After sanding, the "half seeds" obtained were stained with a $10\text{-}3M$ solution of fluorescein dibutyrate (Serva Feinbiochemica, Heidelberg, West Germany) in 80% (v/v) aqueous ethanol (Jensen and Heltved 1982). Visualization of enzyme activity was based upon hydrolysis of nonfluorescent fluorescein dibutyrate to butryate and highly fluorescent fluorescein (λex = 490 nm, λem = 520 nm), a reaction known to be catalyzed by hydrolases, preferentially by lipase (Guilbault and Kramer 1964). The appearance of yellow fluorescent fluorescein in sprouted wheat kernel (Fig. 1) was

TABLE I
Falling Number (sec) of Wheat Samples

	Location					
Genotype	I	II	III	IV	V	VI
A Timgalen	79	164	340	367	493	559
B Kite	206	262	441	506	510	616
C Oxley	126	309	447	475	479	548
D Shortim	68	353	384	441	525	684
E Flinders	329	375	397	455	486	595
F QT 2870	···	400	409	447	468	568

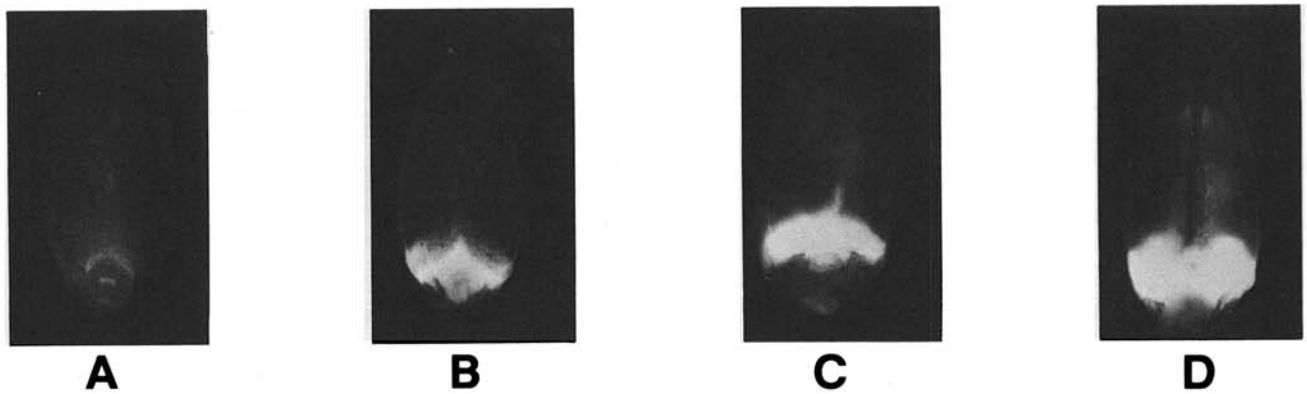

Fig. 1. Longitudinal "half seed" sections of wheat kernels (genotype Shortim) with varying degrees of sprouting. The kernel sections were stained with fluorescein dibutyrate and after 10 min examined in Carlsberg macrofluorescence microscope. Kernel **A,** no indication of sprouting; kernel **B,** incipient sprouting; kernel **C,** moderate sprouting; and kernel **D,** high degree of sprouting.

[1]Carlsberg Research Laboratory, Dept. of Biotechnology, DK-2500 Valby, Denmark.
[2]Queensland Wheat Research Institute, Queensland Dept. of Primary Industries, 13 Holberton Street, Toowomba 4350, Australia.

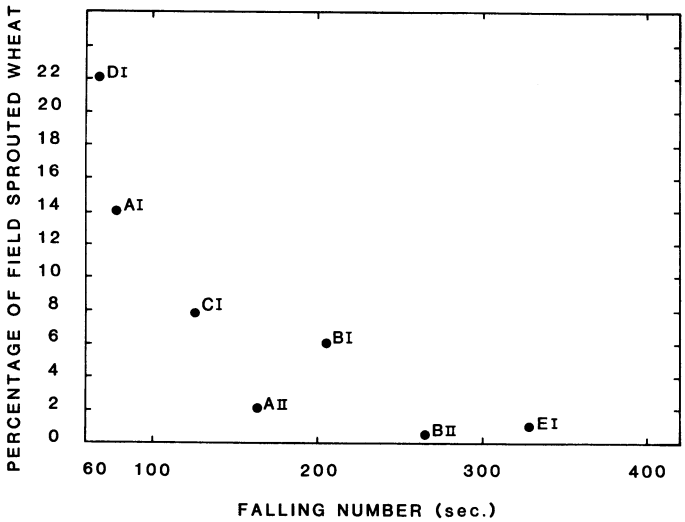

Fig. 2. Percentage of field-sprouted wheat kernels versus falling number. Letters and figures refer to Table I.

evaluated through use of a Carlsberg macrofluorescence microscope (Gibbons 1981) and in a Carlsberg Seed Quality Analyzer. Both instruments were obtained from the Carlsberg Research Center.

RESULTS AND DISCUSSION

The range in hydrolase activity detected in the wheat kernels is illustrated in Fig. 1. Kernel A was practically devoid of fluorescence, thus indicating a low level of activity for enzymes hydrolyzing fluorescein dibutyrate. In kernel B, a distinct yellow fluorescence appeared at the scutellum endosperm interface. Hydrolase activity in kernels C and D gradually spread over the entire endosperm, indicating a high level of enzyme activity in these kernels. The hydrolase activity range shown in Fig. 1 coincides with the germination period of 0–4 days used in the previous study (Jensen and Heltved 1982).

The relation of falling number to the percentages of field-sprouted wheat kernels as assessed by the fluorescence method (N = 200) is shown in Fig. 2. The low falling number as observed for the five varieties grown at location I was clearly reflected by a higher percentage of field-sprouted kernels. In the Shortim sample with a falling number of 68 sec, 22 out of 100 kernels were sprouted. In samples with falling number above 329 sec, no sprouted kernels were detected. A relatively good inverse relation existed between the percentage of sprouted kernels and the falling number value when the falling number values were below approximately 350 sec (Fig. 2).

The present fluorescence technique provides a simple and rapid method for the detection of sprouted wheat kernels. The analysis can be performed within 10 min. Varying degrees of sprouting, particularly incipient sprouting, which is difficult to assess accurately by a visual scoring system, is detected efficiently by the present technique. A further application of the method will soon be published.

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