Preharvest Sprouting in Hard Winter Wheats: Assessment of Methods to Detect Genotypic and Nitrogen Effects and Interactions[1]

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ABSTRACT

A study was undertaken on hard red and white winter wheats (*Triticum aestivum* L.) to determine the effects of genotypes, nitrogen levels, and the interaction of these on preharvest field sprouting. The effects were measured by determining α-amylase activity, protease activity, and falling number. Significant genotypic differences in sprouting were found by all three criteria. Nitrogen effects on sprouting were not significant. Differences in sprouting due to the interaction of genotype and nitrogen levels were shown by falling number only. In general, α-amylase activity and falling number appeared to be better determinants of sprouting damage than did proteolytic activity.

Preharvest sprouting beyond minimal levels detrimentally affects milling and baking qualities of hard wheats (Greenaway 1969, Perten 1964). Sprouting potential is related closely to pericarp color; white wheats are considered to lack the pigmentation that inhibits sprouting in red wheats (Freed et al 1976, McEwan 1976). Frequent rains that caused extraordinary preharvest sprouting in sections of the central Great Plains during 1979 gave the opportunity to assess sprouting damage in two popular hard red winter wheat cultivars, one hard white winter wheat cultivar, and one hard white winter wheat experimental line, all of which differed in susceptibility to preharvest sprouting.

Bhatt et al (1977) and Derera et al (1977) recommended biochemical determinations of enzyme activities and the measurement of falling number to assess sprouting damage in wheat. They and other investigators showed substantial genotypic variation in sprouting susceptibility. Few reports considered the interaction of genotype and environment, including soil nitrogen status. Two recent studies (Hong 1979, Nielsen 1980), however, involved the effects of genotype-environment interactions on sprouting. Hong (1979) found that genotype-location interaction effects were insignificant for sprouting, dormancy, and α-amylase activity, whereas genotype-year interaction effects were more important components of variation among the three traits studied. He also found significant variety-year-location interactions for the three traits, which suggested that breeding for stable sprouting characteristics may be difficult. Nielsen (1980) determined the influence of climatic factors on sprouting in wheat. He found that diurnal temperature fluctuations, daily average temperatures, and precipitation before and after physiological maturity affected sprouting susceptibility. His results indicated a complex effect of climate on sprouting susceptibility.

This article presents results of our study measuring the influence of genotypes, nitrogen fertilizer levels, and genotype-nitrogen interactions on preharvest field sprouting in hard winter wheats and evaluating several laboratory methods for measuring sprouting. The relationship among the methods for measuring sprouting and other grain traits also was determined.

MATERIALS AND METHODS

Wheat Genotypes

The four genotypes used were Newton, which is the most popular hard red winter wheat cultivar in Kansas and is sprouting-resistant; Centurk, another popular hard red winter wheat, which is sprouting-susceptible; Clark's Cream, a sprouting-resistant hard white winter wheat cultivar; and KS73256, a sprouting-susceptible hard white winter wheat experimental line.

The four genotypes were seeded at the University Agronomy Farm, Manhattan, KS, on October 18, 1978. The soil contained medium levels of available nitrogen (19 ppm N) and phosphorus (24 ppm P) and a high level of exchangeable potassium (112 ppm K). Interrow and intrarow spacings were 20 cm and 6 cm, respectively. The experiment used a split-plot design with three replications. Main plots were assigned to the four genotypes, and subplots were assigned to three nitrogen levels—0, 50, and 100 kg/ha—applied in the form of NH_4NO_3 on April 24, 1979. The purpose of the nitrogen fertilizer was to induce different protein levels in the grain so that the relationship between grain protein content and sprouting damage could be determined. Each subplot had six rows 10 m long. The plants were grown under dryland (rainfed) conditions.

Heavy rainfall (13.4 cm) between harvest maturity in early July and actual harvest on July 27 caused differential sprouting. Percentage of sprouting was assessed visually by counting the grains in which the radicle and/or the scutellum had penetrated the pericarp on ten spikes on ten randomly-chosen plants in each plot in one replication. Yield was determined by weighing the grain from a 9.6 m^2 area of each plot and test weight by weighing a 0.47-L (one-pint) subsample of the grain. Grain protein percentage was determined by near-infrared reflectance.

Laboratory Assays

Grain samples were ground through a 0.6-mm sieve and analyzed for enzyme activities (α-amylase and protease) and falling number. α-Amylase activity was determined as described by Mathewson and Pomeranz (1977) with Phadebas tablets as the substrate. Enzyme activity was read as absorbance at 620 nm and expressed in millidextrinizing units per gram per hour. Procedures outlined by Wittenbach (1978) were followed for determining the protease activity of the samples. Azocasein was used as substrate, and enzyme activity was expressed as milliabsorbance units at 340 nm (1 milliabsorbance unit = enzyme assay absorbance × 1,000). Falling number measurements were expressed in seconds (AACC 1976).

Experimental results were analyzed as a split-plot design. Interrelationships among α-amylase activity, protease activity, and falling number as well as those between each of the three criteria and grain yield, grain protein percentage, test weight, and field sprouting score were studied by computing simple correlation coefficients.

RESULTS

Grain yields were significantly higher and protein percentages significantly lower from the hard red wheats than from the hard white wheats (Table I). Test weights did not differ among the genotypes, but sprouting varied as expected, ie, low for Newton and Clark's Cream and high for Centurk and KS73256. Nitrogen fertilizer increased the grain protein percentage of all genotypes

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except Clark's Cream but had no significant effect on grain yield or test weight.

Analyses of variance for α-amylase activity, protease activity, and falling number are given in Table II. Highly significant (0.01 level) genotypic differences existed for the three traits. No differences due to nitrogen treatments were significant for any of the three traits. The interaction of genotypes with nitrogen levels was significant only for falling number.

Mean values of the enzyme activities and of falling numbers are given in Table III. The two sprouting-resistant gentoypes, Newton and Clark's Cream, had low α-amylase activity, whereas the two sprouting-susceptible gentoypes, Centurk and KS73256, had high enzyme activity. Relative levels of α-amylase activity in the four genotypes (Table III), in general, agreed with the visual assessment of field sprouting (Table I). However, no such trend was evident in protease activity determined on the same set of samples. Newton and KS73256 exhibited relatively low protease activity. Centurk and Clark's Cream showed almost equal protease activities, which were significantly higher than those of Newton and KS73256.

Newton had the highest falling number, significantly higher than that of Clark's Cream, which was intermediate. The falling numbers paralleled the low amount of sprouting in both those genotypes. The falling numbers of Centurk and KS73256 were nearly equal and very low, suggesting that both genotypes were extremely susceptible to sprouting.

Differences in falling number due to nitrogen levels were not significant, but the interaction of genotypes with nitrogen levels was highly significant. Newton exhibited greater sprouting resistance by its higher falling number at a nitrogen level of 100 kg/ha than at 0 and 50 kg/ha. The reverse was true for Clark's Cream, in which resistance to sprouting increased with decreasing levels of nitrogen fertilizer. Falling number of the two sprouting-susceptible cultivars, Centurk and KS73256, did not interact with

TABLE I
Effect of Genotype and Nitrogen Fertilizer on Grain Yield, Protein, Test Weight, and Field Sprouting of Wheat

Genotype	Nitrogen Treatments, kg/ha 0	50	100	Genotype Mean
Grain Yield, kg/ha[a]				
Newton	2,352	2,634	2,292	2,426
Centurk	2,486	2,379	2,419	2,428
Clark's Cream	1,626	1,788	1,848	1,754
KS73256	1,331	1,472	1,667	1,490
Treatment mean	1,949	2,068	2,056	
Grain Protein, %[b]				
Newton	10.9	11.8	12.8	11.8
Centurk	11.1	11.7	12.6	11.8
Clark's Cream	12.9	13.5	13.9	13.4
KS73256	11.9	12.3	13.3	12.5
Treatment mean	11.7	12.3	13.2	
Test Weight, kg/hl[c]				
Newton	71.4	69.6	69.4	70.1
Centurk	70.0	68.7	67.3	68.7
Clark's Cream	69.8	68.6	69.0	69.1
KS73256	61.9	61.5	58.5	60.6
Treatment mean	68.3	67.1	66.1	
Field Sprouting, %[d]				
Newton	2	2	0	1
Centurk	17	30	40	29
Clark's Cream	3	20	17	13
KS73256	78	75	80	78
Treatment mean	25	32	34	

[a]Least significant difference ($P = 0.05$) is 437 for gentoypes and not significant for nitrogen rates and the interaction.
[b]Least significant difference ($P = 0.05$) is 0.6 for genotypes, 1.3 for nitrogen rates, and not significant for the interaction.
[c]Least significant difference ($P = 0.05$) is not significant for genotypes, nitrogen rates, and the interaction.
[d]One replication only; visual assessment.

TABLE II
Analysis of Variance for α-Amylase Activity, Protease Activity, and Falling Number

Source	Degrees of Freedom	Mean Squares α-Amylase Activity	Protease Activity	Falling Number
Blocks	2	8,139	1,157	3
Genotypes	3	2,910,737[a]	14,947[a]	88,615[a]
Error	6	14,364	450	110
Nitrogen treatments	2	39,727	785	161
Genotype × nitrogen treatments	6	15,941	1,714	946[a]
Error	16	12,518	476	60

[a]Significant at $P = 0.01$.

TABLE III
Effect of Genotype and Nitrogen Fertilization on α-Amylase Activity, Protease Activity, and Falling Number of Wheat

Genotype	Nitrogen Treatments, kg/ha 0	50	100	Genotype Mean
α-Amylase Activity (mDU/g)/hr[a]				
Newton	21.3	11.3	16.3	16.3
Centurk	305.7	498.7	613.7	472.7
Clark's Cream	66.7	78.7	81.7	75.7
KS73256	1,177.0	1,260.3	1,315.7	1,251.0
Treatment mean	392.7	462.3	506.8	
Protease Activity (milliabsorbance units)[b]				
Newton	39.3	28.3	53.2	40.3
Centurk	93.6	144.8	134.2	124.2
Clark's Cream	136.8	114.1	98.5	116.5
KS73256	47.7	48.5	94.5	63.6
Treatment mean	79.4	83.9	95.1	
Falling Number (sec)[c]				
Newton	259.0	264.7	289.3	271.0
Centurk	67.3	63.0	62.0	64.1
Clark's Cream	188.0	167.0	134.3	163.1
KS73256	62.0	62.0	62.0	62.0
Treatment mean	144.1	139.2	136.9	

[a]Least significant difference ($P = 0.05$) is 207.5 for genotypes and not significant for nitrogen rates and the interaction. Measured in millidextrinizing units per gram per hour.
[b]Least significant difference ($P = 0.05$) is 36.7 for genotypes and not significant for nitrogen rates and the interaction.
[c]Least significant difference ($P = 0.05$) is 18.2 for genotypes, not significant for nitrogen rates, and 6.7 for the interaction.

TABLE IV
Correlation Coefficients Among Agronomic and Grain Quality Traits of Wheat

	Grain Protein	Test Weight	Field Sprouting	α-Amylase Activity	Protease Activity	Falling Number
Grain yield	−0.47	0.67[a]	−0.64[a]	−0.57	0.00	0.37
Grain protein		−0.25	0.12	0.02	0.36	−0.04
Test weight			−0.95[b]	−0.95[b]	0.13	0.61[a]
Field sprouting				0.98[b]	−0.05	−0.77[b]
α-Amylase activity					−0.07	−0.75[b]
Protease activity						−0.44

[a]Significant at $P = 0.05$.
[b]Significant at $P = 0.01$.

nitrogen levels.

Interrelationships among the seven traits are presented in Table IV. Grain yield varied directly with test weight and inversely with grain sprouting percentage. Test weight showed highly significant negative correlation with sprouting percentage and α-amylase activity and positive correlation with falling number. Of the three laboratory methods, α-amylase activity had the highest correlation with field sprouting percentage. Falling number had a smaller, but still highly significant, negative correlation with sprouting percentage. Protease activity and sprouting percentage were not related.

DISCUSSION

The adverse effects of preharvest sprouting on grain yield and test weight were evident. Both consequences were probably related; high respiration rates associated with sprouting[3] consumed accumulated carbohydrates in the grains and decreased their weight. Thus, preharvest sprouting was detrimental to agronomic performance as well as to milling and baking qualities (Greenaway 1969, Perten 1964). Differences in resistance to preharvest sprouting among genotypes were apparent (Derera et al 1977, McEwan 1976). The marked resistance of the white genotype Clark's Cream was particularly notable; this cultivar appears to be a good genetic source of the trait for wheat improvement programs. The other major factor studied, nitrogen fertilizer, had little effect on sprouting (Belderok 1967, Greer and Hutchinson 1945, von Lochow 1952).

The results indicate that preharvest sprouting resistance can be selected in red or white wheats by screening for α-amylase activity and/or falling number. α-Amylase activity appears to be a better criterion than falling number because the former was more stable for the different genotypes over the different nitrogen levels used in this study. Screening the breeding material for both α-amylase activity and falling number as recommended by Derera et al (1977) may be desirable, however, because falling number measures starch degradation in addition to α-amylase activity.

Proteolytic enzyme activity may play a role during germination by effecting the early breakdown and subsequent deterioration of gluten protein in situ (Kruger 1980). Huang (1979) found that matrix protein decreased in sprouted wheats, indicating that proteolytic enzymes break down the matrix protein and thereby produce a loose structure around starch granules, which makes them more accessible to α-amylase attack. Accordingly, one would expect an inverse correlation between falling number and protease activity. This was evident in our study, although the correlation was not statistically significant.

Kruger and Preston (1976), working on protease activity of sprouting-resistant and sprouting-susceptible wheats, concluded that sprouting-resistant wheats occasionally had lower protease activity than sprouting-susceptible wheats did. However, the relationship is not firm, as shown by our results. No definite correlation between sprouting resistance and proteolytic activity has yet been found. Thus, some of the published evidence as well as that obtained from our study indicate that proteolytic activity alone is not a usable criterion for measuring preharvest sprouting in wheat.

The value of time-consuming laboratory determinations of α-amylase and falling number in assessing preharvest sprouting might be questioned in view of the very close associations of these measurements with the visual field sprouting score found in our study. However, visual field sprouting may only efficiently detect sprouting under extraordinary sprouting conditions. Varying degrees of sprouting, particularly incipient sprouting, cannot be assessed accurately by a visual scoring system, and therefore laboratory determinations of α-amylase and falling number are important.

[3]Unpublished data.

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