# Preharvest Sprouting and α-Amylase Activity in Hard Red and Hard White Winter Wheat Cultivars[1]

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## ABSTRACT

Preharvest sprouting beyond minimal levels increases α-amylase activity and decreases functional quality of wheat (*Triticum aestivum* L.) grain. No information is available on the sprouting susceptibility of popular hard red winter wheat (HRWW) cultivars in the U.S. Great Plains or on experimental hard white winter wheat (HWWW) lines being developed for the area. Objectives of our study were to determine preharvest sprouting susceptibility of popular HRWW cultivars and experimental HWWW lines under simulated rain conditions and at geographical areas differing in sprout-inducing environments. HRWW cultivars differed markedly in sprouting and in α-amylase synthesis in sprouted grain, which increased as time between maturity and exposure to simulated rain was increased. Sprouting and α-amylase activity measured by falling number and dye-labeled starch degradation were highly correlated. However, cultivar and temporal deviations from that relationship occurred. HWWW lines were generally more susceptible than were HRWW cultivars, but exceptions occurred and differences were more quantitative than qualitative. An HWWW cultivar, Clark's Cream, was resistant to preharvest sprouting. The simulated sprouting environment clearly identified sprouting-resistant phenotypes; it would be useful for selecting desirable germplasm in wheat improvement programs.

Preharvest sprouting of wheat (*Triticum aestivum* L. em Thell) grain is generally not a serious problem in the Great Plains because of favorable climatic conditions and modern rapid harvest techniques. Consequently, little information is available on the subject, and regional wheat breeding programs devote little attention to preharvest sprouting. However, severe preharvest sprouting in some areas in 1979 and the recent emphasis on developing hard white winter wheats for the region has raised concern about the problem.

Wheat grain dormancy, and therefore sprouting resistance, is closely related to pericarp color. Previous investigations showed that white wheats lack dormancy and are more subject to sprouting than are red wheats (Freed et al 1976, Gfeller and Svejda 1960, McEwan 1980). Increased hydrolytic enzyme activity, particularly α-amylase activity, accompanies sprouting and detrimentally affects milling and baking qualities of wheat when beyond minimal levels (Greenaway 1969, Perten 1964). Breeding programs in regions where preharvest sprouting is more prevalent focus attention on cultivar differences in sprouting resistance, including that associated with differential α-amylase production (Derera et al 1977, Flintham and Gale 1980).

Low α-amylase production is associated by linkage or pleiotropism with the dwarfing gene Rht3 in wheat (Flintham and Gale 1980). The higher yield potential of semidwarf (SD) wheat cultivars (Rht1 and/or Rht2) is increasing their popularity in the Great Plains; they will probably become the dominant plant type in the near future. Although the preharvest sprouting susceptibility of Rht1 and Rht2 SD wheats studied so far does not differ from that of tall cultivars, quantitative differences in the amount of α-amylase they produce were observed (Gale and Marshall 1975).

The present study was undertaken to examine preharvest sprouting susceptibility of hard red winter wheat (HRWW) cultivars in the major wheat-growing area of the Great Plains, to study the relationship between sprouting and α-amylase production in those cultivars, and to determine whether hard white winter wheat (HWWW) is more susceptible to sprouting than is HRWW in the region. Methods of evaluating preharvest sprouting were also compared.

## MATERIALS AND METHODS

### Red Wheats

Eighteen red winter wheat cultivars (Bennett, Buckskin, Centurk, Centurk 78, Cheney, Gage, Hart, Larned, Lindon, Newton, Parker 76, Payne, Sage, Scout 66, TAM W-101, Trison, Triumph 64, and Vona), representing most of the wheat acreage of Colorado, Kansas, Nebraska, Oklahoma, and Texas, were studied. All cultivars are HRWW except the soft red winter wheat cultivar Hart. Lindon, Newton, Payne, TAM W-101, and Vona are SD cultivars. The wheats were grown at the North Agronomy Farm, Manhattan, KS, during the 1978 and 1979 crop years.

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Approximately 1,000 spikes were randomly harvested from drill strips 50 m long when the grain was harvest ripe (< 16% moisture). Spikes were stored at room temperature until subjected to a sprouting environment. Sprout-inducing treatments were no rain and rain at 0, 2, and 4 weeks after harvest. A treatment observation consisted of 20 intact spikes per cultivar replicated three times per treatment date. Spikes were placed in a rain simulator similar to that described by McMaster and Derera (1976). Fifty millimeters of "rain" was applied to the spikes over a 2-hr period at 20°C, and relative humidity in the simulator was maintained at 100% for 48 hr. Spikes removed from the simulator were air dried at 40°C and threshed. Germination percentages of the rain-treated wheat were determined on 100-grain subsamples. Grain was considered germinated if the pericarp over the embryo had ruptured. All samples were ground in a Udy Cyclone mill to pass a 0.01-cm sieve.

### Analytical Methods

α-Amylase activity was determined by two methods. A direct colorimetric method described by Mathewson and Pomeranz (1977) used a dye-labeled starch substrate. Enzyme activity was expressed as millidextrinizing units (mDU) per gram$^{-1}$ per hour$^{-1}$. The second, an indirect method, was a modified falling number determination described by AACC method 56-81B (1972). Sample weight was decreased from 7 to 5 g, and water volume was 20 ml, causing values to be slightly lower than in the standard falling number method.

### White Wheats

Sprouting potential of HWWW was compared with that of HRWW in two studies. In the first study, three HRWW cultivars (Centurk, Newton, and Lancota), one HWWW cultivar (Clark's Cream), and one HWWW experimental line (KS73256, an SD) were grown at the Ashland Agronomy Farm in 1978 and the North Agronomy Farm in 1979. Assessment of sprouting potential and α-amylase production were essentially the same as in the red wheat experiment. In the second study, samples of three HRWW cultivars (Eagle, Newton, and TAM W-101) and two HWWW experimental lines (KS75216—a sister line of KS73256 and Newton—and KS77H2690, a white selection from Eagle) were obtained from seven locations of the Kansas Intrastate Nursery during the 1979 crop year. Samples were analyzed for α-amylase by the colorimetric method as before. Falling number was determined on 7-g samples with 25 ml of water. No sprouting was induced, but some sprouting had occurred naturally before harvest.

### Statistical Analysis

Data were analyzed by analysis of variance procedures of the Statistical Analysis System (SAS 1979). Spearman's coefficient of rank correlations were performed as outlined by Steel and Torrie (1960). All tests were analyzed as completely random designs.

**TABLE I**
**Mean α-Amylase Activities, Falling Numbers (FN), and Germination Percentages of Wheat Cultivars Before and After Simulated Rain Treatment During 1978 and 1979**

| | | | Treatment at Weeks After Harvest Maturity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | 0 | | | 2 | | | 4 | | |
| Cultivar | Amylase (mDU)[a] | FN (sec) | Germination (%) | Amylase (mDU) | FN (sec) | Germination (%) | Amylase (mDU) | FN (sec) | Germination (%) | Amylase (mDU) | FN (sec) |
| Bennett | 32 | 331 | 20 | 108 | 254 | 27 | 274 | 186 | 84 | 13,114 | 60 |
| Buckskin | 36 | 346 | 36 | 233 | 198 | 46 | 1,897 | 64 | 93 | 17,505 | 60 |
| Centurk | 27 | 347 | 66 | 964 | 89 | 92 | 11,465 | 60 | 97 | 27,051 | 60 |
| Centurk 78 | 24 | 349 | 61 | 932 | 97 | 94 | 10,930 | 60 | 98 | 24,044 | 60 |
| Cheney | 20 | 342 | 10 | 55 | 313 | 62 | 2,965 | 62 | 96 | 30,088 | 60 |
| Gage | 24 | 350 | 15 | 68 | 303 | 53 | 4,716 | 65 | 93 | 17,618 | 60 |
| Hart | 24 | 301 | 16 | 55 | 299 | 39 | 1,261 | 123 | 78 | 18,471 | 63 |
| Larned | 24 | 342 | 19 | 47 | 346 | 67 | 4,011 | 64 | 90 | 26,196 | 60 |
| Lindon | 19 | 349 | 7 | 41 | 331 | 15 | 100 | 250 | 70 | 5,159 | 97 |
| Newton | 24 | 313 | 11 | 106 | 240 | 58 | 5,588 | 66 | 92 | 35,388 | 60 |
| Parker 76 | 32 | 351 | 82 | 1,227 | 68 | 87 | 8,674 | 60 | 97 | 11,491 | 60 |
| Payne | 22 | 343 | 16 | 53 | 280 | 63 | 2,702 | 64 | 96 | 12,570 | 60 |
| Sage | 24 | 344 | 63 | 736 | 135 | 76 | 9,307 | 60 | 96 | 30,195 | 60 |
| Scout 66 | 20 | 347 | 13 | 153 | 317 | 57 | 2,080 | 64 | 94 | 21,996 | 60 |
| TAM W-101 | 22 | 338 | 17 | 306 | 209 | 34 | 497 | 194 | 75 | 3,894 | 69 |
| Trison | 13 | 352 | 41 | 70 | 289 | 72 | 747 | 92 | 93 | 4,756 | 63 |
| Triumph 64 | 20 | 338 | 63 | 208 | 202 | 83 | 1,978 | 62 | 92 | 4,567 | 60 |
| Vona | 22 | 325 | 15 | 36 | 340 | 27 | 562 | 155 | 76 | 15,731 | 83 |
| Mean | 24 | 339 | 32 | 300 | 239 | 59 | 3,875 | 97 | 89 | 17,769 | 64 |
| Least significant difference, ($P = 0.05$) | 10 | 16 | 6 | 268 | 40 | 8 | 1,795 | 21 | 5 | 5,810 | 7 |

[a] Millidextrinizing units.

**TABLE II**
**Spearman's Coefficients of Cultivar Rankings Between 1978 and 1979 Means for Three Characteristics by Date of Rain Treatment[a]**

| | Characteristic | | |
|---|---|---|---|
| Week[b] of Simulated Rain | Germination Percentage | α-Amylase Activity | Falling Number |
| Control | ··· | 0.122 | 0.591[c] |
| 0 | 0.704[c] | 0.756[c] | 0.721[c] |
| 2 | 0.635[c] | 0.833[c] | 0.693[c] |
| 4 | 0.499[c] | 0.733[c] | 0.000 |

[a] N = 18.
[b] After harvest.
[c] Significant at $P = 0.01$.

**TABLE III**
**Spearman's Coefficients of Cultivar Rankings Among Three Characteristics by Date of Rain Treatment During 1978 and 1979**

| Week[a] of Simulated Rain | Germination vs α-Amylase | Germination Percentage vs Falling Number | Falling Number vs α-Amylase |
|---|---|---|---|
| Control | ··· | ··· | 0.154 |
| 0 | 0.856[b] | 0.837[b] | 0.900[b] |
| 2 | 0.806[b] | 0.878[b] | 0.896[b] |
| 4 | 0.358[c] | 0.710[b] | 0.737[b] |

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

**TABLE IV**
**Mean α-Amylase Activities, Falling Numbers (FN), and Germination Percentages of Hard White and Hard Red Winter Wheat Cultivars Before and After Simulated Rain Treatment During 1978 and 1979**

| | | Control | | Treatment at Weeks After Harvest Maturity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | | | 2 | | | 4 | | |
| Cultivar or Line | Grain Color | Amylase (mDU)[a] | FN (sec) | Germination (%) | Amylase (mDU) | FN (sec) | Germination (%) | Amylase (mDU) | FN (sec) | Germination (%) | Amylase (mDU) | FN (sec) |
| Centurk | Red | 47 | 346 | 54 | 773 | 123 | 78 | 8,347 | 72 | 94 | 8,564 | 61 |
| Clark's Cream | White | 47 | 342 | 34 | 83 | 315 | 45 | 232 | 249 | 53 | 1,486 | 187 |
| KS73256 | White | 57 | 344 | 77 | 980 | 72 | 86 | 6,342 | 61 | 93 | 8,167 | 60 |
| Lancota | Red | 34 | 344 | 5 | 53 | 339 | 6 | 100 | 351 | 36 | 2,993 | 230 |
| Newton | Red | 36 | 332 | 7 | 90 | 248 | 23 | 775 | 123 | 71 | 7,352 | 93 |
| Mean | | 44 | 342 | 35 | 396 | 219 | 48 | 3,177 | 171 | 70 | 5,712 | 126 |
| Least significant difference, $P = 0.05$ | | NS[b] | NS | 7 | 293 | 27 | 4 | 866 | 19 | 8 | 1,434 | 29 |

[a] Millidextrinizing units.
[b] Not significant.

**TABLE V**
**Means of Germination Percentages, Falling Numbers, and α-Amylase Activities of Five Wheat Cultivars and Lines at Seven Locations of the Kansas Intrastate Nursery During 1979**

| Location | Germination (%) | Amylase (mDU)[a] | Falling Number (sec) |
|---|---|---|---|
| Colby | 0 | 64 | 470 |
| Hays | 0 | 42 | 676 |
| Hesston | 0 | 55 | 569 |
| Hutchinson | 0 | 69 | 641 |
| Manhattan | 0 | 74 | 530 |
| Parsons | 23 | 738 | 371 |
| Powhattan | 13 | 248 | 397 |
| Least significant difference, $P = 0.05$ | ... | 69 | 28 |

[a] Millidextrinizing units.

## RESULTS

### Sprouting Response of Red Wheats

Dry climatic conditions prevailed during late grain ripening in 1978 and 1979. Samples were harvested before frequent rains caused considerable sprouting of unharvested wheat in 1979. No germination was evident in the grain before treatment in the rain simulator either year. α-Amylase and falling number differed significantly among cultivars, but the differences were small and values were within normal ranges (Table I). Simulated rain induced sprouting as measured by visual observations, reduced falling number, and increased α-amylase activity. Analysis of variance showed significant differences among cultivars for all variables.

Rain treatment immediately at harvest caused most sprouting in Parker 76 and only slightly less sprouting in Centurk, Centurk 78, Sage, and Triumph 64. Trison and Buckskin sprouted 41 and 36%, respectively, and the other cultivars sprouted 20% or less.

All cultivars exhibited an after-ripening period by sprouting more as the time of rain treatment after harvest advanced. However, the magnitude of increase in sprouting was genotype-dependent, as reflected by a significant genotype-date interaction. Cultivars that had more pronounced after-ripening periods were Bennett, Hart, Gage, TAM W-101, Vona, and Lindon. Lindon sprouted least of the tested cultivars at harvest maturity and four weeks later. However, longer after-ripening periods generally did not seem to be predicted by low sprouting at harvest maturity.

Nonsprouted cultivars at harvest (controls) had a mean α-amylase value of 24 mDU and mean falling number of 339 sec (Table I). Generally, cultivars did not differ significantly. The tall cultivars Buckskin, Parker 76, and Bennett had the highest α-amylase activity and the SD cultivars Hart, Newton, and Vona had the lowest falling number. The relationship of that dichotomy to the Rht genome is not clear.

The effect of simulated rain on α-amylase activity and falling number was modified greatly by the date of treatment and by cultivar. Simulated rain increased α-amylase activity and decreased falling number. All cultivars produced more α-amylase activity per percent sprouting at successive weeks after harvest maturity. The mean mDU per percent sprouting was 8, 58, and 188 at 0, 2, and 4 weeks, respectively, after harvest. De novo synthesis of α-amylase evidently lagged behind visible sprouting more in partially dormant grain than in nondormant grain.

For selection for sprouting resistance to be effective, phenotypic expression must remain reasonably stable when genotypes are subjected to various environmental conditions. Correlations between the 1978 and 1979 cultivar rankings for each characteristic at each treatment date were highly significant (Table II), confirming that relative performance of cultivars was similar from one season to the next. The decreasing trend in germination and falling number correlation values at successive dates after harvest was caused by the lack of mean separation of cultivars at the later rain dates when all sprouting values were high.

Cultivar rankings for sprouting by the falling number and colorimetric methods correlated highly in rain-treated grain but not in untreated grain (Table III). Both methods generally correlated highly with germination percentage, although less than with each other. Trison and Triumph 64 consistently deviated from the sprouting-α-amylase comparison. Both ranked high in sprouting but were low in α-amylase production.

### Sprouting Response of White Wheats

Sprouting of hard red and white winter wheats under simulated rain is compared in Table IV. Newly harvested grain that was not exposed to simulated rain showed no evidence of sprouting, and α-amylase activity and falling number were similar in all genotypes. Simulated rain increased sprouting and α-amylase activity and decreased falling number progressively as time after harvest increased. The two white wheats differed considerably: KS73256 was extremely susceptible, whereas Clark's Cream sprouted less and had a longer after-ripening period than some of the red wheat cultivars. Clark's Cream also had lowest α-amylase activity and less sprouting and higher falling number than any genotype except Lancota on the last date.

Preharvest sprouting occurred at two of the seven locations of the Kansas Intrastate Nursery during 1979 (Table V). The two locations, Parsons and Powhattan, are on the eastern edge of the major HRWW production area. They typically have more rain and higher humidity than do the more westerly locations during harvest. Sprouting of the three HRWW cultivars, Eagle, Newton, and TAM W-101, was insignificant at Parsons and Powhattan. However, the two white wheat experimental lines, KS75216 and KS77H2690, sprouted at both locations (Table VI). Mean α-amylase activity was higher and mean falling number was lower at

**TABLE VI**
**Germination Percentages, α-Amylase Activities, and Falling Numbers (FN) of Five Wheat Cultivars and Lines After Natural Rain Damage at Parsons and Powhattan, KS, During 1979**

| Cultivar or Line | Grain Color | Parsons | | | Powhattan | | |
|---|---|---|---|---|---|---|---|
| | | Germination (%) | Amylase (mDU)[a] | FN (sec) | Germination (%) | Amylase (mDU) | FN (sec) |
| Eagle | Red | 0 | 92 | 618 | 1 | 65 | 590 |
| Newton | Red | 3 | 144 | 423 | 0 | 65 | 460 |
| Tam W-101 | Red | 0 | 86 | 576 | 3 | 77 | 507 |
| KS75216 | White | 67 | 2,816 | 84 | 45 | 782 | 143 |
| KS77H2690 | White | 45 | 552 | 156 | 17 | 248 | 283 |
| Least significant difference, $P = 0.05$ | | 7 | 462 | 49 | 5 | 152 | 84 |

[a] Millidextrinizing units.

the two locations where sprouting occurred. Similarly, the two white wheats had higher α-amylase activities and lower falling numbers than did the red wheat cultivars at those locations. Experimental line KS75216 was more susceptible than KS77H2690 in terms of all three sprouting measurements.

## DISCUSSION

Bingham and Whitmore (1966) and Derera et al (1977) noted significant cultivar differences in basal α-amylase activity in mature sound grain. Basal α-amylase activity in our study differed more between years than among cultivars. Although falling number was affected by environment, it appeared to be a more consistent characteristic of cultivars and was not related to α-amylase at the level of activity found in sound grain. That might occur because falling number is altered by other grain characters including protein, amylose, and fiber content (Moss 1980, Moss and Kirby 1976).

Simulated rain increased sprouting and allowed phenotypic identification of resistance to sprouting and to α-amylase synthesis. Sprouting and α-amylase activity were positively and highly correlated; however, the high correlation overshadowed temporal and cultivar deviations from the relationship. Enzyme activity was not always directly related to visible sprouting, as shown by α-amylase activity that was lower (per germination percentage) after simulated rain at harvest maturity than at later dates and by differences among cultivars at all dates. Gordon (1979) also noted that de novo synthesis of α-amylase lagged behind visible sprout assessment and that cultivar response in "α-amylase dormancy" varied.

A falling number of near 200 was optimum for flour for bread (Greenaway 1969). Because sound wheats have higher falling numbers than that, malt supplements are commonly added to raise diastatic activity of flour by approximately 100 $mDUg^{-1}$ (Mathewson and Pomeranz 1977). Simulated rain at harvest maturity caused sprouting that lowered all wheat samples three or more grades according to the official U.S. grain standards (USDA 1970). Many of the same samples could be graded sound for breadmaking by falling number or α-amylase dextrinizing units. Trison, with 41% sprouting, 289 falling number, and total α-amylase activity of 70 $mDUg^{-1}$, was the most notable example.

The likelihood of sprouting increased markedly as time after harvest was delayed. Only the highly dormant cultivar Lindon had acceptable α-amylase activity when treated with simulated rain two weeks after harvest maturity. Although sprouting and α-amylase activity were high, differences among cultivars were still evident two and four weeks after maturity. Falling number was less useful for cultivar identification at the later rain dates; it had reached a minimum value for most samples by that time.

White wheats were generally more susceptible to sprouting than the red wheats in these studies. White wheats were not uniformly susceptible to sprouting, however, and they also exhibited an after-ripening period. Differences in dormancy and sprouting resistance between white-grained and red-grained cultivars were more quantitative than qualitative, as reported previously (Freed et al 1976, Gfeller and Svejda 1960, McEwan 1980). The reaction of Clark's Cream was most notable; not only was it moderately dormant at harvest maturity, but it had a very long after-ripening period and low α-amylase synthesis per germination percentage. The dormancy of Clark's Cream was confirmed under natural sprouting conditions (Bhatt et al 1981).

In breeding programs, the number of experimental lines is great and the amount of material available for testing is small. Effective screening criteria must be rapid, precise, and conservative of plant tissue. The method of rain simulation adequately distinguished phenotypes on the basis of sprouting. Given the high correlation between the colorimetric and falling number methods, either would serve to measure α-amylase activity. However, the colorimetric method was much more rapid, used less grain meal, was affected less by other grain traits, and measured a wider range of α-amylase activity. Selecting for both sprouting resistance and low α-amylase production is recommended because the two traits are not mutually inclusive.

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