# PRE-HARVEST AMYLASE ACTIVITY AND SPROUTING IN ROMANIAN WHEAT CULTIVARS

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## ABSTRACT

**High amylase activity and pre-harvest sprouting have become major limiting factors of wheat quality and yield in Romania. A study was conducted on 14 Romanian cultivars grown in yield trials in 10 environments which had favourable conditions for pre-harvest sprouting in 2005. Variations caused by environments and genotypes were very significant and of similar magnitude. Falling number was correlated with total rainfall from June 1 to harvest time. Scores from artificial test on spikes correlated well with percentage of sprouted grains in the field and with falling number. Many new Romanian winter wheat cultivars, obtained using selection based on artificial tests, had a good resistance to pre-harvest sprouting, especially the new, recently registered wheat cultivars, Glosa and Faur, and the perspective line Izvor.**

**Key words**: amylase activity, pre-harvest sprouting, wheat

## INTRODUCTION

Wheat production and quality in Romania is strongly influenced by climatic factors. Among them, rains during maturity, before harvest, can cause an increase of amylase activity in the grains and pre-harvest sprouting, with important negative influence on bread-making quality and even yield.

The enzyme alpha-amylase is synthesized in the aleurone layer and scutellum and released in the endosperm to decompose the starch into sugars available for germination (Lunn et al., 2002). Pre-harvest initiation of this process is harmful for quality by degrading the starch, reducing grain test weight, decreasing the absorption capacity of the flour, the strength of the dough and finally the loaf volume (Humphreys and Noll, 2002).

Pre-harvest sprouting (PHS), following the increase of amylase activity, besides the negative effects on quality, can also cause an yield reduction as a result of metabolizing part of the starch stored in the grains, a decrease of seed germination energy and seedling vigour (Cseresnyes and Săulescu, 1989), and can also favour contamination of the grains with potentially toxin producer fungi and bacteria.

Flour produced from sprouted grains or grains with high amylase activity is considered of low quality and gives bread with lower loaf volume and compact and sticky crumb (Ceapoiu et al., 1984).

Despite the fact that in Romania wheat maturation usually coincides with a dry, less rainy period, pre-harvest sprouting can have important economic consequences. The year 2005, with heavy rains preceding and during harvest, allowed a good characterisation sprouting resistance and amylase activity of Romanian wheat cultivars.

## MATERIAL AND METHODS

Amylase activity was estimated by the Hagberg Falling Number (FN) in 25 winter wheat cultivars tested at the National Agricultural Research & Development Institute Fundulea, the National Research & Development Institute for Potatoes and Sugar Beet Braşov, and the Research Stations Târgu Mureş and Oradea, on two levels of nitrogen fertilization ($N_{100}$ and $N_0$). At Fundulea the yield tests were planted and harvested at two dates, which produced differentiated response to sprouting. Fourteen cultivars were common to the tests performed in all these 10 environments.

Pre-harvest sprouting resistance was estimated by the percentage of sprouted grains in the field and in artificial tests. Artificial tests were performed by rolling 20 entire spikes into moist filter paper. Scores from 1 = no visible sprouting to 9 = complete sprouting in all spikes were given after 10 days.

Test weight was measured in kilograms per hectolitre and physiological maturity estimated in the field as number of days after January 1, 2005. The environment favourability for sprouting was estimated by the total rainfall during the period from June 1 to the harvest day.

ANOVA and correlation analysis were used in analyzing the data.

## RESULTS AND DISCUSSION

ANOVA shows that both weather conditions and genotypes had significant and similar

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contributions to the variation of the amylase activity (Table 1).

*Table 1.* ANOVA for falling number values of 14 winter wheat cultivars in 10 environments

| Source of variation | Degrees of Freedom | Sum of squares | Mean Square | F values |
|---|---|---|---|---|
| Genotypes (G) | 13 | 225313. 8 | 17331. 8 | 9.03*** (2.30) |
| Environ-ment (E) | 9 | 164398. 6 | 18266. 5 | 9.51*** (2.59) |
| G x E inter-action + error | 117 | 224621. 8 | 1919. 8 | |
| Total | 139 | 614334. 2 | | |

Amylase activity was strongly influenced by the total pre-harvest rainfall, which explained 35% of the total variation of the average falling number among the 10 environments (Table 2).

The falling number (FN) gives good indications on starch degrading as a result of α-amylase activity (Hageman and Ciha, 1984).

According to the Institute for Food Bioresources (2002), flour can be considered of good quality if the FN is higher than 200 seconds. In UK good flour is required to have FN higher than 250 seconds (Lunn et al., 2002). Flours having FN lower than 180 sec. are considered inappropriate for baking.

FN averaged over all tested cultivars was higher than 200 sec. in most environments, except Fundulea at the second date of harvest, where, as a result of very high rainfall after physiological maturity average FN was below 180. In this environment very low FN values were registered in cultivars Cãtãlin and Crina (FN = 62 sec.), Fundulea 4 (FN = 63 sec.), Dor (FN = 69 sec.), Lovrin 34 (FN = 71 sec.), and Romulus (FN = 79 sec.). However, in the same environment, several cultivars showed high FN values, indicating low amylase activity, meeting the requirements for bread-making. This was the case of the newly registered cultivar Glosa (FN = 204 sec.) and of the promising line Izvor (FN = 205 sec.).

*Table 2.* Rainfall and falling number averaged over 25 winter wheat cultivars, in 10 environments

| No. | Location and treatment | Rainfall during the period from June 1 to harvest (mm) | Falling number averaged over 25 cultivars (sec.) |
|---|---|---|---|
| 1 | Oradea $N_{100}$ | 130.2 | 264.9 |
| 2 | Oradea $N_0$ | 130.2 | 222.5 |
| 3 | Târgu Mureş $N_{100}$ | 147.0 | 197.3 |
| 4 | Târgu Mureş $N_0$ | 147.0 | 204.6 |
| 5 | Braşov $N_{100}$ | 240.5 | 235.9 |
| 6 | Braşov $N_0$ | 240.5 | 232.7 |
| 7 | Fundulea, first harvest date $N_{100}$ | 239.2 | 245.9 |
| 8 | Fundulea, first harvest date $N_0$ | 239.2 | 207.8 |
| 9 | Fundulea, second harvest date $N_{100}$ | 324.9 | 162.0 |
| 10 | Fundulea, second harvest date $N_0$ | 324.9 | 147.5 |
| | | r = - 0.59 | $R^2$ = 0.35 |

Figure 1 shows the FN averaged over the ten environments for the winter wheat cultivars tested in all locations. Most cultivars had an acceptable average behaviour.

The highest average FN was recorded in the newly released cultivars Glosa, Gruia, Faur, Delabrad and Boema, as well as in the new lines Izvor, Jupiter, Junona, and Jiana, which had average values over 200 seconds.

Glosa and Izvor had average FN higher than 250 seconds and values over 200 sec. even in the most severe environment.

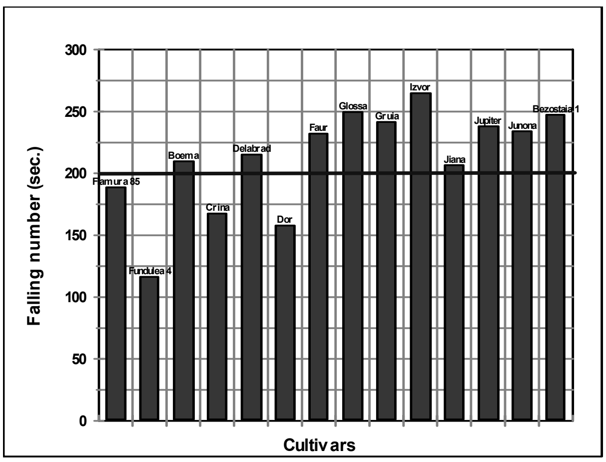


*Figure 1.* Average falling number in some Romanian winter wheat cultivars in 10 environments in 2005

Older cultivars, like Fundulea 4, but also some new cultivars like Dor and Crina had average FN below 180 seconds. Over all, about one third of the tested cultivars had unacceptable FN values (below 180 sec.), while two thirds produced grains meeting baking industry requirements.

Cultivars Fundulea 4 and Dor showed relatively high amylase activity even in environments not favouring pre-harvest sprouting. Apparently these cultivars are characterized by what was described as „late maturity α-amylase activity (LMA)”, i.e. high synthesis of α-amylase in the grain without any sprouting (Mrva and Mares, 2002).

Weather conditions in 2005 lead to pre-harvest sprouting in the field, allowing a good characterization of cultivars in this respect (Table 3).

*Table 3*. Percentage of sprouted grains in the field, in 14 winter wheat cultivars grown in several environments

| Cultivar | Fundulea $N_{100}$ | Oradea $N_{100}$ | Oradea $N_0$ | Tg. Mureş $N_{100}$ | Tg. Mureş $N_0$ | Braşov $N_{100}$ | Average of cultivar |
|---|---|---|---|---|---|---|---|
| Flamura 85 | 1.5 | 8.0 | 6.0 | 5.5 | 5.0 | 15.0 | 6.83 |
| Fundulea 4 | 6.0 | 18.0 | 11.0 | 13.0 | 6.0 | 7.0 | 10.17 |
| Boema | 0.5 | 6.0 | 5.0 | 6.0 | 2.0 | 5.0 | 4.08 |
| Crina | 14.0 | 11.0 | 9.0 | 8.5 | 8.0 | 8.0 | 9.75 |
| Delabrad | 1.5 | 4.0 | 5.0 | 2.0 | 2.0 | 6.0 | 3.41 |
| Dor | 5.0 | 10.0 | 7.0 | 6.5 | 5.5 | 20.0 | 9.00 |
| Faur | 0.0 | 2.0 | 5.0 | 2.5 | 1.5 | 4.0 | 2.50 |
| Glosa | 0.5 | 0.0 | 3.0 | 3.0 | 2.5 | 6.0 | 2.50 |
| Gruia | 7.0 | 9.0 | 6.0 | 3.5 | 1.0 | 2.0 | 4.75 |
| Izvor | 3.0 | 3.0 | 2.0 | 2.0 | 1.0 | 11.0 | 3.67 |
| Jiana | 1.0 | 2.0 | 0.0 | 9.0 | 3.5 | 3.0 | 3.08 |
| Jupiter | 5.0 | 4.0 | 2.0 | 4.0 | 4.0 | 16.0 | 5.83 |
| Junona | 1.0 | 4.0 | 5.0 | 4.5 | 2.5 | 12.0 | 4.83 |
| Bezostaia 1 | 0.5 | 5.0 | 1.0 | 3.0 | 2.5 | 10.0 | 3.67 |
| Average of location | 33.0 | 6.1 | 4.8 | 5.2 | 3.4 | 8.9 | 5.29 |

The data clearly differentiate the cultivar response to sprouting conditions. Best results were seen in the new cultivars Glosa and Faur with an average percentage of sprouted grains of 2.5%, when Fundulea 4 exceeded 10%. Among the cultivars already grown on large areas, the best behaviour was that of the cultivar Boema. It is interesting to notice that nitrogen fertilization slightly increased the percentage of sprouted grains. This is in accordance with the reported stimulating effect of nitrates on germination (Adkins et al., 2002).

Amylase activity, as expressed by the falling number, was significantly correlated with the percentage of grains sprouted in the field (r = - 0.84***), cultivars with lower amylase activity (higher FN) being more resistant to pre-harvest sprouting (Figure 2).

Cultivars with high amylase activity, such as Fundulea 4, Dor and Crina, were susceptible to sprouting, while cultivars with lower amylase activity, such as Glosa and Faur were more resistant to sprouting in the field. This type of correlation has often been reported (Hageman and Ciha, 1984).

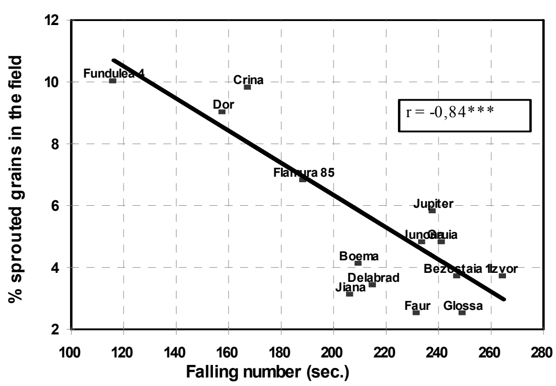


*Figure 2*. Correlation between falling number and seed sprouting percentage in field in 14 wheat cultivars grown in 6 environments

A very significant correlation (r=0.90***) was found between the scores for sprouting in artificial tests, performed at NARDI Fundulea and the percentage of sprouted grains in environments favourable to sprouting (Figure 3). Cultivars such as Holda, Crina, Cătălin, 99654 GP2, Romulus, which were identified in artificial tests as susceptible to sprouting, had higher percentage of sprouted grains in the field, while cultivars scored as resistant, such as Faur, Boema, Glosa, F 98039 G5-1, showed less sprouting in the field. This result is important, as it confirms our screening strategy in breeding for PHS resistance.

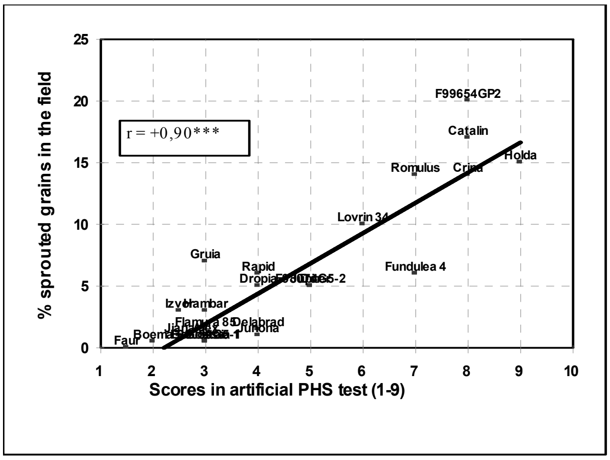


*Figure 3.* Correlation between scores from artificial test of pre-harvest sprouting resistance and seed sprouting percentage in field at Fundulea in 2005

Scores from artificial tests of sprouting resistance were also significantly correlated with the falling number (r = - 0.86 ***), meaning that the artificial tests can allow selection for lower amylase activity, too.

As expected, falling number significantly correlated with the percentage of grains sprouted in the field in all trials, except the one in Braşov (Table 4). This confirms the statement that the correlation between amylase activity and sprouting is not absolute (Hageman and Ciha, 1984).

Falling number significantly correlated with the date of physiological maturity only at Fundulea, in three of the four yield tests, indicating that in these environments later maturing cultivars had lower amylase activity. Obviously this correlation depends on the rainfall distribution. At Fundulea in 2005, rains came when early cultivars were already mature, while later cultivars had not reached the physiological maturity.

Falling number was only rarely correlated with the test weight, a fact that can be explained by the many factors which influence the volumetric weight (shape and size of the grains, diseases etc.).

*Table 4.* Correlation between falling number, percentage of grains sprouted in the field, maturity date and test weight

| Location and treatment | Percentage of grains sprouted in the field | Maturity date | Test weight |
|---|---|---|---|
| FUNDULEA, first harvest date, $N_{100}$ | | + 0.51* | + 0.33 |
| FUNDULEA, first harvest date, $N_0$ | | + 0.41* | |
| FUNDULEA, second harvest date, $N_{100}$ | - 0.74*** | + 0.52** | + 0.40* |
| FUNDULEA, second harvest date, $N_0$ | | + 0.30 | |
| ORADEA, $N_{100}$ | - 0.63** | + 0.12 | + 0.06 |
| ORADEA, $N_0$ | - 0.53** | - 0.11 | + 0.20 |
| TG. MUREŞ, $N_{100}$ | - 0.79*** | + 0.17 | + 0.55** |
| TG. MUREŞ, $N_0$ | - 0.64** | + 0.19 | + 0.31 |
| BRAŞOV, $N_{100}$ | - 0.02 | + 0.04 | + 0.13 |
| BRAŞOV, $N_0$ | | + 0.13 | + 0.09 |

Sprouting resistance has not been a priority breeding objective in regions with continental climate, where rains during harvest are less frequent. Because of that, most cultivars grown in such regions, including the ones grown in Romania, do not posses adequate PHS resistance. The use of Mexican germplasm as a source of dwarfing genes in breeding the modern semi-dwarf Romanian cultivars could only increase the sprouting susceptibility.

The last few years showed that pre-harvest sprouting is increasingly becoming a major problem for wheat production in all regions of Romania. Although management solutions for reducing the harvest duration can help, the only reliable solution for avoiding damages from sprouting and increased amylase activity is to grow cultivars with better sprouting resistance and genetically low amylase activity.

Selection based on artificial tests of sprouting resistance introduced in recent years in the wheat breeding program at NARDI Fundulea lead to the release of new cultivars, such as Boema, Delabrad, Faur and Glosa, with good levels of amylase activity and sprouting resistance.

Further progress can be expected from:

- recombining best existing cultivars and lines, adapted to the Romanian environment;
- using new sources of resistance to PHS, obtained from crosses with *Aegilops squarosa,* which under conditions very favourable for sprouting and with late harvesting had FN higher than 300 seconds;
- improving testing methods and using marker assisted selection, especially for introgression of resistance genes from alien species.

## CONCLUSIONS

The year 2005, with heavy rains at harvest time, allowed a good characterisation of pre-harvest sprouting resistance and amylase activity, as well as a confirmation of artificial tests efficiency.

Environment and genotypes had very significant contributions of similar magnitude to the variation of the falling number.

New cultivars and promising lines, obtained using artificial selection for sprouting resistance, were superior to older cultivars, both for sprouting resistance and amylase activity.

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