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To cite this article: Srbislav Dencic, Ron DePauw, Borislav Kobiljski & Vojislava Momcilovic (2013) Hagberg Falling Number and Rheological Properties of Wheat Cultivars in Wet and Dry Preharvest Periods, *Plant Production Science*, 16:4, 342-351, DOI: [10.1626/tpps.16.342](https://doi.org/10.1626/tpps.16.342)

To link to this article: <https://doi.org/10.1626/tpps.16.342>



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Published online: 03 Dec 2015.



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Hagberg Falling Number and Rheological Properties of Wheat Cultivars in Wet and Dry Preharvest Periods

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Abstract: The effects of dry and wet preharvest periods on Hagberg falling number (HFN), a parameter of α -amylase activity, and rheological properties including farinograph dough development time (FDT), farinograph absorption (FA), resistance to extension (RE), loaf volume (LV) and baking score (BS) were examined in 30 hexaploid wheat (*Triticum aestivum* L.) cultivars originating from 19 countries. The cultivars were grown in the field in 2000 – 2010 and HFN and rheological properties were analyzed for three replicates. The cultivars were divided into three groups according to HFN in the wet preharvest period: HFN below 150 s (group H-1), HFN from 250 to 350 s (group H-2), and HFN over 400 s (group H-3). The cultivars in group H-3 were superior to those in either group H-1 or H-2 in all rheological properties except RE. In the dry preharvest periods, HFN was not correlated with rheological properties, while in the wet preharvest period HFN showed a highly significant positive correlation with FDT and BS. The canonical variate analysis for assessment of the general performance of all cultivars with HFN as the main factor and the other rheological properties as subfactors, indicated that the cultivars Stepnaja 30, Garazinko, Kirac, Klein Forten and Žitarka showed the highest potential regardless of preharvest rainfall amounts. In conclusion, differential genetic expression of resistance to preharvest sprouting, maintenance of low α amylase level, high HFN values, maintenance of rheological properties, and baking performance can be reliably detected and measured under wet preharvest conditions.

Key words: Canonical variate analysis, Hagberg falling number, Preharvest period, Rheological properties, Wheat cultivars.

When it rains before harvest, wheat seeds may begin to germinate on the spike, a phenomenon known as preharvest sprouting. The germination causes an increase in α -amylase (EC 3.2.1.1, α -1,4-glucanohydrolase). The α -amylase is an endo-acting starch-digesting enzyme that cleaves the α -1,4 bonds in amylose and amylopectin. α -Amylase digestion of damaged and gelatinized starch produces maltose and linear and branched dextrans of various sizes. The longer the seed germinates, the larger the amount of α -amylase formed. Hagberg (1961) and Perten (1964) developed the Hagberg falling number (HFN) method as a simple and rapid test for determining α -amylase activity using wheat meal as the substrate. This method has become the international standard (AACC, 1972; ICC, 1995) that is used widely in grain classification and bread-making quality control. The HFN method measures differences in enzyme, substrate and the interaction of enzyme and substrate, and the results are

influenced by genotype and environmental conditions under which the seeds developed and matured (Meredith and Pomeranz, 1985; DePauw et al., 1989; Mares and Mrva, 2008).

Increasing levels of α -amylase result in a decrease in HFN to 60 seconds, beyond which further increases in α -amylase activity cannot be measured. This is reflected in an inverse curvilinear relationship between α -amylase activity and HFN. Wheat seeds have the capacity to synthesize a large amount of α -amylase. The quantity required to reduce HFN below industry reception limits is quite small: about 2 – 3 times the level found in sound seeds (Mares, 1987). Many countries use HFN as an important component of trading specifications. HFN values above 250 or 300 s. (depending on country) are required for seeds to be classified into high-quality grades (Mares and Mrva, 2008).

Wheat genotypes react differently to α -amylase and HFN. Some genotypes are sprouting-resistant and always

have a high HFN, some are sprouting-susceptible with a low HFN, and some genotypes show resistance and a high HFN under certain environmental conditions (temperature and humidity) but susceptibility under the conditions favorable for sprouting (low HFN) (Kulp et al., 1983; DePauw et al., 1989; Barbeau et al., 2006; Biddulph et al., 2008).

By examining the effect of cultivar on various quality parameters in six different environments, Lukow and Mcvetty (1991) found that the variance component for cultivars accounted for 73.1% of the total HFN variation. DePauw and McCaig (1991) reported the variance component for 26 cultivars grown in two environments accounted for 33% of the total components of variation, and heritability for HFN of 0.59.

Temperature (Osanai et al., 2005), application of fertilizers (Kindred et al., 2005; Craven et al., 2007), application of fungicides (Dimmock and Gooding, 2002) and seed size (Evers et al., 1995) have been associated with the variation in grain α -amylase levels and HFN. The weather, especially rainfall in combination with cool temperatures, during preharvest period is the main determinant of low HFN (Kettewell et al., 1999; Yanagisawa et al., 2005; Biddulph et al., 2008).

Amylase and protease activities are often very high in sprouted wheat. These enzymes can cause serious damage to the structural integrity of starch and storage proteins of wheat, making sprouted grains unsuitable for use in food products. Bread made from wheat with elevated α -amylase activity and low HFN had sticky crumbs that led to tearing when the bread was mechanically sliced (Edwards et al., 1989). Low HFN is associated with loss in functional baking quality including reduction in test weight, low milling yield, low absorption, reduced dough strength and loaf volume and poor crumb structure (Derera, 1988; Kruger, 1989).

The aims of this study were to assess the effects of wet and dry preharvest periods on the grouping of wheat genotypes based on HFN value, the relationships between HFN and other bread-making quality traits in grains that ripened under wet and dry weather, and the value of individual wheat cultivars based on HFN and other rheological properties as affected by wet and dry preharvest periods. The experiment was undertaken to obtain comprehensive information on the effects of genotype, environment (year) and their interaction on HFN and various bread-making quality traits, as well as relationships among the analyzed traits (Denčić et al., 2011).

Materials and Methods

1. Plant materials and meteorological data

One hundred and forty wheat genotypes (cultivars and experimental lines) originating from 28 countries were grown on the experiment field of Institute of Field and Vegetable Crops, Novi Sad, Serbia, in the 2000 – 2010. A

subset of thirty varieties was selected for in-depth analysis by HFN and other quality parameters. The basic selection criteria were representation of a global area of winter wheat production and similar time to maturity.

Each year the wheat genotypes were planted in a randomized complete block design with five replicates in a field nursery with a chernozem soil, with about 2.5 to 3.5% organic matter. Field plots of 5 m² with 10 rows spaced 10 cm apart were planted at 550 seed per m². In the beginning of October before planting, the experimental area was fertilized with 50 kg N ha⁻¹, 50 kg P₂O₅ ha⁻¹ and 50 kg K₂O ha⁻¹. The genotypes were planted in mid-October which is the optimal time for winter wheat. Top-dressing was conducted in early February (65 kg N ha⁻¹) and in late March (45 kg N ha⁻¹), the early booting stage. In spring weeds were controlled by application of an appropriate herbicide. Prevalent diseases such as powdery mildew caused by *Blumeria graminis* (DC.) E.O. Speer f.sp. *tritici* Ém. Marchal [syn. *Erysiphe graminis* DC. F.sp. *tritici* Ém. Marchal], leaf rust caused by *Puccinia triticina* Eriks., *Septoria tritici* (perfect state *Mycosphaerella graminicola*) and fusarium caused by *Fusarium graminearum* Schwabe teleomorph *Gibberella zeae* (Schwein. Petch), *F. avenaceum* (Corda ex Fr.) Sacc., and *F. culmorum* (W.G. Smith) Sacc. were controlled with two applications of appropriate fungicides. Field plots were harvested in late June when grain moisture was 14% in all experiment. When the latest cultivar reached maturity, all cultivars were harvested. Normally there was a spread of about 7 days between the earliest cultivar and the latest to mature.

During the 11 years, two years with above average precipitation during late grain filling and harvest and two years with below average precipitation were compared for quality and HFN. The analysis of individual years showed that 2001 and 2010, had extremely high rainfall during the preharvest period, which typically occurs between early May and the end of June (Fig. 1). Rainfall was extremely low in 2000 and 2003 compared with the long-term average (Fig. 2). In the other years, rainfall during the preharvest period was comparable to the long-term average. The temperatures tended to be below average during the wet periods and above average during the dry periods compared with the long-term average (Fig. 1 and 2).

2. Quality assessment

All bread-making quality traits were analyzed in three replicates using the first, third and fifth replications from the field. Wheat samples were milled in a pneumatic laboratory mill MLU 202 (Bühler AG, Uzwil, Switzerland) after tempering to 15% moisture.

HFN was determined with a Falling Number Apparatus 1800 (Perten, Sweden) using the ICC 107/1 method (ICC, 1995).

Flour was tested for dough properties using a

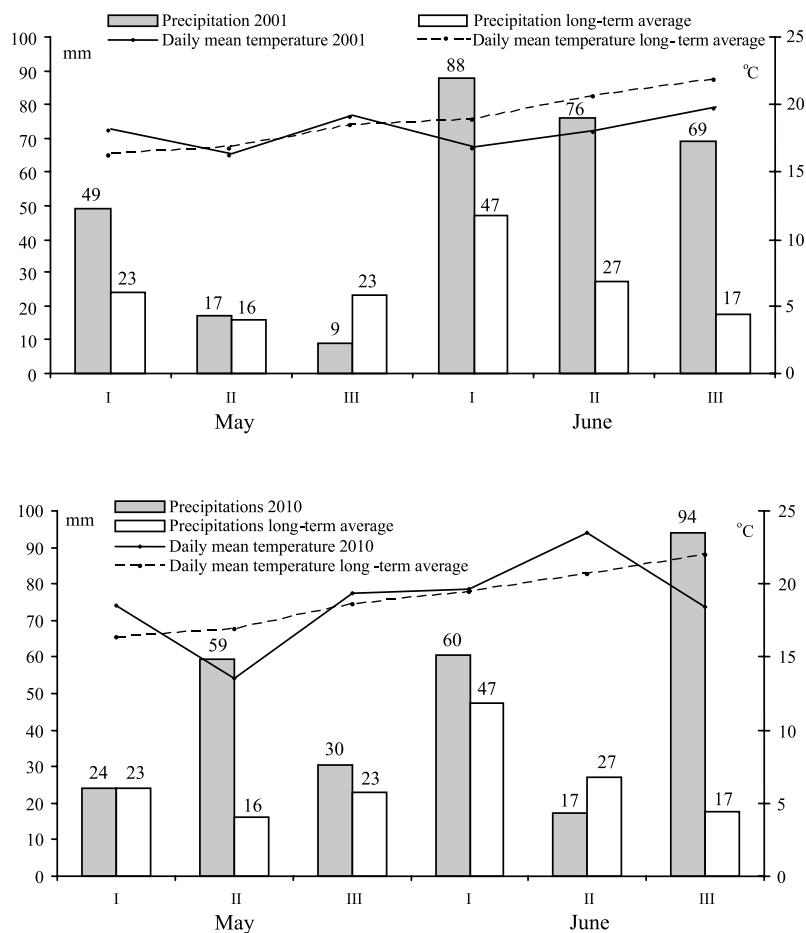


Fig. 1. Precipitation and temperature in years with a wet preharvest period which was sectioned into 10-day periods of the month indicated by Roman numerals.

farinograph (Brabender OHG, Duisberg, Germany) according to the ICC method 115/1 (ICC, 1992). Farinograph absorption (FA) and farinograph dough development time (FDT) were measured.

Extensogram measurements (Brabender OHG, Duisberg, Germany) were done on 100-g flour samples according to the ICC method 114/1 (ICC, 1992). Resistance to extension (RE) at the peak was measured after a rest period of 135 min.

Bread-making properties were evaluated using the standard 350 g pup loaf procedure, a straight-dough procedure using flour, water, salt (2.0%), and yeast (2.0%) with a fermentation time of 3 hr. The loaf volume (LV) was measured by rapeseed displacement. The baking score (BS) (on the scale 0 – 7, where 7 is excellent) was determined as a numerical expression based on the organoleptic assessment of bread crumb, representing the sum of points for elasticity (0.0 – 4.5, where 4.5 is excellent) and pore structure fineness (0.0 – 2.5, where 2.5 is extremely fine).

3. Statistical analysis

Least significant differences (LSD) test was used to

compare the means and interactions between cultivar groups for rheological properties. The cultivars were classified into three groups according to the HFN value obtained in the wet preharvest period. The canonical variate analysis (Jobson, 1992) was used to confirm and verify the classification into these groups and to make the final ranking of the cultivars. Canonical variate analysis is a statistical procedure frequently used in systematic studies to differentiate among n groups. Results are often depicted graphically in bivariate plots containing the scatters of the scores of the n groups on the first two canonical variate axes. These graphs help the investigator to visualize relationships among groups. When constructed appropriately, such graphs are valuable tools in data interpretation (Campbell and Atchley, 1981). In our study, associations between variables (rheological properties) were determined by Pearson's correlation coefficients (Bernard, 1992). All statistical analyses were done with program XLSTAT-Pro (demo version, Version 3.02, 2009).

Results and Discussion

The main objective of the study was to analyze HFN variation per cultivar and per year depending on the

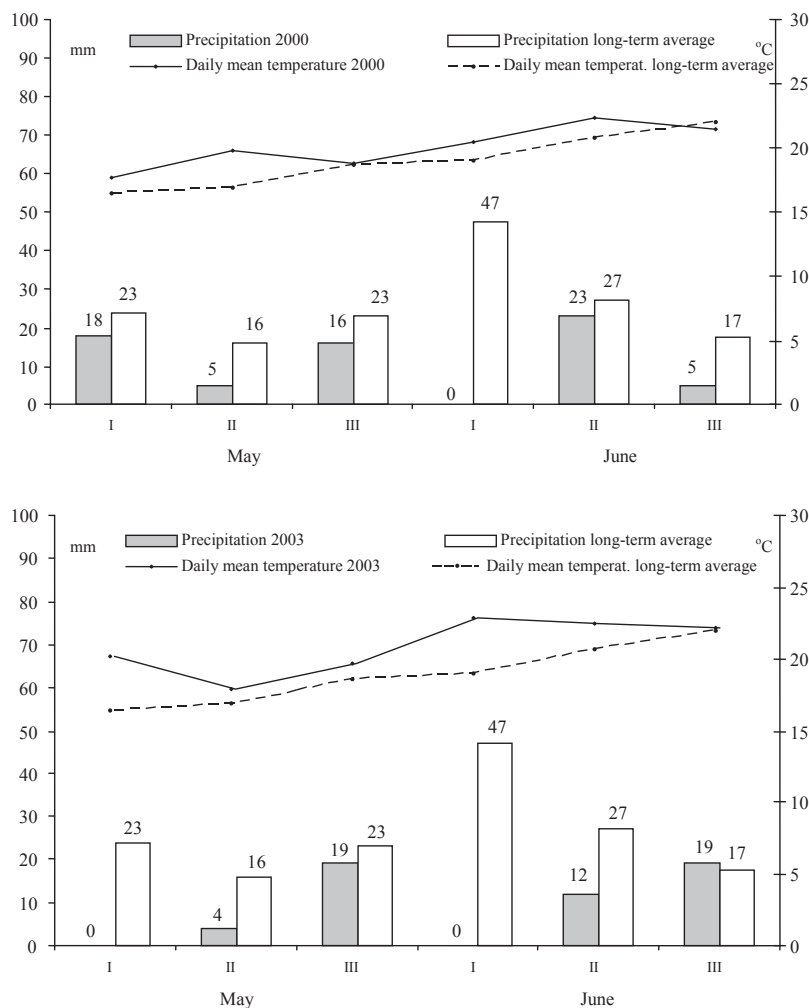


Fig. 2. Precipitation and temperature in years with a dry preharvest period which was sectioned into 10-day periods of the month indicated by Roman numerals.

amount of preharvest rainfall. Attention focused on the possibility of classifying cultivars according to their HFN values and interaction with environment varying in rainfall regime during the grain filling period as well as on the effect of rainfall regime on rheological properties.

Only in the years with a wet preharvest period (2001 and 2010) did the 30 cultivars show significant and non-continuous variation in HFN values, which allowed for clustering of cultivars. Ten cultivars each had HFN below 150 s (H-1), HFN between 250 and 350 s (H-2) and HFN over 400 s (H-3) (Table 1). Such clustering was not exhibited in 2000 and 2003, the years in which the preharvest rainfall was several times below the long-term average (Fig. 2). A similar differentiation of wheat cultivars based on HFN was made by Barbeau et al. (2006) who examined 17 wheat cultivars in three locations. In the location with intense preharvest rainfall, six cultivars had significantly lower HFN than the other cultivars. These cultivars did not differ from the others in the location with a dry preharvest period. Genotypic differences between cultivars in HFN value were also observed by Hareland

(2003) who studied the effect of pearling on HFN, and Craven et al. (2007) who studied the effects of different doses of mineral fertilizers on HFN.

Canonical variate analysis as a type of discriminant analysis was used to assess the reaction of the 30 wheat cultivars to dry and wet preharvest periods. There was no discernible clustering to adequately differentiate the response in the dry period (Fig. 3).

Table 2 shows the variation of the rheological properties in the wet preharvest and dry preharvest periods according to the group (H-1, H-2 and H-3). The average HFN values showed highly significant differences with the cultivar type in the wet preharvest period and with the year, which was expected since HFN was the main criterion for the classification of cultivars. There were no significant differences between cultivars in group H-1 and those in group H-2 in the dry periods. This indicates that the H-1 cultivars were prone to sprouting under increased moisture conditions of wet preharvest periods, which is a trait of low quality wheat considered unfavorable for making food products. These types of cultivars showed the

Table 1. Classification of wheat cultivars according to HFN in wet preharvest period.

Variety group	Cultivar and country of origin ¹	Average HFN in wet years	Average HFN in dry years
Group H-1 Low-HFN cultivars with HFN lower than 150 s	Dobric (BUL)	69	291
	MV 17 (HUN)	91	363
	Balkan (SRB)	107	341
	Maris Huntsman (UK)	110	454
	Condor (AUS)	111	362
	Bazalt (DEU)	112	407
	Lada (CZE)	115	534
	Siete Cerros (MEX)	121	301
	Nahodka 4 (UKR)	131	466
	Jugoslavija (SRB)	131	398
	Average	110	392
Group H-2 Medium-HFN cultivars with HFN between 250 and 350 s	Irnerio (ITA)	263	338
	NS Brkulja (SRB)	263	296
	Cook (AUS)	279	339
	Noe (FRA)	294	372
	MV 20 (HUN)	296	491
	Slavija (SRB)	294	399
	Kavkaz (RUS)	305	417
	Gaboto (BRA)	312	382
	KG 100 (SRB)	334	495
	Florida 301 (USA)	336	417
	Average	297	392
Group H-3 High-HFN cultivars with HFN above 400 s	Žitarka (HRV)	412	406
	Klein Forten (ARG)	434	536
	Arina (SWZ)	439	409
	Garazinko (BRA)	450	529
	Winalta (CAN)	454	421
	Kirac (TUR)	467	490
	Odeskay 66 (UKR)	469	415
	Sardona (SWZ)	471	452
	MV 21 (HUN)	485	607
	Stepnaja 30 (RUS)	497	561
	Average	458	482

¹ARG – Argentina; AUS – Australia; BRA – Brazil; BUL – Bulgaria; CAN – Canada; CZE – Czech Republic; DEU – Germany; FRA – France; HRV – Croatia; HUN – Hungary; ITA – Italy; MEX – Mexico; RUS – Russia; SRB – Serbia; SWZ – Switzerland; TUR – Turkey; UK – United Kingdom; UKR – Ukraine; USA – United States of America.

same clustering in the expression of FDT and FA values. The average values of both traits were significantly higher in the H-3 group than in the H-1 and H-2 groups. The latter groups displayed no significant differences. Similar responses were manifested in both dry and wet preharvest periods. The only difference between these two traits was that FDT was significantly higher in dry than in wet preharvest periods, which was not the case with FA. These results indicated that alpha amylase activity was disrupting the protein starch matrix and resulting in a weakening of

the gluten as measured by FDT. Similar results, i.e., cultivars with HFN values over 400 s were resistant to sprouting (sprout-free), and good quality were reported by Barbeau et al. (2006).

Extensograph results are particularly useful for evaluating dough strength, observing changes in dough properties over an extended time frame, and for characterizing different flour and wheat types. Resistance to extension was the only property that showed no difference either between the cultivar type or between the dry and wet

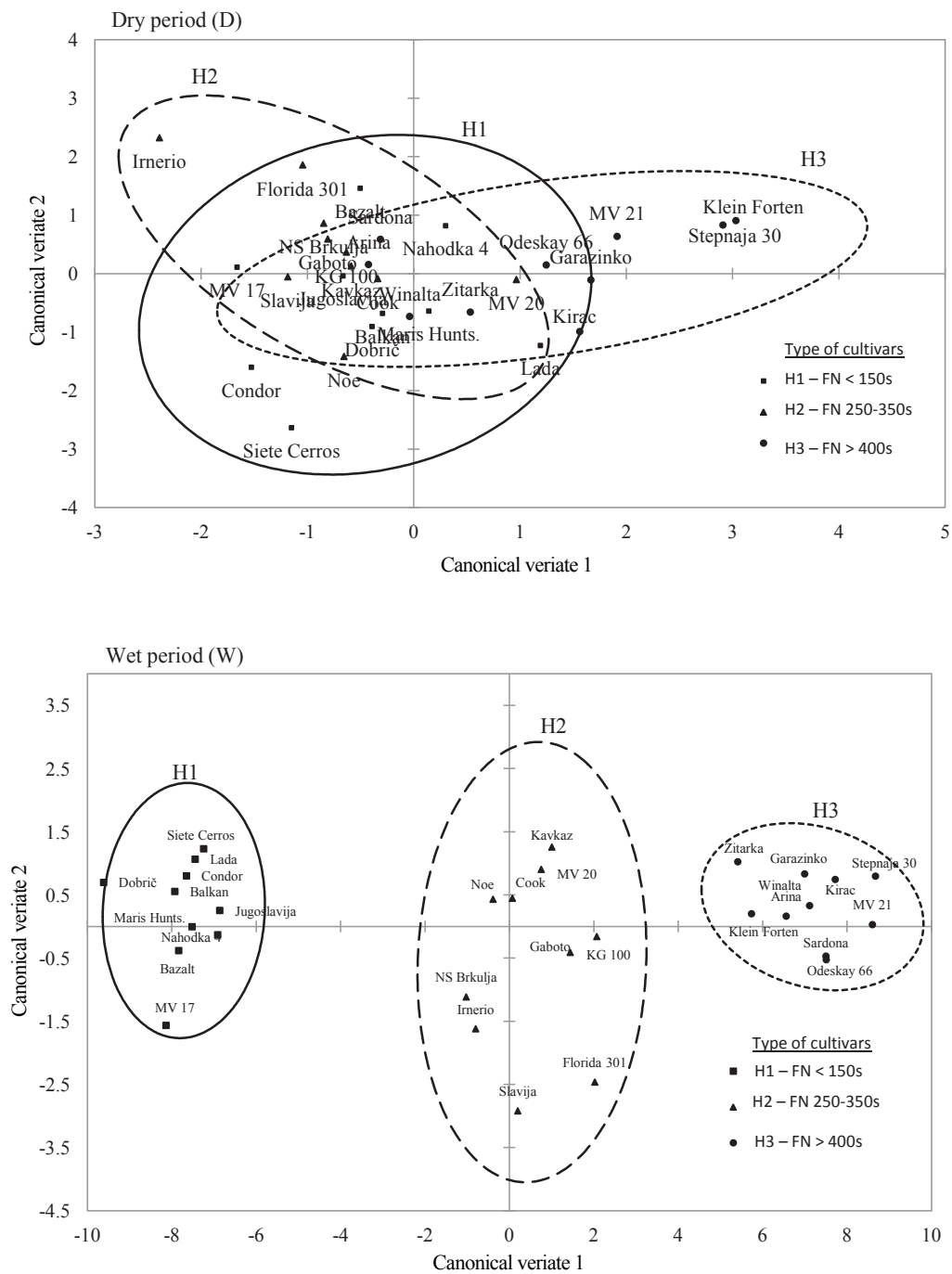


Fig. 3. Canonical variate classification of wheat cultivars according to HFN value in dry and wet preharvest periods.

preharvest periods (Table 2). This was probably due to a high correlation between extensograph properties and flour protein content (Singh and MacRitchie, 2001). The cultivars in group H-3 had a significantly higher LV value than those in group H-1 or H-2, regardless of dry or wet preharvest period (Table 2). A similar situation was manifested with regard to BS, which showed superiority of the cultivars with high HFN values in relation to the cultivars in groups H-1 and H-2. The differences were large in the wet preharvest periods (Table 2).

Relationships between HFN and other rheological properties as well as among rheological properties in dry and wet preharvest periods were evaluated by Pearson's correlation coefficients (Table 3). The HFN values obtained for the dry periods did not correlate with any other rheological property. This is understandable since the high HFN values obtained in the dry periods (low α -amylase activity) did not represent a stress factor that would lead to starch degradation. In the wet preharvest periods, in which HFN values are typically significantly

Table 2. Average and significant differences of rheological properties in different cultivar groups in dry and wet preharvest periods.

Years (A)	Cultivar group (B)			Average	LSD	0.05	0.01
	H-1	H-2	H-3				
Falling number (HFN)							
Dry	392	392	482	422	A ¹	25.1	33.6
Wet	110	297	458	288	B ²	30.8	41.1
Average	251	345	470		AB ³	43.5	58.1
Farinograph dough development time (FDT)							
Dry	4.55	4.45	6.85	5.28	A	0.92	1.22
Wet	2.44	3.49	5.06	3.67	B	1.12	1.50
Average	3.50	3.97	5.95		AB	1.59	2.12
Farinograph absorption (FA)							
Dry	60.04	59.14	62.89	60.69	A	1.35	1.80
Wet	59.90	59.42	61.59	60.30	B	1.65	2.21
Average	59.97	59.28	62.24		AB	2.34	3.12
Resistance to extension (RE)							
Dry	316	386	340	347	A	53	70
Wet	284	384	358	342	B	64	86
Average	300	385	349		AB	91	121
Loaf volume (LV)							
Dry	1225	1194	1344	1254	A	86	115
Wet	1240	1228	1394	1288	B	105	141
Average	1233	1211	1369		AB	149	199
Baking score (BS)							
Dry	3.81	3.22	4.46	3.83	A	0.72	0.96
Wet	3.70	3.98	5.63	4.44	B	0.88	1.18
Average	3.76	3.60	5.04		AB	1.25	1.67

¹ A – Years.² B – Cultivar groups.³ AB – Interaction Year/Cultivar group.

Table 3. Correlation coefficients between rheological properties of 30 wheat cultivars (values of dry years are above the diagonal and those of wet years are below the diagonal).

Trait	HFN ¹	FDT ²	FA ³	RE ⁴	LV ⁵	BS ⁶
HFN		0.31	0.32	-0.02	0.31	0.35
FDT	0.60**		0.31	0.36*	0.54**	0.45*
FA	0.26	0.15		-0.21	0.68**	0.71**
RE	0.30	0.24	-0.24		0.10	0.10
LV	0.34	0.53**	0.52**	0.16		0.86**
BS	0.50**	0.60**	0.58**	0.20	0.89**	

¹ Hagberg falling number.² Farinograph dough development time.³ Farinograph absorption.⁴ Resistance to extension.⁵ Loaf volume.⁶ Baking score.

** and * shows significant difference at 1% and 5% probability level respectively with ANOVA.

Table 4. Classification of 30 cultivars for Hagberg falling number (HFN), farinograph dough development time (FDT), farinograph absorption (FA), extensiograph resistance to extension (RE), baking loaf volume (LV) and baking score (BS) in response to dry and wet conditions during grain filling and preharvest, calculated using canonical variate analysis with HFN as the main factor, FDT, FA, RE, LV and BS as subfactors.

FN classification	Cultivar	Dry year		Wet year	
		Potential	Rank ¹	Potential	Rank ¹
H-1	Dobric	0.34	18	-1.28	20
	Jugoslavija	-0.60	23	-1.38	22
	MV 17	-2.07	28	-3.80	29
	Lada	2.12	5	-0.38	16
	Balkan	1.08	11	-1.34	21
	Bazalt	-1.06	25	-2.42	26
	Maris Hunts	0.34	17	-1.62	23
	Condor	0.49	16	-0.76	19
	Siete Cerros	1.13	10	-0.15	15
	Nahodka 4	1.37	7	-1.95	24
H-2	Imerio	-3.89	30	-2.62	27
	MV 20	0.93	13	0.68	10
	NS Brkulja	-0.97	24	-2.09	25
	Cook	0.88	14	0.20	14
	Florida 301	-2.12	29	-3.30	28
	Kavkaz	-0.40	22	1.27	6
	Noe	1.14	9	0.67	11
	Gaboto	-1.37	27	-0.73	18
	Slavija	-1.18	26	-4.08	30
	KG 100	0.30	19	-0.53	17
H-3	Žitarka	1.27	8	1.46	4
	Klein Forten	3.78	1	0.99	7
	Arina	-0.16	21	0.76	9
	Winalta	0.77	15	1.32	5
	Garazinko	3.08	2	1.76	2
	Kirac	2.39	4	1.74	3
	Sardona	0.30	20	0.53	12
	MV 21	1.72	6	0.98	8
	Stepnaja 30	2.47	3	2.54	1
	Odeska 66	1.00	12	0.45	13

¹Rank indicates cultivar position with regard to all factors.

lower than those in dry periods, positive correlations were found between HFN and FDT. This indicates that, under conditions of moisture stress occurring in the preharvest period, cultivars with high HFN (sprout-free) maintained low levels of amylolytic and proteolytic activities which resulted in maintenance of dough strength. This was further confirmed by BS, the final expression of bread quality, which also was positively correlated with HFN (Table 3). Quite similar results were reported by Singh et al. (2001) who studied the effect of sprouting conditions on functional and dynamic rheological properties. Significant positive correlations between the HFN and

several farinograph-measured properties, such as dough stability and farinograph time to breakdown, were found by Hareland (2003).

The other rheological properties exhibited the expected mutual relations, and their values were similar in wet and dry preharvest periods (Table 3).

When canonical variate analysis is applied, the initial classification according to HFN, ANOVA could be extended to other rheological traits, FDT, FA, RE, LV and BS. Table 4 shows the final classification of the cultivars. As the data obtained from canonical variate analysis gave a combined reaction of HFN, farinograph dough

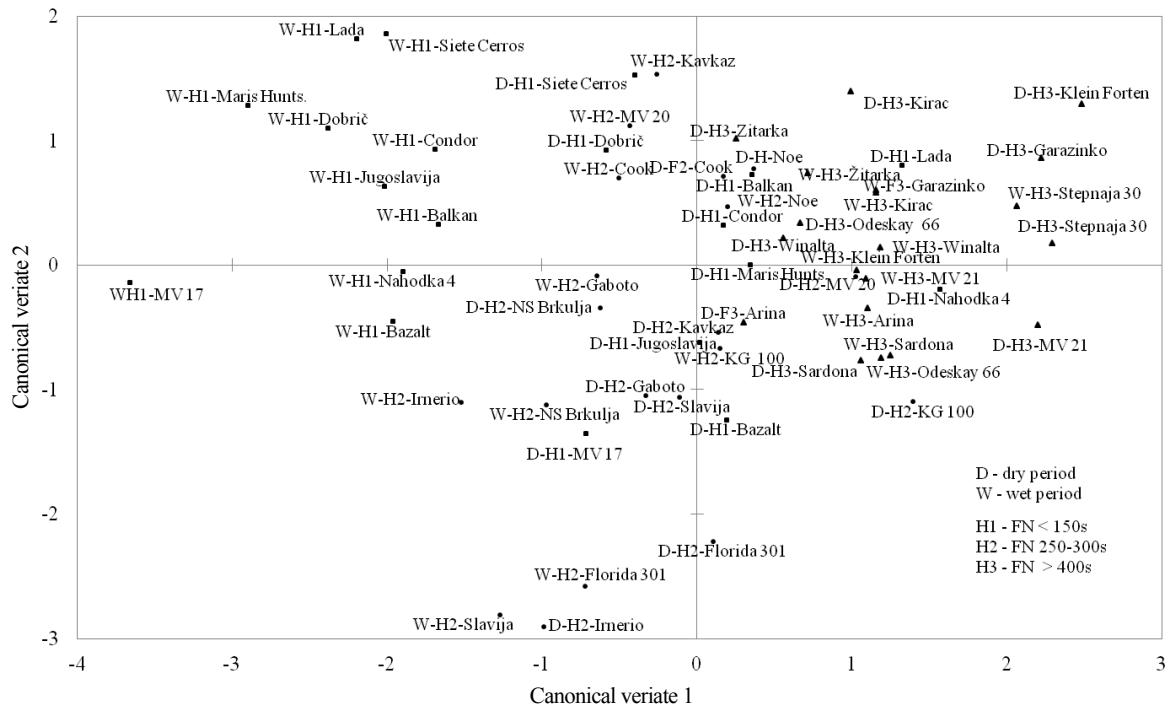


Fig. 4. Canonical variate analysis of the HFN responses of 30 wheat cultivars in dry and wet preharvest periods.

development time, farinograph absorption, resistance to extension, loaf volume and baking score to dry and wet preharvest periods, the initial grouping was duly altered. Since the study focused on the HFN response to dry and wet preharvest periods, three main response groups (low, medium and high) were maintained. The combined performance of each cultivar is indicated with the use of the canonical variate means used to plot each cultivar on the canonical variate analysis graph (Fig. 4). When x and y axis coordinates were added for each cultivar, potential response values were obtained (Table 4). A more negative value means poorer performance of the cultivar for the combined factors. If the value was closer to zero, a more average response was obtained. Finally, the more positive the value obtained, the higher the performance of the cultivars for the analyzed factors. Typical examples of negative response were the cultivars Imerio, MV 17 and Slavija, found in the left quadrant (Fig. 4), with negative values in both wet and dry preharvest periods. Their reaction showed that, regardless of the rainfall level in the preharvest period, these cultivars have low HFN and FDT values. In contrast, the cultivars Stepnaja 30, Klein Forten and Garazinko, whose values for both wet and dry preharvest periods were in the upper right quadrant, had positive HFN and FDT values (Fig. 4).

Table 4 shows the cultivars according to HFN as the main criterion, but combined with the other rheological properties, FDT, FA, RE, LV and BS. In the wet preharvest periods, HFN was highest in the Russian cultivar Stepnaja 30 (2.54), followed by the Brazilian cultivar Garazino

(1.76) and the Turkish cultivar Kirac (1.74). These cultivars also performed well in the dry periods, i.e., they retained good rheological quality even in years with abundant rainfall during the grain filling period. Biddulph et al. (2008) also reported that some cultivars maintain high HFN (over 300 s) even in locations and rainy periods in which intensive preharvest sprouting occurred. HFN was high in the cultivars Lada (2.12), Balkan (1.08), Nahodka 4 (1.38) and Siete Cerros (1.13) in the dry preharvest periods, which indicated that rheological properties were good under such conditions, while in the wet preharvest periods they had a negative value and poor rheological properties (Table 4). The cultivars Imerio, Florida 301, MV 17, Slavija and Bazalt had the lowest potentials in both wet and dry preharvest conditions (Table 4). In the study of Barbeau et al. (2006), the cultivars Recital and Hayne showed similar reactions in HFN and bread making quality traits in wet and dry preharvest periods. Biddulph et al. (2008) studied the preharvest sprouting, dormancy, HFN and other properties of wheat cultivars under different conditions of moisture/drought stress at the stage of grain filling. They classified cultivars into the strongly dormant genotypes that retain HFN over 300 s with up to 70 mm of rain before harvest, partially dormant genotypes whose HFN value was slightly lower but not less than 250 s, under conditions of moisture stress; and non-dormant genotypes, whose HFN value was significantly below 250 s under moisture stress conditions.

Like most properties, HFN was clearly affected by genotype, environment and their interaction. Which of

these factors will prevail depends on the variability of the set of cultivars used, the amount of rainfall and temperature during the preharvest period. Differential genetic expression of resistance to preharvest sprouting, maintenance of low α amylase, high HFN values, maintenance of rheological properties, and baking performance is reliably detected and measured under wet preharvest conditions.

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