EFFECTS OF GENOTYPE, NITROGEN FERTILIZER AND WATER STRESS ON MIXING PARAMETERS IN WHEAT (*TRITICUM AESTIVUM* L.)

Gabriela Stanciu[1], Amalia Neacşu[1]

ABSTRACT

Dough mixing properties, which are important in determining wheat processing and end-use quality, are known to be influenced by both genetic and environmental factors. We analyzed some Reomixer mixing parameters of 26 breeding lines grown in 2007, in four contrasting environments (with and without nitrogen fertilization, under water stress or irrigated). Nitrogen supply had large and significant effects on grain protein concentration and on all mixing properties, except extensibility. Effects were particularly large on dough strength and mixing requirements, and therefore on estimated bread volume. Nitrogen fertilization proved to be necessary for obtaining high quality wheat, even on a relatively fertile soil. Strong water stress, which induced poor plant growth, reduced grain protein concentration and affected all mixing parameters, except „initial slope". Observed significant interactions between genotypes and both N and water availability may open perspectives for identifying future cultivars with increased quality tolerance to variation in N and water supply.

Key words: mixing parameters, wheat, nitrogen fertilization, water stress.

INTRODUCTION

Dough mixing properties are important in determining wheat processing and end-use quality. The quantity and quality of flour proteins, control to a large extent the time needed to mix to maximum dough resistance („mixing requirements"), the maximum resistance observed („dough strength"), as well as the extensibility of the dough and the rate of dough resistance decrease by overmixing („dough stability" or „mixing tolerance") (Uthayakumaran et al., 1999).

The effect of flour proteins characteristics on mixing properties can be best determined by recording dough behavior during mixing, using a mixograph type device (Finney and Shogren, 1972), among which, the Reomixer „provides practical, rapid small-scale wheat protein quality measurements derived from dough mixing characteristics" (Anderson, 2004).

Mixing parameters can be used for predicting bread volume (Wikstrőm and Bohlin, 2007) and are themselves important objectives in breeding for improved wheat processing quality. A high bread volume was related to higher values for both resistance (strength) and extensibility (Antes and Wieser, 2001).

Dough properties are known to be influenced by both genetic and environmental factors (Zhu and Khan, 2001).

This paper analyzes the effect of genotype, nitrogen fertilizer and water stress on several mixing parameters in 26 lines from the NARDI Fundulea wheat breeding program, grown in four contrasting environments.

MATERIAL AND METHODS

Twenty six winter wheat lines, bred by NARDI Fundulea breeding program and not previously selected for mixing parameters, were grown in 2007 in four contrasting environments: irrigated, with and without nitrogen fertilization, and non-irrigated, with and without nitrogen fertilization, on a moderately leached chernozem formed on loess, with 2.8% organic matter in the arable layer. Severe drought was recorded in 2007 during the whole vegetation period. The four environments are best described by the average values across genotypes for grain yield and protein content (Table 1).

Table 1. Average grain yield and protein content in the four environments

No.	Environment	Grain yield averaged across genotypes (kg ha^{-1})	Protein content (%) averaged across genotypes
1	Irrigated, with nitrogen fertilization	6080	15.4
2	Irrigated, without nitrogen fertilization	5230	11.8
3	Dryland, with nitrogen fertilization	4083	13.1
4	Dryland without nitrogen fertilization	3631	10.1

[1] National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania

Nitrogen fertilization increased yield by 850 kg ha^{-1} under irrigation and by 452 kg ha^{-1} on dryland, while water stress under dryland reduced yield by 1957 kg ha^{-1} with nitrogen fertilization and by 1599 kg ha^{-1} without nitrogen fertilization.

On the other hand, nitrogen fertilization increased protein content by 3.6 units under irrigation and by 3.0 units on dryland, while poor plant development under dryland reduced protein content by 2.3 units with nitrogen fertilization and by 1.7 units without nitrogen fertilization.

Ten grams flour samples of all lines from the four environments were analyzed with Reomixer, following manufacturer's instructions.

The Reomixer, manufactured by Reologen i Lund AB, conforms at large to the AACC Mixograph standard, and the mixing curves produced agree well with the results of the classical pen recording National Mixograph (Bohlin, 2007). The Reomixer measures the torque by detecting the deflection of a lever arm constrained by a pair of stiff springs. This deflection is measured by a non-contacting sensor. The software provided by the manufacturing company allows for determining 13 mixing parameters describing specific characteristics of the mixing curve, plus three integrating (integrated height to peak - IHTP, area below and area within the mixing curve) and one calculated parameter, the estimated bread volume (BV).

Neacşu et al. (2008) found that most information contained in this large number of mixing parameters can be condensed by the following five parameters, which describe the basic rheological aspects of dough development and are most appropriate for use in breeding:

- initial slope („initslope") describing the water absorption phase**;**
- development time, or time to peak („peaktime"), describing the mixing requirements of the dough;
- peak height („peakheight") describing the dough strength or elasticity;
- dough breakdown („breakdown"), describing the dough stability or tolerance to overmixing;
- final width („endwidth"), describing mainly the dough extensibility.

The definitions of these mixing parameters are presented in figure 1 (after Bohlin, 2007 and personal communication).

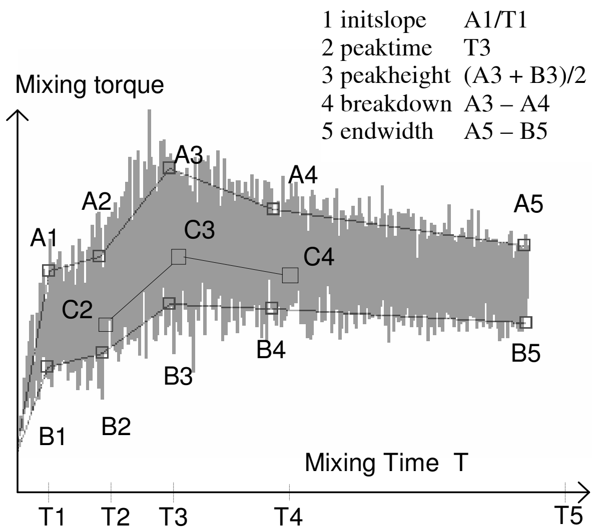


Figure 1. Definition of Reomixer mixing parameters

We used these five parameters, plus one calculated parameter „BV", the bread volume estimated using formulae included in the Reomixer software.

ANOVA model I, with three fixed factors was used to analyze the results obtained for every parameter. Individual variance components were calculated for each factor, for two-factor interactions and the rest, and their share from their total was expressed as percentage, to illustrate their relative contribution to the overall variation.

RESULTS AND DISCUSSION

Genotypes significantly influenced the variation of all studied parameters, except „peak height" and estimated bread volume, while nitrogen fertilization had significant effects on all parameters, except „end width" (Table 2). Water availability had significant effects on all parameters, except „initial slope".

Interactions between genotypes and fertilization and between genotypes and water availability were significant for all parameters, being especially large for parameters where average effects of N fertilization or water availability were not significant („peak height" and „initial slope" respectively).

Table 2. Variance components expressed as percentage of total variance of several mixing parameters

Parameter	Source of variation				
	Genotypes	N fertilization	Genotypes* N fertilization	Water avail-ability	Genotypes* Water availability
Initial slope	28.80*	12.96*	29.88*	1.33ns	24.76*
Peak time	42.14**	13.56*	15.44*	14.98*	12.92*
Peak height	3.59 n.s.	48.52**	12.62*	10.22*	13.38*
Breakdown	27.96*	31.72**	10.63*	18.02*	10.91*
End width	41.84**	1.92 n.s.	17.54*	9.99*	27.77*
Estimated bread volume	3.12 n.s.	55.36**	11.31*	18.4*	11.51*

*) significant at P<0.05 level ns) not significant at P>0.05 level
**) significant at P<0.01 level

As Zhu and Khan (2001) also pointed out different dough mixing properties showed differences for relative influences of genotype and environment. Particularly dough strength, described by the parameter „peak height", was influenced to the largest extent by nitrogen availability, while mixing requirements, described by the parameter „peak time" was mostly determined by the genotype. Lack of nitrogen fertilization had only a small influence on the value of „initial slope" on average for all genotypes, reducing it more under water stress (Table 3). This is probably related with lower protein content which reduced water absorption. However, best genotypes were able to reach high values of initial slope, even without fertilization. Water stress increased the „initial slope" when nitrogen was applied, but reduced it slightly when wheat was grown without fertilization.

Table 3. Average effect of N fertilizer and water availability on the parameter „initial slope"

	Average for all genotypes		Best genotypes		Worse genotypes	
	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer
Irrigated	7.36	7.11	8.77	9.85	5.90	5.20
Water stressed	7.85	6.98	9.42	8.77	5.91	4.28

Mixing requirements were increased when no N fertilizer was applied, insufficient nitrogen increasing the „peak time" by 16% under irrigation and by 22% under water stress (Table 4). An explanation of the very long time for dough development observed with less available nitrogen can be the variation of protein contents (see Table 2), a lower content, caused by lack of fertilization and in our experiment also by water stress, making the formation of the gluten network during mixing more difficult.

Both insufficient N availability and water stress caused large decreases of dough strength on average for all genotypes (of 25% under irrigation and 33% under water stress), as well as for the strongest and weakest genotypes (Table 5). However, even under N fertilization and good water supply genotypes with weak gluten could not reach satisfactory values of peak height. Genotypes with the strongest dough could reach high enough values of dough strength, even without N fertilization or under water stress.

Table 4. Average effect of N fertilizer and water availability on mixing requirements, described by the parameter „peak time"

	Average for all genotypes		Genotypes with highest mixing requirements		Genotypes with lowest mixing requirements	
	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer
Irrigated	3.46	4.10	5.13	6.90	2.27	2.20
Water stressed	4.18	5.36	7.18	9.67	2.33	2.92

Table 5. Average effect of N fertilizer and water availability on dough strength, described by the parameter „peak height"

	Average for all genotypes		Genotypes with strongest dough		Genotypes with weakest dough	
	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer
Irrigated	8.03	6.05	9.35	8.72	6.74	4.53
Water stressed	7.24	4.82	8.93	7.13	5.19	3.42

On average for all genotypes and for both most tolerant and less tolerant to overmixing genotypes, grains produced without N fertilizer or under water stress had smaller values for the „breakdown" parameter (Table 6). This is however related to the smaller peak height values, and therefore does not reflect an improvement in dough properties.

Table 6. Average effect of N fertilizer and water availability on dough stability, described by the parameter „breakdown"

	Average for all genotypes		Genotypes with highest breakdown		Genotypes with lowest breakdown	
	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer
Irrigated	1.91	1.19	3.42	2.95	0.74	0.32
Water stressed	1.36	0.72	2.66	1.38	0.40	0.01

On average for all genotypes influence of N fertilization on dough extensibility was small and not significant, but water stress increased the parameter „end width" (Table 7). However, interaction of genotypes with both N fertilizers and water stress was important reflecting large genetic differences in cultivar response to insufficient nitrogen or water availability.

Table 7. Average effect of N fertilizer and water availability on dough extensibility, described by the parameter „end width"

	Average for all genotypes		Genotypes with highest extensibility		Genotypes with lowest extensibility	
	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer
Irrigated	1.81	1.97	2.81	2.99	0.93	0.86
Water stressed	2.13	2.22	2.72	3.93	1.00	0.84

The strong negative influence of the absence of N fertilization and of water stress on bread volume (Table 8) reflects the effects of these factors on most important mixing parameters, and mainly on dough strength. Estimated bread volume was decreased by lack of N fertilization with 26% under irrigation and with 33% under water stress. This influence seems to be smaller in the best genotypes and very strong in the weak gluten ones, therefore offering some hopes of selecting genotypes more tolerant to the effect of deficient N nutrition on bread quality.

Table 8. Average effect of N fertilizer and water availability on estimated bread volume

	Average for all genotypes		Best genotypes		Worse genotypes	
	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer	Fertilized	Without N fertilizer
Irrigated	1411	1049	1651	1482	1207	803
Water stressed	1229	818	1508	1273	911	598

Our results illustrate the importance of N fertilization for obtaining wheat of high bread-making quality, even on a relatively fertile chernozem soil. The negative effects of low nitrogen supply are particularly important for „organic agriculture”, where lower soil fertility cannot be corrected by applying mineral fertilizers. It is also expected that future elevated CO_2 concentrations will exacerbate the deleterious effects of low soil nitrogen on grain quality (Kimball et al., 2001).

In this context, exploiting the observed interactions of genotypes with N fertilization and identification of genotypes that are less afecte by a lower N availability may become very important. Figure 2 exemplifies mixing curves of several genotypes with differential response to low nitrogen (Figure 2).

The negative influence of water stress on processing and bread-making quality in our study was somewhat unexpected. Generally drought, which usually is manifested during the grain filling period, reduces more accumulation of starch than of protein, causing a relative increase of protein concentration, with positive effects on quality. Obviously, in 2007 drought had earlier effects on the whole plant growth and development, causing an unusual reduction of protein concentration and of related mixing properties.

On the other hand it is possible that stress induced modifications in the ratio of protein components. Antes and Wieser (2001) demonstrated that maximum resistance of dough and bread volume was strongly increased by HMW subunits and decreased by LMW subunits. On the other hand, Blumenthal et al. (1993) stated that gliadin synthesis continues at a greater rate than glutenin synthesis during a period of heat stress, and consequently the mature grain has a higher ratio of gliadin : glutenin and produces weaker dough.

CONCLUSIONS

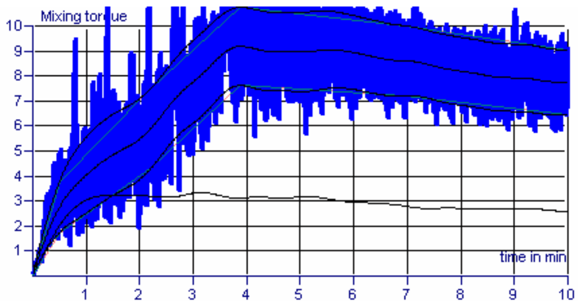
Nitrogen supply had large and significant effects on grain protein concentration and on all mixing properties except extensibility. The effects were particularly important on dough strength and mixing requirements, and therefore on estimated bread volume. Nitrogen fertilization proved to be necessary for obtaining high quality wheat, even on a relatively fertile soil.

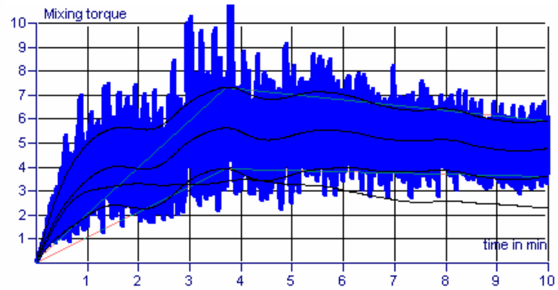
Strong water stress, which induced poor plant growth, reduced grain protein concentration and affected all mixing parameters, except „initial slope”.

Observed significant interactions between genotypes and both N and water availability, may open perspectives for identifying future cultivars with increased quality tolerance to variation in N and water supply.

Fertlized with 100 kg N/ha No N fertilizer

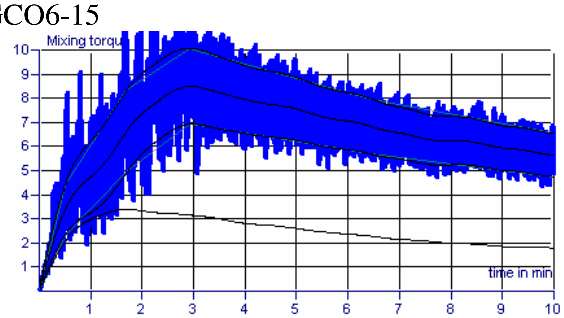
GCO2-7

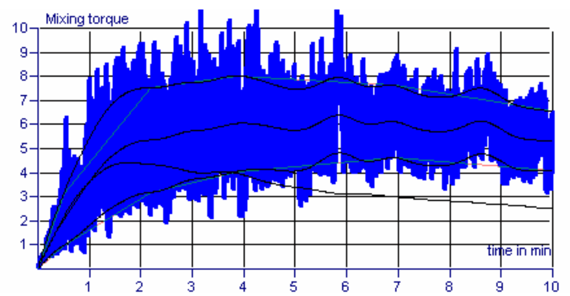




Protein = 15.0% Peak height = 9.19 Peak time = 3.88 Protein = 11.1% Peak height = 5.63 Peak time = 4.05

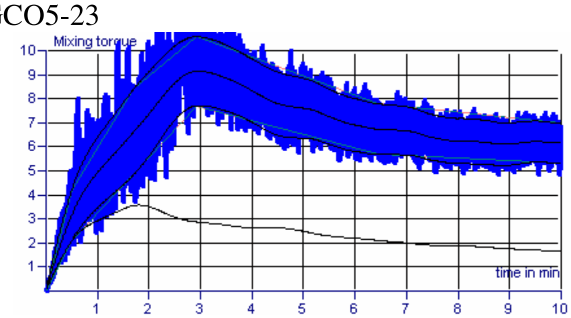
GCO6-15

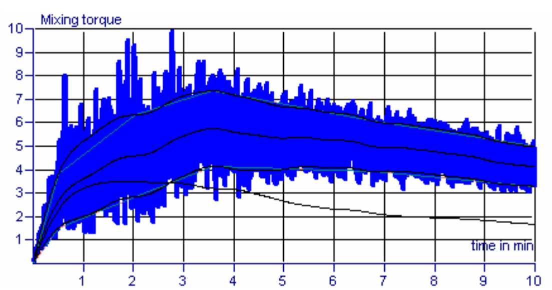




Protein = 14.4% Peak height = 8.50 Peak time = 3.00 Protein = 11.3% Peak height = 6.05 Peak time = 3.70

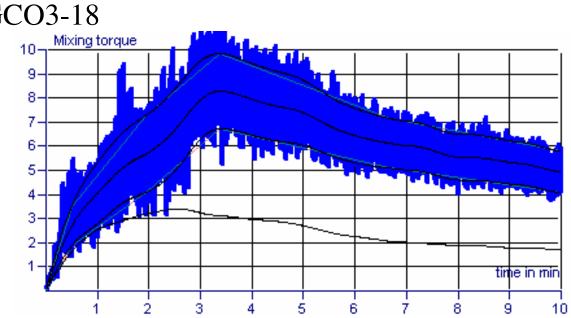
GCO5-23

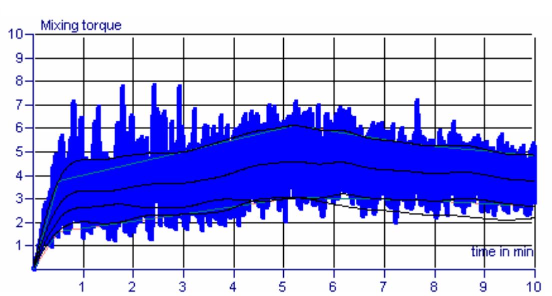




Protein = 15.1% Peak height = 9.14 Peak time = 2.92 Protein = 11.6% Peak height = 5.76 Peak time = 3.20

GCO3-18





Protein = 15.5% Peak height = 8.28 Peak time = 3.38 Protein = 11.0% Peak heigh = 4.59 Peak time = 5.40

Figure 2. Mixing curves of several genotypes grown with and without N fertilization

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