# CHARACTERIZATION OF "TURDA" MAIZE GERMPLASM FOR THE CHEMICAL COMPOSITION OF THE GRAIN

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## ABSTRACT

Evaluation of maize grain quality traits across several genotypes is essential to determine the potential of maize for value-added products. The diversity of maize uses requires appropriate quality characteristics. The objectives of this study were: 1) to evaluate the potential of "TURDA" maize germplasm according to its grain quality traits, such as: protein, oil, fiber, ash, and starch concentrations; 2) to estimate the extent of phenotypical variability and correlations for various quality components and 3) to formulate a selection criterion in a breeding program. To examine phenotypical diversity in grain content, a total of 754 maize samples were evaluated for their grain quality attributes: 265 local populations (landraces); 59 synthetics/composites and 430 "TURDA" inbred lines. Inbred lines were on average the most divergent in grain starch concentration (range value 19.9) as compared to landraces (range value 11.8) and synthetics (range value 12.5). The grain oil and ash content showed high variability among the genotypes. The mean starch concentration of inbred lines had the highest value 67.5%, comparatively with local population 64.9% and synthetics 65.9%. CV values for kernel content reflect a lower diversity for starch and protein concentration and a high diversity of inbred lines for fat and fiber concentration and a high diversity of inbred lines for fat and minerals concentration. Grain starch content was negatively correlated with protein, oil, fiber and ash contents, while protein, oil, fiber and ash contents were, in most cases, positively correlated between themselves.

Key words: maize germplasm, phenotypical diversity, grain chemical composition

### **INTRODUCTION**

Maize is one of the most important grain crops in Romania, with over 2 million hectares in production. This crop is an integral part of Romanian agriculture and has a potential to compete with its multi-products.

In Romania, and in almost all of the European maize-growing countries, the diffusion of maize hybrids, possessing a vield, caused a progressive superior substitution of local populations. Therefore, the genetic variability of the cultivated maize germplasm was reduced over the past five decades, in term of both number of alleles and genetic diversity across hybrids (Reif et al., 2005). The necessity to collect and maintain the traditional maize landraces has emerged for the first time in past decades, to avoid a significant loss of the maize genetic variability in Europe. In different countries, collections of populations (landraces, local varieties and so on) were activated (Lavergne et al., 1991; Berardo et al., 2009).

Because maize is a relevant food source, the quantification of the nutritionally important grain constituent role is important for the best exploitation of the different genotypes. In this context, the traditional germplasm represents a good source of genetic variability to explore and may help to identify the most suitable materials for the development of more nutritious foods.

Specifically, different industries have different requirements of maize for their particular use. The wet milling industry would like soft starch, and low protein content, while hard starch is required for dry milling and for masa production. The feed industry would gain value from maize with increased energy content, i. e. maize with higher oil content, and from increased protein content and a better

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amino acid balance. Genetic variability that can be used for modifying maize grain composition to satisfy all of these requirements has been frequently reported among strains (Smith, 1990). However, it is necessary to further explore germplasm and genetic variability for such quality-related traits and their association with grain yield and other yield attributes.

Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in maize improvement strategies, as maize germplasm could be easily managed, using recurrent selection method (Lavergne et al., 1991; Mohammadi and Prasanna, 2003). Many have documented genetic studies and phenotypic variability for grain composition traits in maize (Whitt et al., 2002; Has et al., 2004; Uribelarrea et al., 2004; Duarte et al., 2005; Pollak and Scott, 2005; Reynolds et al., 2005; Berardo et al., 2009).

"Turda" - Romanian maize germplasm, consisting of local populations, varieties, synthetics and single-crosses, double-crosses, and three-way hybrids, has large phenotypic and genetic variability. Genotype germplasm sources range from very early to late and from dent to flint grain characteristics (Grecu and Legman, 1994; Has et al., 2004).

The objective of this study was to evaluate the variability existing for some chemical components of the grain in a large range of "Turda" maize germplasm and to identify genotypes that could be interesting in term of breeding for nutritional value.

## **MATERIAL AND METHODS**

### Maize samples

Maize samples used in this study consisted of 754 accessions from "Turda" germplasm collection, among which there were 265 local populations (landraces), collected in different Romanian regions (Transylvania and Moldavia); 59 synthetics/composites among which 30 synthetics created at ARDS Turda and 29 synthetics acquired from different countries (Spain, Italy, Germany, USA -University of Minnesota, University of Pennsylvania); 430 "Turda" inbred lines. The local populations used in this study have been originated in more stages:

- after middle part of the XVII<sup>th</sup> century, maize was introduced in south and east part of Romania from Turkey (flint type);

- in west part of Romania (Transylvania region) maize was introduced in the first part of XVIII<sup>th</sup> century from Italy (flint type).

- In the last part of XIX<sup>th</sup> century and first part of XX<sup>th</sup> century, maize was brought from USA and Argentina, especially dent type (Has et al., 2006).

All local populations (landraces), synthetics and inbred lines are currently used in the framework of breeding and genetic program at the Agricultural Research and Development Station Turda – Romania (ARDS Turda). The studied genotypes differed by germplasm source, grain type, maturity classification (very early, early, intermediate and late) and grain appearance and color.

### **Experimental designs**

These genotypes were grown at the Agricultural Research and Development Station Turda – Romania (Transylvania region), in 2006.

Each group of genotypes was grown in separate but adjacent trials. Experimental plots were 2-rows, 5 m - long, with 0.7 m spacing between two rows without replications. Plant densities averaged 60,000 plants/hectare in each trial.

At least six plants in each experimental plot were sib-pollinated by pollen from the same plot to avoid xenia effects. Approximately five hand-pollinated ears per row were harvested, after physiological maturity, and bulked for chemical analysis, i.e. protein, fat, starch, fiber, and ash.

In addition, 50 grains from the middle of each plot were removed and used for measuring moisture concentration.

For each plot, a representative 50 g sample of the grain was ground, and the concentration of starch, protein, oil, fiber and ash in the ground (flour) sample was determined with a Dickey-John Instalab 600 near-infrared reflectance analyzer, after curve calibration.

#### Statistical analysis

All grain physical quality tests were performed in duplicate, and the mean value was analyzed statistically. Analyses of variance (ANOVA) using a one-factor model without replications were done for each trait and for each group of genotypes (Ceapoiu, 1968). Pearson's correlation coefficients were computed to express the relationship among characters.

## **RESULTS AND DISCUSSION**

#### **Description of variability**

In all trials, coefficients of phenotypic variation were over 5% for most grain components (Table 1); they were higher for

percentage of oil (12.3 to 21.2%), fiber (10.5 to 18.9%) and ash (51.1 to 88.2%).

Although, there is little variation in the percentage of starch in the germplasm studied here, there appears to be differences in the percentage of recoverable starch in these materials. In the same Table 1 it was also evident that local populations showed starch contents ranging between 57.1% and 68.9%. The range of variation observed for synthetics was larger than in local populations, ranging between 60.1% and 72.6%.

Among synthetics some interesting forms with high level of starch content were identified: Tu Comp. A (RRS) (1) (71.8%), Tu Comp. B (RRS) (1) (69.6%), Tu 5D (RRS) (69.6%), Tu Comp. A (RRS) (10) (69.5%) (Table 2).

Table 1. Means values, range of variation, and coefficients of variation (CV) for grain content in "Turda" germplasm

	The second se	Grain concentration in:				
Trait		Starch	Oil	Protein	Fiber	Ash
Germplasm	Range			%		
Local Populations	Minimum	57.10	3.80	11.20	3.30	0.03
(Count = 265)	Mean	64.90	5.40	13.70	5.30	2.30
	Maximum	68.90	9.10	15.60	7.30	7.20
	Variance	3.81	0.44	0.71	0.62	1.38
	Standard Deviation	1.95	0.66	0.84	0.78	1.17
	Standard Error	1.95	0.04	0.05	0.78	0.07
	Confidence Level (95.0%)	0.23	0.08	0.10	0.09	0.14
	CV%	3.00	12.30	6.20	14.90	51.10
"Turda" Synthetics	Minimum	60.10	3.50	11.70	3.60	0.01
(Count = 59)	Mean	65.90	5.40	13.60	5.40	2.10
	Maximum	72.60	7.30	14.80	6.70	5.80
	Variance	6.86	0.48	0.62	0.32	2.24
	Standard Deviation	2.62	0.79	0.69	0.57	1.50
	Standard Error	0.34	0.09	0.10	0.07	0.19
	Confidence Level (95.0%)	0.68	0.21	0.18	0.15	0.39
	CV%	4.00	14.70	5.10	10.50	70.30
Inbred lines	Minimum	52.80	2.40	10.80	2.30	0.01
	Mean	67.50	4.20	13.40	4.90	1.60
(Count = 430)	Maximum	72.70	8.00	14.80	7.50	10.60
	Variance	7.73	0.79	1.17	0.85	2.04
	Standard Deviation	2.78	0.89	1.08	0.92	1.42
	Standard Error	0.13	0.89	0.05	0.04	0.07
	Confidence Level (95.0%)	0.26	0.08	0.10	0.09	0.14
	CV%	4.10	21.20	8.00	18.90	88.20

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Table 2. Local populations and synthetics with increased p	per se values for their quality grain content

Genotype	Protein	Oil	Starch %	Fiber	Ash	Grain type	
		Local n					
Local populations   Acatari/02 14.8 6.6 60.1 6.1 3.6 Flint + Sem							
Apoldu de Sus/01	14.0	6.7	62.2	6.5	4.9	Flint	
Baita Cainelui de Sus/99	14.2	6.1	64.0	6.7	3.1	Semi-dent	
Berind CN26-84/99	13.4	6.2	63.7	5.5	3.2	Flint + Semi-flint	
Beriu (sugary)/99	11.3	9.1	57.1	4.7	7.2	Sugary	
Blaj (Verza)/01	14.6	7.3	59.3	6.6	5.9	Flint	
Bradu B-18/01	13.8	6.2	63.4	6.1	3.4	Semi-dent	
Castori/03	14.2	6.6	61.8	6.3	4.5	Semi-flint	
Campeni/01	13.9	6.5	63.0	6.6	4.1	Semi-dent	
Carnesti/01	15.0	6.9	59.5	6.6	4.9	Flint	
Coldau/01	14.2	6.1	62.9	6.3	3.4	Flint	
Cornesti/01	14.0	6.2	61.7	5.3	3.9	Flint	
Danes/01	14.9	6.6	61.4	6.9	4.2	Semi-flint	
Dumbravita/03	14.4	6.1	63.2	6.6	3.9	Semi-flint	
Feldioara/01	15.0	6.2	62.8	7.0	3.4	Semi-flint	
Geoagiu/01	15.3	6.1	62.0	6.8	2.9	Semi-flint	
Ghiula/04	15.2	6.7	60.2	7.0	5.5	Flint	
Gurghiu/04	14.6	6.2	61.3	5.9	4.6	Semi-flint	
Hadareni/01	14.5	6.3	62.4	6.7	3.4	Semi-flint	
Iclod/01	15.1	7.0	60.3	7.2	5.0	Semi-flint	
Ighiu/01	14.9	6.3	62.3	6.9	3.8	Semi-flint	
Lujerdiu/04	13.0	6.6	61.8	5.3	5.7	Flint	
Marunt Alb de Virstea/99	13.6	6.3	62.4	5.1	3.9	Flint	
Mihaiesti CN-8/99	13.7	6.4	63.5	6.5	4.0	Flint	
Ohaba/03	13.1	6.8	61.9	5.4	4.7	Semi-flint	
Rodna/01	14.6	6.5	62.3	7.0	4.0	Flint	
Salva/01	15.5	7.1	59.3	7.2	4.9	Semi-flint	
Sarmisegetuza/01	14.7	7.1	60.4	7.3	5.0	Flint	
Satu Lung/01	15.6	6.7	60.2	7.1	4.4	Semi-flint	
Sanpetru de Campie/01	14.1	6.2	63.5	6.5	3.8	Flint	
Santana de Mures/01	14.1	6.3	61.6	5.5	3.6	Flint + Semi-flint	
Secuieni/01	14.2	6.3	62.2	6.0	3.8	Flint	
Stanceni/03	12.6	6.1	63.5	4.8	3.4	Flint	
Susenii Bargaului/01	14.7	6.4	61.2	6.3	3.7	Flint	
Sona/01	14.7	6.4	62.4	7.1	3.7	Dent	
Telciu/01	13.7	6.2	63.2	5.9	3.9	Flint	
Uriu Ilisua/03	13.6	6.6	61.9	6.0	3.4	Semi-flint	
Vanători/01	14.2	7.1	60.1	6.6	5.1	Flint	
Zetea (B145-84)/99	13.6	6.4	62.2	5.4	4.0	Semi-flint	
Synthetics							
Tu Syn 1	13.2	7.1	60.9	5.2	4.6	Flint	
Tu Syn 2	13.8	7.0	60.1	5.6	4.8	Flint	
Tu Syn (3) (per se) (1)	13.7	7.3	60.8	6.3	4.9	Flint	
Tu 6I (RRS) (5D)	13.3	6.3	63.1	5.3	3.7	Flint	
Tu 2I (RRS) (5D) (1)	14.8	6.1	61.9	5.7	3.5	Flint	
Syn 54 Marano - Italia	13.5	6.5	62.6	5.4	4.4	Flint	
Syn 55 Marano - Italia	13.6	6.4	61.3	4.6	3.7	Flint	
Syn 57 Marano - Italia	14.1	6.8	61.8	6.2	5.8	Flint	
Syn 66 Marano - Italia	13.1	6.1	63.3	4.9	3.5	Flint	
Coruna Early – Spania	14.1	6.4	62.8	6.1	4.4	Flint	
Sarria	13.8	6.3	64.3	6.4	4.8	Flint	
Coruna Prolific Syn	14.3	6.4	61.8	6.0	3.7	Semi-flint	

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Inbred lines were, on average, the most divergent in grain starch concentration (range value 19.9) as compared to landraces (range value 11.8) and synthetics (range value 12.5) (Figure 1). About 100 genotypes were characterized by high starch content, with an increased *per se* value. Some of them are "Turda" inbred lines that were identified with high starch content (>71%) in grain (Table 3). Most of these inbred lines are characterized by dent or semi-dent grain type.

Among "Turda" inbred lines, some interesting forms with high level of starch content were identified: TC 384AcmsC (72.5%), TC 384AcmsT (72.2%), TE 210 (72.1%), TC 378 (72.0%).

All these genotypes characterized by high starch grain content can be used as high starch maize parents in a breeding program. Either pedigree selection or recurrent selection could be used to further increase the percentage of starch in grains.

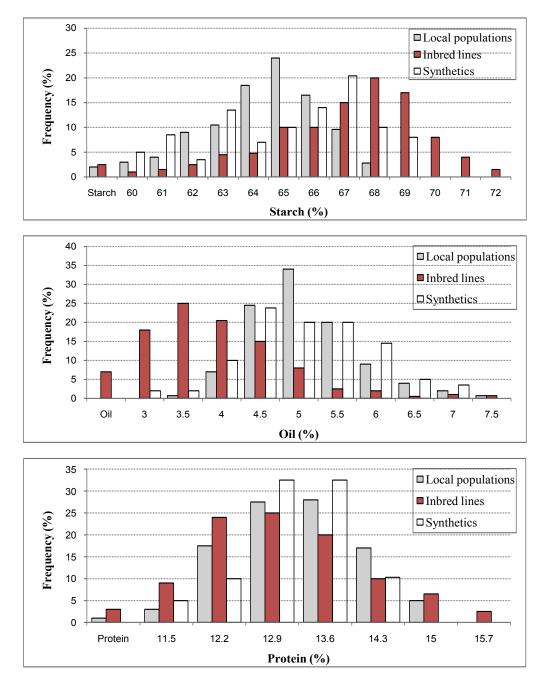


Figure 1. Frequency distribution of the three groups of genotypes by their starch, oil and protein concentrations

The oil percentage ranged from a low level of 2.4% (inbred lines) to a high level of 9.1% (local populations) (Table 1). Local populations showed an average oil concentration of 5.4%, ranging between 3.8% and 9.1%. Among local populations some interesting forms with high level of oil concentration were identified: Blaj (Verza)/01 (7.3%), Iclod/01 (7.0%), Salva/01 (7.1%), Sarmisegetuza/01 (7.1%), and Vanatori/01 (7.1%) (Table 2).

The data about synthetics showed a range among the genotypes for oil concentration from 3.5% to 7.3%. A similar range of variation (5.26 and 7.17%) was observed by Berardo et al. (2009) in a collection of 93 landraces. High oil concentrations were found in the following synthetics: Tu Syn 1 (7.1%), Tu Syn 2 (7.0%) and Tu Syn (3) (*per se*) (1) (7.3%). All these genotypes characterized by high oil grain content can be used as high oil maize source material in a breeding program. These high oil local populations and synthetics have a large reduction in the starchy endosperm (Table 2) and most of them are characterized by flint or semi-flint grain type. For this germplasm Smith (1990) supported that pedigree selection has been used to develop some elite high oil lines.

Inbred lines showed the lowest mean value for oil percentage among the genotypes analyzed. Some of inbred lines were identified with a high concentration in oil (Table 3). All these genotypes characterized by high oil grain content can be used as high oil maize parents in a breeding program.

Table 3. "Turda" inbred lines with increased per se values for their quality grain content

	<u>O</u> min	Grain content				
Inbred line	Grain	Protein	Oil	Starch	Fiber	Ash
	type	%				
T 169acmsC	Dent	11.7	3.6	71.3	3.9	0.5
TC 182	Flint	12.8	2.6	71.9	4.1	0.3
TD 246	Dent	10.8	4.2	71.3	4.8	1.0
TD 270 Nrf C	Dent	12.0	3.0	71.6	3.6	1.0
TD 270 cmsC	Dent	11.4	3.4	71.4	3.7	1.2
TD 276	Semi-dent	12.4	3.8	71.1	5.2	0.9
TE 210	Dent	11.7	3.4	72.1	4.7	0.8
TC 321	Dent	12.1	3.5	71.4	4.7	0.1
TC 330A	Semi-dent	13.0	2.4	71.8	3.4	0.2
TC 354	Semi-dent	12.6	3.6	71.2	4.4	0.2
TC 362	Dent	12.7	3.9	71.5	5.4	0.2
TC 374	Semi-dent	13.5	3.6	71.2	3.2	0.2
TC 378	Semi-dent	12.9	2.5	72.0	3.8	0.3
TC 384A Nrf	Dent	11.7	3.1	71.7	3.6	1.9
TC 384A cmsC	Dent	11.8	2.9	72.5	3.8	1.1
TC 384 A cmsT	Dent	12.4	2.9	72.2	4.1	1.5
TC 384 B	Semi-dent	12.7	2.5	71.4	3.4	0.8
TD 375	Semi-dent	12.2	3.1	71.9	4.6	0.7
TE 325	Dent	12.8	3.2	71.4	4.8	1.0
TA 439	Dent	13.2	2.7	71.3	4.1	0.6
TC 344A	Dent	15.2	7.6	58.1	7.2	5.5
TC 334	Dent	15.1	7.5	59.0	7.5	6.8
TC 106	Flint	16.4	7.5	55.1	7.1	8.0
TC 375	Dent	14.7	7.1	60.3	7.3	4.3
T 442	Flint	15.6	7.2	56.1	6.2	6.6
TC 336	Flint	15.3	6.8	59.1	6.6	6.9
TC 221	Flint	15.4	6.7	58.6	6.5	6.3

Analyses of protein showed that the percentage ranged from a low level of 10.8%

for inbred lines to a high level of 15.6% for local populations. Some of local populations

were identified with high grain content in protein and oil too (Table 2): Carnesti/01 (15.5% protein and 6.9% oil), Ghiula/04 (15.2% protein and 6.7% oil), Iclod/01 (15.1% protein and 7.0% oil), Salva/01 (15.5% protein and 7.1 oil), Satu Lung/01 (15.6% protein and 6.7% oil). Work at the University of Illinois has also shown that protein varied from 8 to 11% in maize (Smith, 1990).

The mean values recorded for fiber content were found in the range of 2.3% to 7.5%. The following inbred lines (Table 3) exhibited maximum grain fiber and oil content too: TC 334, TC 375, TC 344A and TC 106. Mean values for grain ash content ranged from 0.01% to 10.6%. Some genotypes, such as: TC 382 (10.6%), TA 25 (9.6%), TC 106 (8.0%), TC 336 (6.9%) exhibited high grain ash contents (Table 3).

The local populations showed a larger variability and higher oil concentration (max value = 9.1%) when compared to inbred lines (max value = 8.0%).

According to table 1 and figure 1, CV values for grain content reflect:

- lower diversity for starch and protein concentration: 3.0 to 4.1%, respectively 5.1 to 8.0% for all germplasm analyzed;
- medium diversity for oil (local populations = 12.3% and synthetics = 14.7%) and fiber concentration;
- high diversity for oil (inbred lines 21.5%) and minerals concentration for all genotypes analyzed.

# Phenotypic correlations in the three groups of genotypes

Starch content was negatively and significantly correlated with protein, oil, fiber and ash per grain content for all groups of germplasm analyzed (Table 4).

The results showed that an increase in starch content may ultimately decrease protein, oil, fiber and ash content, so breeding for high starch genotypes requires a moderate balance among these quality grain traits. The results are in accord with Saleem et al. (2008).

The data presented in table 4 indicated that grain oil content was positively and

significantly correlated with protein, fiber and ash contents. The results showed that an increase in oil content may also increase protein content, so breeding for high oil and high protein genotypes may be made simultaneously.

Table 4. Phenotypic correlations among grain
quality traits in maize

Trait	Starch	Protein	Oil	Fiber			
	Local populations						
Protein	$-0.50^{\circ}$	-					
Oil	$-0.87^{0}$	0.20*	-				
Fiber	$-0.58^{\circ}$	0.73*	0.58*	-			
Ash	$-0.80^{\circ}$	0.18*	0.90*	0.52*			
	N = 265						
	Synthetics						
Protein	$-0.46^{\circ}$						
Oil	$-0.93^{\circ}$	0.22					
Fiber	$-0.32^{\circ}$	0.59*	0.38*				
Ash	$-0.87^{0}$	0.22	0.92*	0.30*			
N = 59							
Inbred lines							
Protein	$-0.62^{\circ}$	-					
Oil	$-0.85^{\circ}$	0.39*	-				
Fiber	$-0.52^{\circ}$	0.68*	0.66*	-			
Ash	$-0.66^{\circ}$	0.11	0.69*	0.28*			
N = 430							

\* = Significant at 5% level of probability

Negative and significant correlation was found between ash and starch contents in all genotypes analyzed. The results showed that the breeding for high ash content may cause a significant decrease in grain starch content.

# The interest of this material for a resources program

The results of this study emphasized a great variability in the 3 groups of genotypes. As these groups represented only a small part of the material available at Turda - Romania for a resources program, one can imagine the amount of variability which could be used by breeders. And as expected from a large phenotypic pool of variability, the variability for *per se* performances revealed by local population and synthetics was large enough (Lavergne et al., 1991).

# The structure of the variability following in the geographic origin

For all characters, the variation was very continuous in the whole material: and no separated groups of local populations were observed. This result is consistent with those of Brandolini (1971) who suggested that successive introductions of American hybrids in Europe had led to homogeneous European maize. Also, Pavlicic (1971) observed a great similarity among flint maize varieties, independent of the origin.

#### CONCLUSIONS

The screening of Turda - Romanian maize germplasm revealed the presence of a wide phenotypic variability for oil, fiber and ash concentration.

Although there is little variation in the percentage starch among normal germplasm, there appears to be differences in the percentage of starch in these materials.

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Maize local populations and synthetics with high oil content in grains are available that can be used as source material for recurrent selection for increased oil content. Pedigree selection can also be used to develop elite high oil lines. Inbred lines showing the highest mean values for oil percentage could be used in combination with normal elite lines to produce hybrids with increased oil content.

The results showed that an increase in starch content may ultimately decrease protein, oil, fiber and ash contents, so breeding for high starch genotypes requires a moderate balance among these quality grain traits. Positive and significant correlations were found between oil protein contents. Consequently, and an increase in oil contents may increase also protein contents, so breeding for high oil and high protein genotypes may be made simultaneously. Negative and significant correlation was found between ash contents and starch con-tents at all genotypes analyzed. Therefore, the breeding for high ash contents level may cause significant decrease in grain starch content.

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