Scientific Papers. Series A. Agronomy, Vol. LVIII, 2015
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

# PROTEIN, STARCH, AMINO AND FATTY ACIDS CONTENT IN CORN HYBRIDS DEVELOPED AT NARDI FUNDULEA IN NATURAL CLIMATIC CONDITIONS

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***Abstract***

*Corn is one of the most important crops in Romanian agriculture. Cultivated area varies little from year to year, ranging between 2.7 to 3.0 million hectares. Corn has the greatest production potential and also the widest range of uses of all the grain crops. The NARDI-Fundulea's program for improving protein quality in corn hybrids was initiated in 1967 when first source of genes responsible for the synthesis of lysine and tryptophan amino acids were introduced, namely, opaque two (o2) and floury-2 (fl 2). Currently, at NARDI, there is an intensive breeding program aiming the create valuable hybrids for both productive potential and quality traits through the use of valuable genetic resources (genes) for high protein content, improving local populations, and inducing endosperm modifier genes in opaque hybrids. The analysis for essential amino acids, protein content and protein quality, made between 2011 and 2013, has shown that the average protein content was 9.77% with a maximum of 10.6% in Olimpius corn hybrid. The average amino acid content was 7.53%, with a maximum of 8.41% in F 165/11 hybrid. The average content of essential amino acids was 2.71%; average lysine content 0.26%.Next to high content of protein and essential amino acids NARDI's corn hybrids are rich in fatty acids. Average fat content in top hybrids was 4.53%. F 376 and Mostiştea corn hybrids had the highest fatty acids content compared to the average (F 376 (5.00%) and 4.93% Mostiştea).*

***Key words****: hybrid corn quality, protein content, starch, fatty-acids, essential amino acids.*

## INTRODUCTION

Maize (*Zea mays* L.) is the crop with highest production at world level and it occupies the third place (after wheat and rice) in the top of cultivated surfaces. The surface cultivated with maize is increasing yearly due to multiple uses of this crop: as food for humans and animals, pharmaceutical industry, composite materials but especially due to increasing demand as bio-fuel. Maize is considered as one of the most important energetic crops because it is able to accumulate high quantities of carbohydrates.

According to FAO data, 20-22% of the world maize production is used for human nutrition and 70-72% is consumed as forage due to the high coefficient of dry matter conversion of maize into milk meat and eggs.

Corn had a high percentage of essential amino acids, especially threonine, valine and phenylalanine but it is poor in lysine and tryptophan. This problem was partially solved by the discovery and use for improvement of protein quality of the opaque-2 and floury-2 genes (Mertz et al., 1964; Salamini et al., 1983). These genes are modifying the zein ratio from endosperm in favor of lysine and tryptophan.

At NARDI-Fundulea, the maize breeding program was focused on two main objectives: rising of yield potential and improvement of grain quality. At the beginning the autochthon genetic material (local populations and

Romanian corn varieties with good grain quality) was used, but, during 1970-1980 period foreign genetic sources were used especially for opaque-2 and floury-2 genes.
The aim of our current researches includes creation of productive hybrids, with high protein and amino and fatty acids content based on identification of new sources for genes implied in synthesis of these compounds.

## MATERIALS AND METHODS

In order to release valuable hybrids to farmers, in terms of quality, we test in the maize breeding field of NARDI-Fundulea about 150 to 200 new hybrid combinations every year. During 2011 to 2013 more than 400 hybrids and 100 inbred lines and local populations were analyzed.
All analyzed samples were from NARDI's corn breeding field and from contest and orientation comparative maize cultures in which no irrigation and open pollination conditions were applied. We note that the years 2011 and 2013 were normal in terms of climatic conditions and were very favorable for maize culture. The agricultural year 2012, was very dry during the critical silking and kernel forming phase that occurred in July; that month that was practically rainfall free (2 mm) during which, drought and heat affected the kernel development with negative impact on the crop amount but grain's quality index wasn't badly affected. Before sowing time, around 80-90 kg (active substance) of nitrogen and ammonium nitrate fertilizer was applied for maize; phosphorus was applied to wheat (previous crop).
The analysis for the protein, fatty acids and starch content was performed at NARDI's Laboratory for Grain Quality. The analysis for amino acids content was performed at NIBAN-Baloteşti (National Institute for Biology and Animal Nutrition), at the Chemical Analysis and Nutritional Physiology Laboratory.
The equipment used was a Surveyor Plus HPLC system, Thermo Electron, equipped with PDA detector.

## RESULTS AND DISCUSSIONS

*Enhancing of the content and quality of protein was a priority objective.*
Increasing of protein content and quality has been a priority in maize breeding program at NARDI-Fundulea. Initially were used local populations, especially local populations from Moldova with a protein content of between 10.8 to 14.6% (Gologan et al., 1966). After the discovery of the favourable effects of the gene opaque-2 (02) by Mertz et al in 1964, the breeding activity was focused on this direction.
Increased protein content and quality led to a decline in production due to a negative correlation yield/ quality. According to Alexander (1988) breeding activity has made a significant progress in simultaneous improvement of both traits (high yields with up to 12% protein content).
The hybrids analyzed during the years 2011-2013 are all authorized hybrids some of them consecrated by farmers use other just officially recorded.
The three years average of protein content for all the 15 tested hybrids was 9.77%, this average was higher in 2011 when it exceeded 10.2% (Table 1). The hybrids Olimpius, F322 and Milcov exceeded significantly the experimental average with more than 7.5%.
The lowest protein content among the test hybrids was recorded for F 475 M with an average of 9.13%. The grains of this hybrid presented the highest content in starch.
Since 1967, at NARDI-Fundulea were introduced the first sources for opaque-2 mutant genes from USA, Italy and France in order to improve the quality of corn protein.
The first hybrids with opaque gene had a farinaceous endosperm and they were more susceptible to pathogens and attacks of insect pests. It was necessary a selection for

endosperm texture modifying genes (Cosmin et al., 1974; Neguț et al., 1982). Only breeding for hard endosperm and high lysine / protein ratio can lead to competitive hybrids both quantitatively and qualitatively.

Table 1. Protein content (%) of some corn hybrids created in NARDI-Fundulea

Fundulea 2011-2013, rainfed

| No. CRT. | HYBRID | PROTEIN % | | | X | % | DIFF. | SIGNIF. |
|---|---|---|---|---|---|---|---|---|
| | | 2011 | 2012 | 2013 | | | | |
| 1 | Olimpius | 11.3 | 10.4 | 10.1 | 10.60 | 108.5 | 0.83 | ** |
| 2 | F 322 | 11.1 | 10.2 | 10.3 | 10.53 | 107.8 | 0.76 | ** |
| 3 | Milcov | 10.5 | 11.3 | 9.7 | 10.50 | 107.5 | 0.73 | ** |
| 4 | F 540 | 11.0 | 10.3 | 9.8 | 10.36 | 106.0 | 0.59 | * |
| 5 | Paltin | 10.7 | 9.1 | 9.9 | 9.90 | 101.3 | 0.13 | |
| 6 | Crişana | 9.5 | 9.6 | 10.5 | 9.87 | 101.0 | 0.10 | |
| 7 | F 376 | 10.7 | 9.4 | 9.2 | 9.77 | 100.0 | 0.00 | |
| 8 | Rapsodia | 10.3 | 9.8 | 9.0 | 9.70 | 99.3 | -0.07 | |
| 9 | Mostistea | 10.4 | 9.1 | 8.9 | 9.47 | 96.9 | -0.30 | |
| 10 | Iezer | 10.3 | 9.1 | 8.9 | 9.43 | 96.5 | -0.34 | |
| 11 | Generos | 9.1 | 9.9 | 9.3 | 9.43 | 96.5 | -0.34 | |
| 12 | Campion | 9.4 | 9.3 | 9.5 | 9.40 | 96.2 | -0.37 | |
| 13 | Neptun | 9.6 | 9.3 | 9.1 | 9.32 | 95.5 | -0.44 | |
| 14 | Olt | 9.7 | 9.0 | 9.1 | 9.27 | 94.9 | -0.50 | |
| 15 | F 475 M | 9.6 | 9.0 | 8.8 | 9.13 | 93.4 | -0.64 | 0 |
| | **Exp. mean** | **10.2** | **9.6** | **9.5** | **9.77** | **100.0** | **0.00** | |
| | LSD 5 % | | | | | | 0.53 | |
| | 1% | | | | | | 0.70 | |
| | 0.1 % | | | | | | 0.92 | |

A new stage of improving quality protein in maize kernels is represented by the discovery of the gene dzs10 (Zarkadas, 1997; Olsen et al., 2003). The gene dzs10 induce a high methionine content. The maize kernels rich in essential amino acids are more efficient in human food and animal feed.

Worldwide, the most developed program for improvement of quality protein in maize was conducted at CIMMYT. For countries of South America and Africa, where maize is used as staple food, specialists of CIMMYT promoted maize hybrids with the endosperm modifying genes and also rich in essential amino acids.

Up to now, were isolated about 500 naturally occurring amino acids. After biochemical and physiological importance they have in human body there are essential amino acids: threonine (Thr), valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met); semi- essentials amino acids: serine, arginine, cystine, tyrosine; and non-essential amino acids:, aspartic acid, glutamic acid, glycine and alanine.

Essential amino acids are produced only in plants, animals being forced to extract them from food. Threonine is an amino acid indispensable for normal growth process and metabolism of proteins; phenylalanine is required for the memory process and normal intellectual activity; izoleucine is required for synthesis of haemoglobin. Lysine contributes to the development of tissues and synthesis of vital substances as: antibodies, hormones,

enzymes; tryptophan prevents pellagra, it is indispensable for a normal intellectual activity and it contributes in decreasing of the intensity of the depressive states. Methionine decreases the amount of blood cholesterol, protects the kidneys, it is natural chelating agent for heavy metals.

The protein and essential amino acids contents (according to the analysis performed at NIBAN-Baloteşti) of some new hybrids released at NARDI-Fundulea is presented in Table 2.

The mean protein content was 9.8 with a maximum of 10.8% for hybrid F 150/11. The average percentage of total amino acids content was 7.53 %, with a maximum of 8.41% for the hybrid F165/11. The average percentage of essential amino acids content was 2.71%, with a maxim for hybrid F 39/11 (3.04%).

The average lysine percentage was 0.26%, with a minimum of 0.22% recorded for hybrid F 100/11 and maximum of 0.29% for hybrid F 13616 A/08 (Table 2).

The natural variability of oil content in maize is 3.5-6.0% (Alexander, 1988 b). Most of the oil is contained in the embryos. An efficient method for rising the oil content is the selection of hybrids with larger embryos. A genotype is considered rich in oil if the oil percentage is above 6%.

The quality of the maize oil is very good and it contains: 0.8 to 12.7% palmitic acid; 1.3 to 4.3% of stearic acid; 20.0 to 24.4% oleic acid and linolenic acid 51-60%, it is rich in vitamin A and E, and it is cholesterol free.

Table 2. Essential amino acids and protein contents (in %) to some new hybrids released at NARDI-Fundulea. Fundulea 2013, rainfed

| Hibryd | Protein | Total amino acids | Sum of essential amino acids | Including | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Thr | Val | Phe | Isl | Leu | Lys | Met |
| | % | % | % | % | % | % | % | % | % | % |
| F 133-08 | 8.8 | 7.48 | 2.72 | 0.35 | 0.36 | 0.35 | 0.23 | 1.06 | 0.23 | 0.14 |
| F 100-11 | 8.3 | 6.91 | 2.52 | 0.32 | 0.33 | 0.32 | 0.21 | 0.98 | 0.22 | 0.14 |
| F 13616 A/08 | 10.3 | 8.26 | 2.95 | 0.47 | 0.39 | 0.39 | 0.26 | 1.00 | 0.29 | 0.15 |
| F 39-11 | 10.4 | 8.23 | 3.04 | 0.46 | 0.38 | 0.38 | 0.25 | 1.14 | 0.28 | 0.16 |
| F 67-11 | 10.3 | 7.24 | 2.62 | 0.48 | 0.31 | 0.31 | 0.24 | 0.81 | 0.27 | 0.15 |
| F 150-11 | 10.8 | 7.97 | 2.88 | 0.48 | 0.34 | 0.34 | 0.29 | 0.89 | 0.28 | 0.15 |
| F 165-11 | 10.4 | 8.41 | 3.13 | 0.51 | 0.35 | 0.35 | 0.24 | 1.24 | 0.25 | 0.17 |
| F 238-11 | 9.7 | 6.97 | 2.47 | 0.42 | 0.30 | 0.30 | 0.21 | 0.84 | 0.23 | 0.14 |
| F 31-11 | 9.7 | 6.95 | 2.43 | 0.40. | 0.31 | 0.31 | 0.21 | 0.78 | 0.27 | 0.14 |
| F 474-11 | 9.3 | 6.83 | 2.39 | 0.38 | 0.29 | 0.33 | 0.21 | 0.80 | 0.24 | 0.14 |
| ***Mean*** | ***9.8*** | ***7.53*** | ***2.71*** | ***0.43*** | ***0.36*** | ***0.36*** | ***0.24*** | ***0.96*** | ***0.26*** | ***0.15*** |

In Romania, the food industry cover the demand for maize oil from the embryos resulted from the de-germination process of maize kernels used for producing maize flour meal, flaked maize and starchy products.

Although NARDI-Fundulea hasn't a special maize breeding program to improve oil content, the corn hybrids registered of our institute have a high oil content (4.53% on average) (Table 3). Hybrids F 376 (5%) and Mostiştea (4.93%)

significantly exceeded the average of the studied hybrids.

Starch is the most important component of maize endosperm. The starch content of the endosperm can be up to 80-90%.

Worldwide, more than 75% of the starch is extracted from corn, in USA is percentage rise up to 95% (Wurzberg, 1986). High starch content of corn grain made possible the development of a specific industry around it. Development of starch industry has led to specialization in maize breeding: for starch with high amylose content it is used the mutant *ae* (White, 1994) and for amylopectin-rich starch it is used the mutant *wx* (Fergason, 1994).

Table 3. Oil content (%) for some registred maize hybrids created at NARDI –Fundulea

Fundulea 2011-2013, rainfed

| HIBRYD | OIL (%) | | | MEAN | % from general mean | Diff. | Signifficance |
|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2013 | | | | |
| F 376 | 5.5 | 4.6 | 4.9 | 5.00 | 110.4 | 0.50 | ** |
| Mostiştea | 5.2 | 4.7 | 4.9 | 4.93 | 108.8 | 0.40 | * |
| Campion | 5.3 | 4.5 | 4.7 | 4.83 | 106.7 | 0.30 | |
| Iezer | 4.6 | 4.5 | 5.0 | 4.70 | 103.8 | 0.17 | |
| Crişana | 4.8 | 4.4 | 4.9 | 4.70 | 103.8 | 0.17 | |
| Paltin | 4.7 | 4.6 | 4.4 | 4.57 | 100.9 | 0.04 | |
| F 540 | 4.4 | 4.6 | 4.7 | 4.57 | 100.9 | 0.04 | |
| Milcov | 4.5 | 4.6 | 4.6 | 4.57 | 100.9 | 0.04 | |
| Olt | 4.4 | 4.3 | 4.6 | 4.43 | 97.8 | -0.10 | |
| Rapsodia | 4.4 | 4.2 | 4.7 | 4.43 | 97.8 | -0.10 | |
| Olimpius | 4.2 | 4.4 | 4.6 | 4.40 | 97.1 | -0.13 | |
| F 322 | 4.2 | 4.2 | 4.4 | 4.27 | 94.5 | -0.26 | |
| F 475 M | 4.4 | 3.8 | 4.5 | 4.23 | 93.3 | -0.30 | |
| Generos | 4.1 | 3.9 | 4.3 | 4.10 | 90.5 | -0.43 | 0 |
| Neptun | 3.9 | 4.1 | 4.3 | 4.10 | 90.5 | -0.43 | 0 |
| ***Media*** | ***4.6*** | ***4.4*** | ***4.6*** | ***4.53*** | ***100.0*** | ***0*** | |
| DL 5% | | | | | | 0.35 | |
| DL 1% | | | | | | 0.46 | |
| DL 0.1% | | | | | | 0.61 | |

The corn hybrids rich in amylose have a specific market because of high amylose starch demand from textile industry and fabrication of waterproof cellophane films required by the packaging industry.

Rich corn amylopectin was discovered in a population of corn in China, the endosperm of this population is dull with a waxy aspect - called for this reason *waxy*. Corn rich in amylopectin is used in dairies and cheese, paper and textile industries.

In Romania, there are particular concern for improving the quality of starch at ARDS Turda (Haş et al., 2004).

The *amylacea* variety is used as a genetic source for raising the starch content in maize hybrids.

The hybrids created and tested at NARDI Fundulea in 2011-2013 had an average of starch content of 71.33% (Table 4). A higher percentage of starch 72.2% was recorded in 2012, a very dry year, which shows that under water and heat stress, the starch anabolism is

less affected than anabolism of proteins and fats.

Although there are no statistically significant differences among hybrids in terms of starch content, however, one may remark hybrids F 475 M (72, 63%) and Generos (72.1%).

Table 4. Starch content (%) for some corn hybrids released at NARDI -Fundulea

Fundulea 2011-2013, rainfed

| HYBRID | STARCH (%) | | | MEAN | % | Diff. | Significance |
|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2013 | | | | |
| F 475 M | 72.1 | 73.5 | 72.3 | 72.63 | 103.3 | 1.30 | |
| Generos | 72.1 | 72.9 | 71.3 | 72.10 | 101.1 | 0.87 | |
| Rapsodia | 71.9 | 72.3 | 70.8 | 71.66 | 100.5 | 0.33 | |
| Olt | 71.1 | 72.1 | 71.5 | 71.56 | 100.3 | 0.23 | |
| Iezer | 71.4 | 72.5 | 70.7 | 71.53 | 100.3 | 0.20 | |
| F 376 | 71.1 | 72.0 | 71.0 | 71.36 | 100.0 | 0.00 | |
| Crişana | 70.6 | 72.8 | 70.5 | 71.33 | 100.0 | 0.00 | |
| F 322 | 70.4 | 72.4 | 71.0 | 71.33 | 100.0 | 0.00 | |
| Mostistea | 70.0 | 72.8 | 70.9 | 71.23 | 99.8 | -0.10 | |
| Olimpius | 70.2 | 71.8 | 71.2 | 71.10 | 99.7 | -0.23 | |
| Paltin | 70.0 | 71.2 | 71.7 | 71.00 | 99.5 | -0.33 | |
| F 540 | 70.5 | 71.8 | 70.8 | 71.00 | 99.5 | -0.33 | |
| Campion | 70.6 | 72.0 | 69.9 | 70.83 | 99.3 | -0.50 | |
| Neptun | 69.1 | 72.5 | 70.6 | 70.70 | 99.2 | -0.63 | |
| Milcov | 70.9 | 70.4 | 70.6 | 70.60 | 98.9 | -0.73 | |
| ***Mean*** | ***70.8*** | ***72.2*** | ***71.0*** | ***71.33*** | ***100.0*** | ***0.00*** | |
| DL 5 % | | | | | | 1.33 | |
| DL 1 % | | | | | | 1.79 | |
| DL 0.1 % | | | | | | 2.35 | |

## CONCLUSIONS

The average protein content of studied maize hybrids was 9.77% with values varying from 9.13 and 10.6%.

The average total amino acids percentage was 7.53% and that of essential amino acids was 2.71%.

The average oil content was 4.53%, with values varying from 4.10 to 5.00%

The average starch content was 71.33%.

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