RESEARCH ON TETRAPLOID MAIZE WITH *OPAQUE-2* ENDOSPERM

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Maize (*Zea mays* L.) is considered a diploid species (2x=20). In the experimental polyploid series of maize, the only forms of practical importance are tetraploids (4x = 40). The first tetraploid forms of maize were obtained by L. F. Randolph in 1932 by temperature shock. These maize cytotypes have valuable morphological, physiological and biochemical traits but show reduced fertility, slower growth, longer growing season, low productivity, which make them uncompetitive with diploids. However, scientists use them in different research programs regarding genetic variability, inbreeding and heterosis, genomic imprinting, gene expression, dosage effects etc. Also, some researchers from Russia succeeded in obtaining tetraploid maize populations on a broad genetic base, including introgression of teosinte, that have high productivity, protein content and are drought resistant.

At the State Agrarian University of Moldova are carried out experiments with the aim of using polyploidy and kernel mutant genes in improving the quality of grain proteins. In this paper we present the results on induction and study of tetraploid forms of maize which contain the *opaque-2* gene (*o2*) that determines a high content of lysine in the protein of the kernel.

The research was carried out during 2010-2014. The genetic stocks used in the experiments consisted of a single-cross maize hybrid with increased protein and lysine content Chişiniovschi 307PL with *o2* gene in the endosperm, single-cross hybrid Porumbeni 331Mrf, and the tetraploid synthetic B with normal endosperm. Tetraploidy was induced by colchicine treatment of 0,15% concentration. Newly created tetraploid forms of maize were studied on the morphological, cytological and biochemical levels. Biochemical analysis was performed by infrared spectroscopy. Protein amino acid content was determined by ion exchange chromatography on an automatic aminoacid analyser T339M.

Application of the colchicine allowed obtaining chimeric plants characterized by different degrees of morphosis. Heterogeneous pollen size served as an important criterion in selecting valuable plants. Self-pollination of these plants generated chimera ears with various classes of kernels, distinguished by size and extent of development of the endosperm. Using these criteria we selected tetraploid grains that differed by size, color and weight, which was 30% higher than diploid grains. After selecting tetraploid grains, next step was the verification of chromosomes number to avoid the cases with diploid or aneuploidy grains. Tetraploid plants obtained from selected grains were shorter than the original diploid forms, had a thicker stem, shorter internodes, fewer panicle branches with central branch thicker and longer. Stomata sizes of tetraploid forms were on average 20-25% higher than in the diploid but, less per unit of surface. Pollen from tetraploid forms was larger, but with a low degree of fertility. Tetraploid grain mass is greater than the diploid, but greatly varies.

In order to determine gene heredity in tetraploid forms we applied the hybridological method by crossing the *o2* tetraploids with the synthetic population B, which has vitreous endosperm. As a result of the analysed tetraploid cobs obtained in the second generation (F_2) by self-pollination of a duplex *O2O2o2o2* we obtained an empirical 34,33:1 segregation, which is close to the theoretical 35:1. Application of the χ^2-test generated a value $\chi^2 = 0.055$, which showed that the *o2* gene inherits at random chromosomal segregation model.

Biochemical analyzes performed on the material under study revealed some essential differences between diploid and tetraploid forms. Protein level in tetraploid *o2* grain was on average 17% higher than in the diploid mutant form Chişiniovschi 307 PL and 30% higher than in the diploid hybrid Porumbeni 331 MRf. No differences were found between *o2* tetraploids and the tetraploid synthetic B. The content of lipids in tetraploid mutant grains was lower than that of diploid mutant form, practically at the level of normal diploid grains, but no essential differences were noticed as regard to cellulose content. The analysis of amino acids content in the protein of diploid and tetraploid grains containing *o2* gene, revealed that with increasing ploidy level there was a tendency to increase the content of aspartic acid, threonine, serine, glutamic acid, tyrosine, alanine, reducing content of proline, glycine, valine, cysteine, leucine, isoleucine, histidine, arginine. Experiments with different doses of *o2* gene in grain endosperm showed that in the case of six *o2* recessive alleles, the lysine content was higher than all other doses of diploid and tetraploid levels.