

USE OF BIOFORTIFICATION PRINCIPLES IN DEVELOPING A MAIZE GERMPLASM COLLECTION

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Abstract: *This study explores the application of biofortification principles in developing a maize genetic collection with specific germplasm aimed at improving nutritional traits. The main objective was to evaluate and classify 174 maize genotypes based on their genetic potential for enhanced nutrient content using traditional and modern breeding tools. The research was conducted at the Laboratory of Applied Genetics and Biotechnology, Technical University of Moldova. A comprehensive methodological framework was employed, including classical selection, induced mutagenesis, polyploidization, haploidization, and molecular diagnostics. The results revealed a significant proportion of genotypes carrying key endosperm mutations such as *o2*, *su1*, *wx1*, and *fl2*, with 31% of the collection exhibiting double recessive combinations. Additionally, 9 lines and 15 hybrids were successfully converted into tetraploids. The study confirms the potential of these genotypes as valuable breeding material for biofortification. The genetic collection developed represents a strategic resource for enhancing the nutritional quality of maize and contributes to advancing sustainable agriculture and food security.*

Keywords: *biofortification, endosperm mutations, genetic diversity, maize, polyploidy.*

INTRODUCTION

In the last decade, a new research direction has emerged in modern agricultural biology — biofortification. Biofortification is considered a set of scientific methods and technologies aimed at improving the nutritional qualities of cultivated plants using breeding methods and a range of biotechnologies [1,2]. Today, biofortification is recognized as part of the sustainable development strategy (contributing to the UN Sustainable Development Goals – SDGs) [3]. The leading research strategy in biofortification is characterized by an interdisciplinary approach, combining modern breeding-genetic and physiological-biochemical methods used to intentionally modify and/or enhance the nutritional qualities of key agricultural crops at various levels [4,5]. In this context, there is a strong link,

primarily, between biofortification and genetics, which can be clearly substantiated by the following points:

1. The foundation of biofortification lies in identifying and utilizing plant genotypes that naturally contain higher levels of desirable nutrients and critical micronutrients. This requires extensive research into genetic diversity within and between related species [4,6]. Advanced genetic methodologies, in combination with principles of metabolic engineering and bioinformatics, play a crucial role in identifying such "wild" or traditional genotypes [7].

2. Traditional breeding methods are fully applicable to solving many biofortification problems. For example, by crossing and selecting plants with desirable traits (e.g., in maize — high iron or provitamin A content), breeders can develop new varieties with improved nutritional value [1,7]. This process is based on the genetic laws of inheritance. However, it can be lengthy and may not always guarantee the desired result, as selection for one trait can affect other genes (pleiotropy and genetic correlations).

3. At the modern level, solutions to biofortification challenges involve targeted genome modification (introducing genes from other organisms responsible for synthesizing or accumulating specific nutrients into plant genomes) or genome editing (introducing precise DNA changes through insertion, deletion, or relocation of fragments using specifically engineered endonucleases) [2].

4. The acceleration of the breeding process aimed at achieving the main goals of biofortification can also be successfully implemented through marker-assisted selection (MAS). Thanks to advances in gene and QTL mapping, molecular markers can be used for the rapid and efficient selection of plants with desired biofortification-related genes [7].

In recent years, maize biofortification research has been actively developed at the Laboratory of Applied Genetics and Biotechnology, Technical University of Moldova — the successor of the former Department of Plant Breeding and Genetics led by Academician Andrei Pali. Building on national and international achievements in maize biochemical breeding, this work forms a foundation for advancing biofortification in Moldova [8–11]. While biofortification requires a comprehensive approach integrating agronomic and breeding considerations, this study focuses specifically on applying its principles to develop a maize germplasm collection with targeted genetic traits.

MATERIALS AND METHODS

The subject of this study was an active maize germplasm collection comprising 174 genotypes, developed at the Laboratory of Applied Genetics and Biotechnology, Faculty of Agricultural, Forestry, and Environmental Sciences, Technical University of Moldova. The selection of optimal biofortification methods

followed a systematic approach, taking into account the nature of the targeted traits, technological feasibility, as well as temporal and economic constraints.

Priority has been given to plant breeding methods closely aligned with biofortification objectives, including traditional Mendelian selection, spontaneous and induced mutagenesis, polyploidization, haploid techniques, heterosis breeding, molecular diagnostics based on storage protein profiles, and biochemical characterization of the maize genotypes.

RESULTS AND DISCUSSIONS

Maize genotypes in the specific germplasm collection were classified according to the following criteria:

A. *Donor-originator centers*

B. *Taxonomic characteristics (subspecies)*

C. *Endosperm structure genes*

D. *Ploidy level.*

A. The maize genotype collection was developed using materials from the following breeding centers: Republic of Moldova (National Seed Research and Production Center; successor of the State Agrarian University of Moldova — Faculty of Agricultural, Forestry, and Environmental Sciences, Technical University of Moldova); Ukraine (Yuriev Institute of Plant Production, Kharkiv); Russia (Krasnodar Agricultural Research Institute named after P. P. Lukyanenko, now part of the National Grain Center); Croatia (EU Seeds Gallery — International online seed store [12]). Selection and systematization principles of the materials from these originators are presented in sections B, C, and D.

B. In addition to the maize types traditionally used in Moldovan breeding and adapted to local agroclimatic conditions — including flint, dent, sweet, starch, waxy, popping, and pod corn — the collection also includes several subtropical forms. These forms, visually characterized by Figure 1, carry genetic traits associated with enhanced carbohydrate metabolism, photoperiod sensitivity typical of subtropical maize, lodging resistance, and anthocyanin pigmentation. The latter is particularly relevant for classical genetic studies using morphological markers.

C. A clear indicator of the targeted development of foundational breeding material with specific maize germplasm—serving as a basis for experimental biofortification efforts in Moldova—is that 118 out of the 174 genotypes in the collection are mutant lines with known endosperm structure genes. Figure 2 illustrates a pie chart summarizing the distribution of these mutations.

The chart is divided into two major categories: monogenic mutants (69%) and double recessive mutants (31%). Among the monogenic forms, the genotypic distribution is as follows: *o2* — 48%, *su1* — 22%, *wx1* — 18.5%, *fl2* — 9%, *su2* — 2.5%.

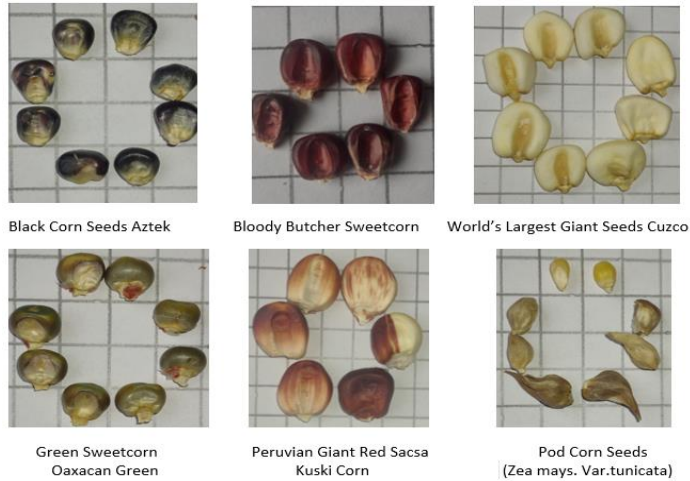


Figure 1. Visual phenotype of subtropical maize forms included in the working genetic collection with specific germplasm (on a 10mm grid).



Figure 2. Pie chart illustrating the percentage of maize mutants by endosperm structure genes in the working genetic collection.

Within the double recessive category, the combinations are distributed as: *wx1y1* — 68%, *o2wx1* — 11%, *o2su2* — 8%, *o2sh2* — 5%.

Additionally, 8% of the genotypes possess the *R1-nj* anthocyanin pigmentation marker, used for the identification of in vivo induced haploids in maize. This supports the dual focus on nutritional enhancement and doubled haploid technology in the breeding program.

D. The development of the maize germplasm collection also provided the opportunity to expand into polyploid breeding. As a result, nine genotypes—comprising both normal lines and mutant forms—were successfully converted into tetraploids. As illustrated in Figure 3A, 56% of these tetraploid lines carry the *o2* gene, 11% carry *fl2*, while the remaining 33% represent tetraploid analogs of standard diploid lines. In addition to these, eighteen Moldovan maize hybrids from the FAO 100–400 maturity groups were also converted into tetraploid forms. These

hybrids served as source material for the development of fifteen new tetraploid populations. According to Figure 3B, 87% of these populations carry the *o2* gene, 7% carry *fl2*, and 6% combine the *su1o2* double recessive mutations.

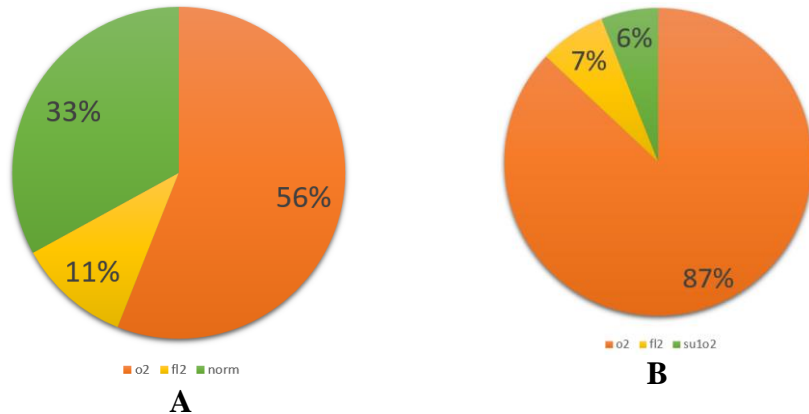


Figure 3. Sector charts showing: A – percentages of normal and mutant lines converted to tetraploids; B – tetraploid populations developed from hybrid combinations

E. The maize germplasm collection has been approached innovatively through several integrated research directions. Over 170 genotypes derived from various hybrids have undergone dihaploid induction to accelerate trait fixation and genetic uniformity. Comparative analyses have been conducted between classical heterotic groups—such as BSSS, Iodent, Lacaune, and Lancaster—and new heterotic patterns emerging from specific germplasm tailored to biofortification goals. The overarching focus remains on enhancing biochemical trait expression, supported by advanced diagnostic methods — biochemical, physiological, and molecular — employed within the Laboratory of Applied Genetics and Biotechnology at the Technical University of Moldova.

CONCLUSIONS

It has been confirmed that the active maize germplasm collection is strategically oriented toward the effective application of biofortification principles, contributing to sustainable agriculture and food security. A detailed analysis reveals that most genotypes—developed through spontaneous mutagenesis, polyploidization, and haploidization—contain specific germplasm and constitute a valuable genetic resource. This collection supports both the development of theoretical frameworks for advanced biofortification diagnostics and the practical implementation of complex breeding strategies, offering a solid foundation for addressing current challenges in maize biofortification.

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