

**IMPLEMENTATION OF MAIZE GENETIC RESOURCES IN DROUGHT
TOLERANCE AND GRAIN QUALITY IMPROVEMENT AT MAIZE RESEARCH
INSTITUTE "ZEMUN POLJE".**

Violeta ANDJELKOVIC*, Dragana IGNJATOVIC – MICIC, Snezana MLADENOVIC
DRINIC, Jelena VANCETOVIC¹

¹Maize Research Institute „Zemun Polje“, Belgrade, Serbia
(Corresponding author: avioleta@mrizp.rs)

Abstract

The world population is expected to reach eight billion by the year 2020 and food grain production will have to be doubled from the current level of about five billion tons per year. With global climatic changes drought has become the most important limiting factor for crop production worldwide. It has been estimated that approximately US \$10 billion of primary food production is lost annually because of insufficient rainfall or lack of rain. The necessity to obtain global food security increased importance of plant genetic resources for agriculture. The treasure of genetic material contained in landraces, crop wild relatives and other wild species provides plant breeders with options to develop more productive crops, adapted to changing environments. Prebreeding activities include introduction, evaluation and improvement of germplasm resources for use in conventional breeding. Conservation and exploitation of maize accessions are the most important tasks. Maize Research Institute genebank with more than 6000 accessions is among the ten largest collections in the world. Testing these accessions under controlled drought stress and in temperate climate resulted in core collection formation. The chosen accessions were further tested for grain quality (protein, oil and starch content), as well as for the presence of *opaque2* mutation and tryptophan content which are the basis for developing quality protein maize. Selected genotypes could be used for creation of new hybrids with higher yielding potential, quality and adaptability.

Key words: *drought, food quality, genebank, prebreeding, Zea mays.*

Introduction

Plant diversity, kept in genebanks worldwide, provides valuable sources needed for future challenges, such as climatic changing, occurrence of new diseases or increased food demands (Araus et al., 2008). Considering that global food security depends on few major crops (rice, wheat and maize) conservation of their diversity is one of the main tasks in agriculture nowadays. Although a huge number of maize accessions are stored within gene banks worldwide there is still a lack of diversity in the conventional breeding programs (FAOSTAT, 2010). Usually the size of germplasm collections limits their utilization in plant breeding research. Identification of genotypes with favorable traits is very hard, expensive and long term process and breeders are rather focused on their already adapted working collections (Marshall, 1989).

Maize is the third most important crop for mankind and very likely that by 2025 it will become the crop with the greatest production globally (Rosegrant et al., 2008), due to growing demand for dairy and meat products in developing countries, as well as, for animal feed and industrial raw material in developed countries. Maize grain is complete in macro (starch, oil and proteins) and micronutrients (carotenoids, tocopherols, minerals, phytic acid,

anthocyanins etc.) compared to other cereals. However, chemical composition, biological and nutritional value of maize grain is depending on both, genetic and environmental factors. Characterization of genetic diversity of maize landraces aids more efficiently exploring the allelic variation for genetic improvement of economically desirable traits as grain quality traits. In this context, the landraces 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.

The single most important abiotic factor limiting maize production is drought. At the same time, increasing human population requires increase in food production. Therefore, there is a great need for maize genotypes that yield better and more stable in both optimal and drought environments. However, selection of genotypes with tolerance to drought in the field and stable yield is very difficult to obtain due to fluctuation of agroecological and climatic conditions (Frova et al., 1999).

In Serbia, maize is the most important crop, grown at more than one million hectares annually. Drought occurs in two to three years out of five, resulting in large annual yield fluctuations. Optimal rainfalls for maize growth range from 500 to 700mm, but in Serbia the precipitation sum during the growing season varied from 278.9mm in 2011 to 458.1mm in 2010. Breeding maize hybrids which have drought tolerance is one of the possible approaches to solving this problem. However, a longer period of breeding hybrids and lower efficiency for incorporation of polygenic traits, such as drought, is making this task very difficult.

A project on identification of new sources of drought tolerance among more than 6 000 genebank accessions at Maize Research Institute "Zemun Polje" has been initiated in 2007. Creation of core collection for drought tolerance was the aim of the project and the results of further testing of grain quality within chosen genotypes are presented in this paper.

Material and methods

All accessions from Maize Research Institute gene bank collection (about 6000) were screened under controlled water stress in Egypt, at Sids Agricultural Research Station (150 km south of Cairo). A total of 672 accessions (10.5%) were selected on the basis of ASI, bareness, seed set and grain filing for further experiments (Brown, 1989). Chosen genotypes were tested during the following season in Egypt, as well as, in two locations in temperate zones (Zemun Polje and Macedonia); 41 (13 landraces, 13 introduced populations and 15 inbred lines) genotypes were selected on the basis of previously observed traits, as well as, for ear, cob and kernel weight (Babić et al., 2011). The seeds of 41 accessions were multiplied and grain quality (protein, starch and oil content) was analysed by Near Infrared Spectroscopy (Infratec 1241 Grain Analyser, Foss Tecator, Sweden).

The 13 landraces were also surveyed for the possible presence of *opaque2* mutation through kernel modification assessment, content of the essential amino-acid tryptophan, quality index of the protein and SSR analysis. Kernel modification was visually assessed using light table, according to the scoring scale from 1 (completely translucent, with no opaqueness) to 5 (completely opaque). Modification score 2 is given to the kernel which is 25% opaque, while scores 3 and 4 are given to 50% and 75% opaque kernels, respectively.

For biochemical analyses each genotype was presented with 30 randomly chosen kernels. Tryptophan content was determined using the colorimetric method of Nurit et al (2009), while the protein content was determined by the standard Kjeldahl method based on nitrogen determination as explained in Vivek et al. (2008). The protein was estimated from the nitrogen value as % protein = % nitrogen x 6.25 (conversion factor for maize). Quality index (QI), defined as tryptophan to protein ratio in the sample was calculated as $QI = 100 \times \text{tryptophan content in the sample} / \text{protein content in the sample}$.

SSR analysis was done with the phi057 primer specific for the *opaque2* gene. The amplification reaction was carried out in 20µl reaction volume containing 1x enzyme buffer, 3mM MgCl₂, 200µM dNTPs, 0.25µM primers, 1.25U *Taq* polymerase and 50ng of DNA. The amplification profiles followed were: an initial denaturation at 94°C/2min, followed by 40 cycles each of denaturation at 94°C/1min, annealing at 60°C/2min and extension at 72°C/2min, with final extension at 72°C/10min. Amplified fragments were first separated on 4% agarose gels in TBE buffer. After electrophoresis gels were stained with ethidium bromide and photographed.

Results and discussion

Pre-breeding is the best link between genetic resources and breeding programmes and encompasses the identification of desirable traits and/or genes from the non-adapted, as well as, from the adapted material, that will be under certain selection pressure (Nass and Paterniani, 2000). A result is formation of new populations for breeding purposes. The aim of pre-breeding activities is the establishment of core collections that represent genetic diversity with the minimum repetitiveness. The core usually encompasses approximately 10% of the collection what makes almost 70% of its genetic variability (Brown, 1989).

Assigning of maize genotypes into heterotic groups has been the key to the economic success of the crop because it allows the exploitation of heterosis, particularly for grain yield (Reif et al., 2005). Heterotic groups are crucial for the utilisation of germplasm for developing hybrids with better performances (Eberhart et al., 1995). Beside drought tolerance, the information about combining ability and classification into heterotic groups was necessary for further utilization of the core collection (Table 1). Based on good general combining ability, 41 genotypes were further selected and among them, six accessions produced superior crosses with three lines representing different heterotic groups (Andjelkovic and Ignjatovic-Micic, 2012).

Table 1. Combining ability and the genetic relatedness with observed heterotic sources

Origin	Number of accessions	Good combining ability
BSSS	1	Lancaster, independent
Lancaster	10	BSSS, independent
Lancaster, BSSS	7	independent
unknown	6	BSSS, Lancaster, independent
independent	6	BSSS, Lancaster
independent, BSSS	2	Lancaster
independent, Lancaster	9	BSSS
inapplicable	10	None

Maize landraces, due to their high genetic variability, are an important source for breeding programmes aimed at improving nutritional and health traits of maize grains (Uarrota et al., 2011). Unfortunately, studies on the chemical composition of maize accession are still scarce in literature. Typical kernel composition values for the yellow dent maize on a dry matter basis are 71,7% starch, 9,5% protein, 4,3% oil, 1,4% ash, and 2,6% sugar (Watson, 2003). The results in this experiment differ from these values, but there was no significant difference between the three groups (Table 2). The average values of starch content were somewhat lower and oil values in the range compared to the commodity yellow dent maize.

However, protein content was higher in all the genotypes analyzed, meaning that these genotypes could be a good source of proteins in quality breeding programs.

Table 2. Macronutrient composition of the analyzed accessions

Macronutrient Content (%)	Inbred lines (mean)	Introduced populations (mean)	Landraces (mean)
Oil	4,25	4,66	4,30
Protein	12,98	12,18	11,86
Starch	68,30	69,26	68,71

The presence of *opaque2* specific allele was found in 11 genotypes (Illustration 1). In one genotype it was absent (genotype 7) and in another (genotype 6) the allele pattern was unspecific. Genotype six will be further analyzed on individual samples and other specific *opaque2* primers.

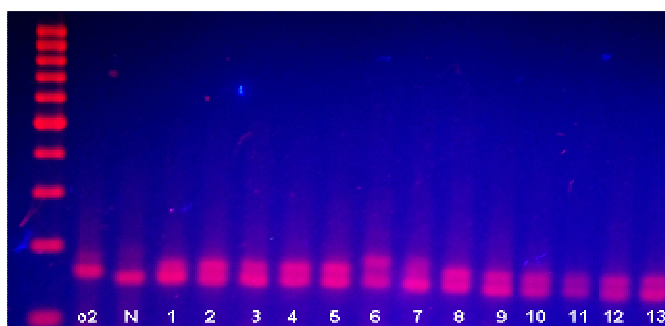


Illustration 1. Electrophoregram of SSR analysis with phi057 (maize landraces). o2 –positive control for *opaque2* allele, N – negative control for *opaque2* allele, 1 to 13 – analyzed landraces.

The results of kernel modifications, tryptophan content and QI are given in Table 3. All the values were compared with threshold limits for quality protein maize given in Vivek et al. (2008). High tryptophan content (over 0.075) was found in nine genotypes. Genotype 7 which lacked the *opaque2* allele had low tryptophan content (0.065). QI was generally low and its improvement could be the main task in using these genotypes as a source of high quality protein. Three genotypes were singled out (genotypes 2, 5 and 9) as potentially outstanding sources of protein quality. They were with good kernel modifications and high tryptophan content, indicating the presence of good modifier genes necessary for developing quality protein genotypes with good agronomic performances (Krivanek et al., 2007).

Table 3. Kernel modifications, tryptophan content, protein content and quality index (QI) of the analyzed landraces

Accession	Kernel modifications (%)		Tryptophan content (%)	Protein content (%)	QI
	Good (type 1 and 2)	Bad (type 3, 4 and 5)			
1	87	92	0.072	13.55	0.53
2	169	92	0.077	13.52	0.57
3	244	76	0.079	13.57	0.58
4	632	70	0.081	11.79	0.69
5	1513	86	0.080	12.51	0.64
6	1720	84	0.073	12.20	0.60
7	1855	95	0.065	14.20	0.46
8	2005	74	0.086	13.33	0.65
9	2013	92	0.081	11.66	0.69
10	2217	74	0.081	12.86	0.63

11	2236	84	16	0.072	11.16	0.65
12	1961	76	24	0.082	11.90	0.69
13	2033	78	22	0.081	12.11	0.67

Conclusion

The chosen accessions could broaden the genetic base of the present breeding material and most probably new elite inbred lines with increased drought tolerance and/or protein quality could be developed. The core collection may represent the base for new generations of maize ZP hybrids of higher yielding potential, grain quality and adaptability.

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