

UDC 575: 633.15

Original scientific paper

BREEDING OF MAIZE TYPES WITH SPECIFIC TRAITS AT THE MAIZE RESEARCH INSTITUTE, ZEMUN POLJE

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Pajić Z. (2007): *Breeding of maize types with specific traits at the Maize Research Institute, Zemun polje*. – Genetika, Vol. 39, No. 2, 169 - 180.

Maize is primarily grown as an energy crop, but the use of different specific versions, such as high-oil maize, high-lysine maize, waxy maize, white-seeded maize, popping maize and sweet maize, is quite extensive.

Speciality maize, due to its traits and genetic control of these traits, requires a particular attention in handling breeding material during the processes of breeding. It is especially related to prevention of uncontrolled pollination. In order to provide successful selection for a certain trait, the following specific procedures in evaluation of the trait are necessary: the estimation of a popping volume and flake quality in popping maize; the determination of sugars and harvest maturity in sweet maize; the determination of oil in selected samples of high-oil maize types, and so forth.

Breeding programmes for speciality maize, except high-amylose maize, have been implemented at the Maize Research Institute, Zemun

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Polje, Belgrade, for the last 45 years. A great number of high-yielding sweet maize hybrids, popping maize, high-oil and high-lysine, flint and white-seeded maize hybrids were developed during this 45-year period. Auspicious selection and breeding for these traits is facilitated by the abundant genetic variability and technical and technological possibilities necessary for successful selection.

Key words: Breeding maize, high-lysine maize, high-oil maize, white maize, sweet maize, popping maize.

INTRODUCTION

A great diversity of morphological, physiological and biochemical traits of maize, together with a very broad adaptability and genetic variability provide the development of types with various purposes in the process of selection. Maize is primarily grown as an energy crop, but the following different types with specific traits are also grown: high-lysine maize, high-oil maize, white-seeded maize, sweet maize and popping maize.

Due to particular traits and the mode of genetic control of these traits, maize with specific traits and purposes requires special attention in the course of breeding, seed and commercial production. It is particularly related to prevention against uncontrolled pollination. In order to provide successful selection for specific traits, certain evaluation procedures of the traits the selection is performed for are necessary, such as: the determination of the popping expansion and flake qualities in popping maize; the determination of the sugar content and other properties important for sweet maize; the determination of the oil content in the selection samples in high-oil types of maize, etc.

As a result of genetic variability of different properties of maize grain, quantity and quality of certain grain components can be significantly altered by selection. It provides, within certain limits, "designing" of new types with specific traits and for various purposes.

HIGH-LYSINE MAIZE

About 20%, i.e. 80% of the total grain proteins are found in the germ, i.e. the endosperm of the common maize, respectively. Endosperm proteins are of a low biological value, in contrast to germ proteins whose quality approaches to the ideal nutritive value. The discovery that the significantly improved amino acid content of maize grain protein had been controlled by two genes - *opaque-2* (*o2*) and *flury-2* (*fl2*) was a great contribution to the improvement of maize grain proteins (MERTZ *et al.*, 1964; NELSON *et al.*, 1965; VASAL S.K., 2001).

The introduction of the *o2* gene is the most markedly manifested in the drastic reduction of the zein fractions by about 50% and also in the simultaneous adequate increase of the relative amount of other fractions. This is the essence of the increased value of the *o2* protein type. The *opaque-2* gene, as a recessive gene, can express its effects only in the homozygous state, (DUMANOVIĆ and PAJIĆ, 1998).

The programmes on the development of *opaque2* hybrids with improved quality proteins initiated in Serbia during the mid-1960's were among the very first programmes in the world. The greatest attention in these initial programmes was paid to the conversion of elite commercial inbred lines. A certain number of successful combinations was developed with yields lagging behind the best yielding commercial hybrids by a few to 15%. The yield of one of the best hybrids - ZP 704o2 was practically competitive with the top yielding commercial common types of maize. The interest in these o2 types lessened during the second half of the 1970's. This was caused by certain limitations of the o2 types, such as: yield reduction in relation to the common type of maize; unfavourable grain phenotype; increased grain moisture at harvest; higher grain susceptibility to diseases and pests; increased risk of grain injuries during different sorts of handling, etc. (PAJIĆ *et al.*, 2000).

HIGH-OIL MAIZE

Maize is, essentially, a carbohydrate plant, and therefore it is a main source of energy in human and animal nutrition. Beside a carbohydrate component, standard grain quality maize contains about 10% of proteins and approximately 4.5% of oil. Maize grain oil is mostly accumulated in the germ (about 80%). Maize oil is considered to be one of the most qualitative plant oils for human food. High quality of maize oil, as well as, very pronounced lack of edible plant oils, resulted in the initiation of projects aimed at the oil content increase in maize grain.

High-oil maize hybrids with about 9% of oil and grain yields comparable with the yields of the elite hybrids of standard grain quality have their place in the maize production. Some advantages of high-oil hybrids are as follows: (1) high-oil hybrids are advantageous in nutrition of domestic animals; (2) high-oil hybrids are advantageous in the industrial processing due to greater amounts of oil obtained per area unit; (3) the same amounts of oil of high-oil maize per area unit as other oil plants indicate to the advantage of maize, as oil is only a by-product of maize, (MIŠOVIĆ *et al.*, 1990).

Genetic variability of the oil content in maize grain. The existence of satisfactory genetic variability is the first prerequisite for successful selection for a given trait. The information on genetic variability of the oil content in maize grain are abundant, and studies are numerous, but the experiment initiated by HOPKINS in 1896 at the University of Illinois, in Urbana, USA, is considered to be one with the widest scope. DUDLEY and LAMBERT (1992) summarised the results of that experiment after 90 generation of selection. The experiment was initiated with the white-seeded variety "Burr White". The original variety had only 4.7% of oil and 10.9% of proteins. Selection was performed in four different directions - the increase of the oil content, the decrease of the oil content, the increase of the protein content and the decrease of the protein content. The oil content after 90 cycles of selection increased from the initial 4.7% to about 20% in the IHO type. The average increase of the oil content amounted to 0.19% cycle⁻¹. After 90 cycles, genetic gain in the IHO type, expressed by the additive genetic standard deviation,

amounted to $22\sigma_A$. The value of the additive genetic standard deviation amounted to $6\sigma_A$ in the IHO group. The most important conclusion of this experiment is that genetic variability was not exhausted even after 90 cycles of selection, as well as, that multiple increase of the oil content in maize grain is possible. Changes in the grain weight and the starch content in these experiments are interesting, as they may be related to the grain yield of high-oil types.

Maize breeding for high oil content. Any maize type with an oil content not lower than 6% is a high oil type of maize. During the 1980's, a greater number of high-oil ZP maize hybrids with yields equal to or insignificantly lower than those of the best yielding commercial hybrids of standard grain quality, was developed. Table 1 presents comparative data for four versions - standard, high-oil, high-lysine and waxy - showing that yields of three speciality maize hybrids could be compared with yields of the best commercial hybrids of that time, (DUMANOVIĆ, 1995).

Table 1. Yield ha^{-1} of grain, energy, protein, useable protein and oil of a standard grain quality maize hybrid and its versions (after DUMANOVIĆ, 1995)

Hybrid	Grain (t ha^{-1})	Energy (MJ ha^{-1})	Protein (kg ha^{-1})	Useable protein (kg/ ha^{-1})	Oil (kg/ ha^{-1})
ZP 704u	10.3	19,943	913	521	864
ZP 704 _{o2}	9.5	17,707	822	537	469
ZP 704 _{wx}	10.1	19,652	925	541	451
ZP 704 St.	10.2	19,297	932	546	545

Deriving of high-oil synthetic populations. The high-oil maize breeding programme of the Maize Research Institute, Zemun Polje, encompassed not only the development of high-oil maize inbred lines and hybrids, but it also initiated a long-standing programme on high-oil populations deriving. Among other populations, a special attention was paid to the development of the population DS7u, derived by combining a certain number of the local, Canadian and US inbreds and hybrids and the population YuSSS originating from the US Iowa Stiff Stalk Synthetic BSSS(R)C5. The procedure applied in the development together with selection methods have already been described (DUMANOVIĆ, 1995). Selection was performed over nine cycles. After nine cycles of selection, the oil content increase amounted to 8.5 absolute percents or on the average to 0.94 absolute percents cycle⁻¹ in the population DS7. The corresponding values in the population YuSSS amounted to 8.18, i.e. 0.91, respectively (Figure 1). Based on progress pertaining to the oil content increase over cycles of selection, it is clear that both synthetics maintained satisfactory genetic variability relating to the oil content, and

therefore a satisfying response to selection can be expected in the subsequent cycles of selection.

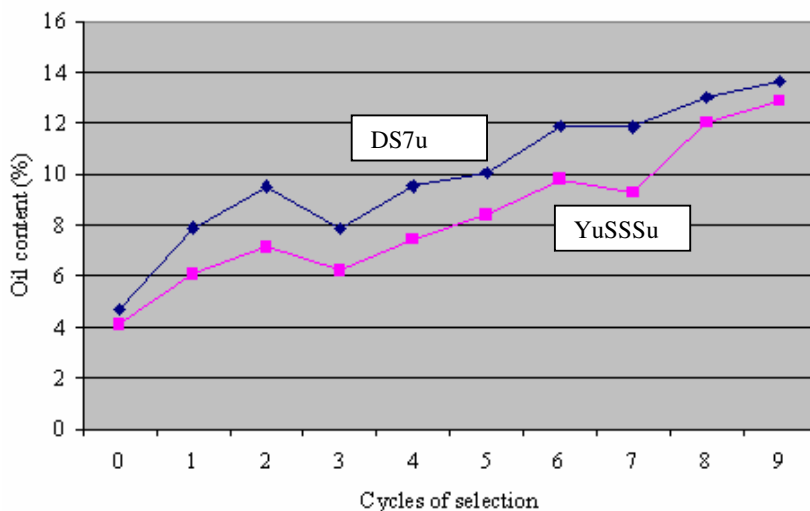


Figure 1. Increasing in oil content in DS7u and YuSSSu synthetic populations during nine cycles of selection (DUMANOVIĆ, 1995)

Effects of pollen from cross-pollinated plants on oil content. Effects of pollen from cross-pollinated plants, or xenia effects, have been intensively observed within maize breeding programmes for the high oil content. This means that a change in the oil content is affected by pollen from cross-pollinated plants in the year of pollination. The practical system, established at the Pfister Hybrid Corn Company, Illinois, USA, was designated as "top cross". The implementation of such a system requires two genetically different genotypes sown simultaneously on the same plot. An elite F₁ hybrid of common maize with incorporated male sterility, widespread in the region, is used as one of the parents (female component). The other parent (male component) is a type with a high oil percentage in the grain. The seed of both parents is mixed. The ratio of parental components amounts to 90-92% (female component) : 8-10% (male component). The yield of pollinator plants can be lower than the yield of female component plants, hence the total yield can be somewhat lower than when the whole crop is represented by the female component. According to available data, yield of such a crop is comparative with yields of the top yielding commercial hybrids, while the oil level was higher by about 75% than the oil content in common type of maize, (DUMANOVIĆ, 1995; LAMBERT, 2001).

WHITE-SEEDED MAIZE

White-seeded maize is a type of maize with the endosperm of the clear white colour without any pigments that produce the yellow colour. The white colour is controlled by a recessive gene *y*. If a dominant allele *Y* is present, instead of the recessive one, the kernel colour is yellow. Modern technological processing of white kernels requires that anthocyanin pigments producing yellow, red or blue colours must not be even traceable. The cob must be of a pure white colour.

The importance of white-seeded maize has been changing gradually. Differences over countries and various cultures are significant. Yellow-seeded maize is absolutely predominant. However, white-seeded maize is a common type of maize in large areas of Africa, South and Central America.

Hereditary character of endosperm white colour. The pure white colour of kernels on the white cob is a desirable trait for products obtained by dry milling. Such desirable vitreous kernels of a pure white colour on a white cob have been bred.

Self-pollinated varieties-populations, as sources of the breeding material, were the most important in the initial stages and back crosses in later stages of the white-seeded inbred lines development. Unlike the development of yellow-seeded maize inbred lines, back crosses had a very important role in the development of white-seeded maize inbred lines. This was the case due to a fact that many favourable properties had already been incorporated into yellow-seeded inbreds, (PONOLEIT, 2001).

Possibilities of white endosperm maize breeding. Recycling of white endosperm elite inbreds has a greater role in the maize breeding programmes. Some breeders of white-seeded maize support a broader use of yellow endosperm inbreds and their recognised heterotic pairs, which adapted for utilisation with the white breeding material, can be precious in the development of new white endosperm elite inbreds. There are not so many pronounced heterotic pairs as in case of yellow endosperm maize, (DUMANOVIĆ and PAJIĆ, 1998).

Utilisation of white endosperm maize. The utilisation of white endosperm maize has been significantly changing over the last few decades - it increased in food or some industrial products. Therefore, certain properties of kernel quality (impeccable white colour, large, uniform, high density kernel, easy pericarp-endosperm separation, white cob) have become so important that the attention paid to it during selection has equalled the attention paid to yield. Moreover, storage conditions of kernels until used are also important for quality of white-seeded maize.

SWEET MAIZE

Sweet maize is used as human food at the milk stage of the endosperm development when the kernel is soft, succulent and sweet. The highest number of commercial sweet maize hybrids is based on one or several recessive alleles that change the endosperm carbohydrate composition.

Forty years ago, a *sugary (su)* allele at the chromosome 4 was used to define sweet maize. However, the homozygous recessive *su* gene has its

limitations, and the major one is a very short period when kernels remain at high quality. The identification of endosperm additional genes causing a higher level of sugar, as well as, prolonged period of kernel peak eating quality led to utilisation of these genes (*sh2*, *fl1*, *fl2*, *ae*, *se*, etc.) in the development and improvement of sweet maize (LAUGHNAN, 1953).

The initial inbred lines were derived from open-pollinated varieties whose number was great at that time. Superior inbred lines had often been used as parental components for several hybrids, while hybrids were used for further development of new inbred lines. Such a process resulted in a narrow genetic diversity.

Genetic base of sweet maize. Fourteen among many known mutants affecting the endosperm development had been studied in order to be used in sweet maize breeding, while eight have been used in breeding practice (COE *et al.*, 1988; BOYER and SHANNON, 1984). Sweet maize is harvested 20-24 days after pollination and mutants with *bt*, *bt2*, *sh*, *sh2* and *sh4* gene contain two to three times more sucrose than the mutants with the *su* endosperm. Due to a high level of sugar, sweet maize hybrids based on these genes are named supersweets or extrasweets. At harvest, hybrids, based on these mutants, contain 4-8 times more total sugars than maize of standard grain quality (HOLDER *et al.*, 1974). Sucrose with an insignificant share of maltose, glucose and fructose, is predominant sugar in standard sweet maize grain. Sweet maize hybrids with a high sugar content in their grain have numerous advantages, but also some disadvantages. A great disadvantage is germination poorer than in *sugary* (*su*) hybrids and the seed production that is much more difficult. Hybrids with the *sh2* gene are acceptable from the point of view of their softness and consistency at the milk stage, (TRACY, 2001).

Germplasm sources and breeding methods of sweet maize. Methods to be applied in a sweet maize breeding programme depend on specific aims of such a programme and the available breeding material. Recurrent selection is broadly used in the sweet maize improvement. The back introduction of new genes into elite inbred lines of *sugary* versions, often in both parents, is the typical pattern of the initial development of varieties based on novel endosperm mutants. Although yield is always important, the meaning of "yield" varies from a market to a market - the number of ear per hectare is important for markets using ears, while, grain yield is important in tinning of cut kernels, (TRACY, 2001).

Improvement of selected populations. The population YUZP I *su* was developed from 16 early maturity inbred lines. The other population YUZP II *su*, is of a later maturity and was derived from 15 inbred lines. Each of these populations has its own internal structure, i.e. each line included into the population is a sub-population. The selection procedure is based on recurrent selection, four years cycle¹, with phenotypic selection within sub-populations (ear appearance and size, kernel appearance, colour and size), (PAJIĆ, 1990; PAJIĆ *et al.*, 1994; DUMANOVIĆ and PAJIĆ, 1998).

Hybrid development. In comparison to other types of maize, the evaluation span of sweet maize hybrids yield and quality is very limited - only 5 to 7 days. Many traits can be quickly, efficiently and subjectively evaluated. In order to estimate sugar content, tenderness and succulence of grain specific equipment is necessary. Since these analyses are expensive and lengthy they are usually performed at the last stage of the testing programme.

Sweet maize hybrids are based on one or several defect endosperm mutants, and therefore a high quality seed production is much more difficult than the seed production of other maize types. These mutants are enzymic "injuries" in the pathway of starch synthesis, which change the endosperm carbohydrate composition and result, almost in all cases, in the decreased starch level. Mature kernels are wrinkled, more angular and brittle than the kernels of standard quality maize, and are susceptible to breakage (MARSHALL, 1987; PAJIĆ and RADOSAVLJEVIĆ, 2003).

Sweet maize in Serbia. During the 1970's, sweet maize breeding and selection was initiated at the Maize Research Institute, Zemun Polje. Twenty six sweet maize hybrids of different growing periods have been released. Table 2 presents yields and the organoleptic evaluation (taste, aroma, colour, sweetness and consistency of grain) of local ZP sweet maize hybrids, (PAJIĆ *et al.*, 2005).

Table 2. Yield and organoleptic properties of released ZP sweet maize hybrids

Hybrid	Assessment of quality	
	Ear yield(t ha ⁻¹)	Total points (100%)
ZPSC 213 su	11,5	96
ZPSC 301 su	13,0	77
ZPSC 451 su	11,7	84
ZPSC 501 su	11,9	91
ZPSC 504 su	10,8	96
ZPSC 321 su	12,1	81
ZPSC 311 su	10,9	85
ZPSC 401 su	12,8	78
ZPSC 231 su	12,2	84
ZPSC 111 su	9,7	76
ZPSC 324 su	13,1	79
ZPSC 425 su	13,6	78
ZPSC 531 su	17,4	89
ZPSC 391 su	13,1	91
ZPSC 424 su	16,9	95
ZPSC 411 su	17,1	94

*Source: Commission for the Variety Releasing, Serbia

POPPING MAIZE

Popping maize is a special type of flints. A major trait that distinguishes popping maize from other types of maize is a formation of a large flake after kernel popping as a response to the heat treatment.

The popping maize breeding programme was initiated in Serbia by the Maize Research Institute, Zemun Polje, during the 1960's (MIŠOVIĆ *et al.*, 1990). The hybrids ZPTC 610k and ZPSC 641k were the first hybrids developed in Serbia and introduced into the production. They were released in 1983.

Kernel structure of popping maize. Popping maize differs from standard grain quality maize in the kernel shape, size and structure. There are two types: pearl-shaped and rice-shaped popping maize kernels. Similarly to other cereals the kernel of popping maize contains three major structural parts: pericarp, germ and endosperm. The pericarp, a firm protective coat enclosing the kernel, directly takes part in the popping action. Furthermore, the pericarp is an important factor for popping maize quality. The kernel endosperm is composed of hard (corneous) and soft (floury) endosperm. Corneous or hard endosperm consists of compact polygonal starch granules, without intergranular spaces. Popping maize kernels are largely composed of hard endosperm, (HOSENEY *et al.*, 1983).

The popping mechanism in popping maize kernels. During the heat treatment of popping maize kernels the pericarp behaves as a vessel under a pressure. Popping takes place at an inner kernel temperature of about 177°C. The overheated water in the kernel transforms into steam and provides the force that expands the grain and breaks the pericarp. The studies show that there are two parameters, pericarp damage and a high percent of soft endosperm, with extremely adverse effects on popping volume. The optimum grain moisture content at popping varies over genotypes, but usually ranges from 12% to 15%, (HOSENEY *et al.*, 1983). The grain of popping maize has to be dried carefully in order to prevent injuries in the pericarp and cracks in the endosperm, (PAJIĆ *et al.*, 2006).

Popping maize breeding. The source germplasm in popping maize breeding programmes is not significantly genetically divergent and its agronomic traits are not so good, as in germplasm of common maize (ZIEGLER, 2001). Progress in the improvement of agronomic traits of popping maize is slower than in standard grain quality maize.

The popping volume is the most important parameter of quality and principal trait distinguishing popping maize from other types of maize. It is controlled by four or five dominant genes (quantitative trait), and a greater number of recessive genes, which contribute to other properties, such as flake shape and tenderness and pericarp dispersion at popping (ASHMAN, 1983). The standard industrial measure for the determination of the popping volume is $\text{cm}^3 \text{g}^{-1}$. The metric weight volume tester (MWVT) is the industrial instrument with which this property is measured.

Traits of local popping maize hybrids. Popping maize hybrids developed at the Maize Research Institute, Zemun Polje, Belgrade, do not lag in neither yield nor the popping volume behind the best foreign popping maize hybrids. All local hybrids

belong to the yellow pearl-shaped kernel popping maize type. Table 3 presents yields and popping volumes of local ZP popping maize hybrids.

Table 3. Yield and popping volume of released ZP popping maize hybrids

Hybrid	Yield (t ha ⁻¹)	Popping volume (cc g ⁻¹)
ZPTC 610 k	6.9	38
ZPSC 611 k	6.4	42
ZPSC 601 k	4.9	43
ZPTC 501 k	5.3	37
ZPSC 622 k	5.2	43
ZPTC 615 k	4.9	39
ZPTC 619 k	5.2	42
ZPTC 621 k	5.1	40
ZPSC 622 k	5.8	41
ZPSC 614 k	5.2	41
ZPSC 616 k	5.4	42

*(Source: Commission for the Variety Releasing, Serbia)

Received June 22nd, 2007

Accepted August 17th, 2007

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OPLEMENJIVANJE SPECIFIČNIH TIPOVA KUKURUZA U INSTITUTU ZA KUKURUZ „ZEMUN POLJE”

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Izvod

Kukuruz se gaji prvenstveno kao energetski usev, ali se dosta široko koriste i različiti specifični tipovi poznati kao: visokouljani, visokolizinski, šećerci, kokičari i kukuruz belog zrna.

Imajući u vidu zadovoljavajuću genetičku varijabilnost različitih svojstava zrna kukuruza, kompozicija zrna se selekcijom može značajno menjati u pogledu količine i kvaliteta pojedinih sastavnih delova. To omogućava da se u određenim granicama "dizajniraju" novi tipovi specifičnih svojstava potrebnih za određena namene.

Specifični tipovi kukuruza, zbog određenih osobina i načina genetičke kontrole tih osobina, zahtevaju posebnu pažnju tokom procesa oplemenjivanja. To se posebno odnosi na sprečavanje nekontrolisanog oprašivanja. Da bi se obezbedio uspeh u selekciji na određena specifična svojstva potrebni su i određeni postupci radi ocenjivanja tih svojstava na koja se vrši oplemenjivanje, kao npr.: određivanje stepena kokičavosti i kvaliteta kokica kod kokičara; određivanje sadržaja šećera i dužine intervala za berbu šećerca; određivanje sadržaja ulja u selekcionim uzorcima kod visokouljanih tipova, i slično.

Program oplemenjivanja kukuruza specifičnih svojstava uspostavljen je u Institutu za kukuruz "Zemun Polje"- Beograd, pre 45 godina. U toku 45-godišnjeg perioda realizovan je veći broj visokoprinosnih i visokokvalitetnih hibrida kukuruza za specijalne namene: visokolizinskih (o2) -5; visokouljanih (u) -8; belih (b) -7; šećeraca (su) -28 i hibrida kukuruza kokičara (k) -11.

Primljeno 22. VI 2007.
Odobreno 17. VIII 2007.