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ROLE OF GENETIC RESOURCES IN DIVERSITY INCREASMENT OF COMMERCIAL MAIZE HYBRIDS

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Abstract

Although breeders have almost unlimited access to diversity of maize germplasm, economic aspects and competition among seed companies impose a broad use of one or a small number of the first-ranked hybrids. Monitoring the genetic diversity available to farmers is important, because plant breeding practices, the registration procedures, and the marketing of new varieties can cause potential genetic erosion and consequently a potential increased genetic vulnerability of cultivated varieties. It is estimated that approximately 3000 inbreds in the hybrid combinations are annually tested at the Maize Research Institute (MRI), Zemun Polje. Nevertheless, each year, only one to two hybrids enter the commercial production.

The assessment of the genetic diversity that exists in the available germplasm is fundamental in the improvement of agricultural plants. Within the MRI gene bank collection a special attention is paid to former Yugoslav landraces (2217), which could be a source of early maturity, bigger ear length, a greater number of kernel rows, larger kernel length and greater kernel weight. Moreover, they can be a source of natural resistance to diseases, pests and abiotic stress or a source of different specific traits. Recently, studies carried out at the Maize Research Institute, Zemun Polje, have been done on the following properties of collected germplasm: tolerance to drought, tolerance to herbicides, new sources of cytoplasmic male sterility (CMS) and new sources for increasing the content of available phosphorus in maize kernels.

Keywords: *genetic erosion, landraces, maize hybrids, new variability*

Introduction

Maize, as a cultivated crop, unlike wheat or rice has no obvious wild relative. Therefore, the development of maize, as a cultivated species, has been a mystery for such a long time. Today, one annual Mexican teosinte is considered the direct ancestor of today's maize. In the process of domestication, the artificial selection through the rapid phenotypic transformation of wild grass teosinte (*Zea mays* ssp. *parviglumis*) has led to the development of the modern maize plant (*Zea mays* ssp. *mays*). Although, modern maize breeders have achieved enormous yield increase in the process of the development of hybrid species, the maize development by Indians remains the greatest breeding accomplishment (Beadle 1980). In the period of several millennia prior to the arrival of Colombo, natives used to cultivate all more important forms of maize. These forms exist even today. The process of domestication itself has resulted in a certain loss of maize diversity. Based on nucleotide polymorphism this loss has been estimated to approximately 30% (Goloubinoff et al., 1993; Hilton and Gaut, 1998). Regardless of a certain loss of diversity, maize is a species that is characterised with tremendous morphological and biological diversities. Maize is distributed from 58^{o} north latitude through moderate, subtropical and tropical regions down to 40^{o} south latitude. In the region of the Andes it grows up to 1016 m above sea (Grant et al. 1963). From the aspect of the conservation of maize diversity, maize is one of the best conserved field crops. It is estimated that the plant gene banks worldwide encompass more than 60,000 accessions.

With the discovery of the New World, maize has quickly become a crop of the essential importance for people who previously were not familiar with such a species. The European maize growing regions, including the territory of the former Yugoslavia, had especially favourable conditions for the growth and development of maize. The development and the improvement of this crop in our regions have, to a large extent, kept up with the maize improvement in developed countries, particularly in the USA. Hybrids have eventually taken priority over other maize types in the commercial production. On the other hand, attention has been paid to conservation and preservation of old landraces with the aim to conserve the diversity of the species (Babic et al., 2012b). The rapid replacement of local varieties by hybrids makes it imperative for the European material to be collected and preserved before this irreplaceable germplasm source is lost (Edwards and Leng, 1965). Therefore, the Maize gene bank has been established within the Maize Research Institute, Zemun Polje.

Although breeders have almost unlimited access to diversity of maize germplasm, the system that delivers the products of plant breeding reduces the diversity of cultivated hybrids leading to an increased genetic vulnerability. The principal problem arises from the use of homozyogous inbreds as parental components of hybrids. If a certain single-cross hybrid is more superior (more yielding) than another single-cross hybrid, economic aspects and competition among seed companies impose a broad use of one or a small number of the first-ranked hybrids.

In the second phase of hybrid breeding, new inbreds were primarily developed by a second cycle of breeding from crosses among elite inbreds within heterotic groups. Moreover, outstanding elite lines were shared as parents of different commercial hybrids. In combination with intensive selection this is expected to result in a reduced genetic diversity in the breeding pools but even more seriously in the hybrid varieties grown by farmers. The risk of genetic erosion does not only depend on plant breeding practices but also on the system that delivers the final products of plant breeding to the market. This includes the regulations to register new varieties and the marketing of registered varieties. Statutory testing of new varieties is required to register them on the national lists (VCU, DUS-UPOV). Afterwards, their acceptance by farmers depends on the amount and quality of the marketing effort of breeding companies but also on further series of voluntarily recommended lists based on regional trials. Consequently, only a few of the registered varieties are grown on a large scale.

There is another sector, which at first glance, has nothing to do with the decrease of genetic variability of maize, while in fact is very important. Namely, the situation within maize breeding has drastically changed during the last 50-60 years in relation to the participation of the private and public sectors in maize breeding. Public sector breeders developed many inbreds through the 1980s. Today, maize breeding is dominated by the private sector. Why is it important, from the perspective of cultivated maize varieties, that there is public breeding? Genetic diversity is the "raw material" of selection and is critically important to maintaining long-term selection progress. The continuing development of an expended germplasm base is a natural role for the public sector. Education of future plant breeders is the most frequently cited role of public maize breeding programs. Secure long-term financial support for genetic resource conservation is fundamentally important. It would be a catastrophic error of monumental proportions if genetic resources that could be more effectively identified and accessible in the future as complex genetics are increasingly well understood have in the meanwhile been left to disappear or to die.

Trends in genetic diversity among maize cultivars

Detailed information about a reduction in genetic diversity could help to emphasize the importance of identifying germplasm sources for broadening the elite breeding pools. Therefore, this issue is a goal of significant genetic studies not only in the USA but also in Europe. Upon maize introduction to the countries of the Old Continent, two events in the recent history of maize breeding had a major impact on the genetic diversity among and within cultivated varieties in Central Europe (Reif et al., 2005). First, the transition from Open Pollinated Varieties (OPVs) to hybrids occurred in the 1950s. Second, during the 1980s there was a shift in cultivation of top- or double-cross hybrids to three-way or single-cross hybrids. With advent of first maize hybrids, in 1933 in the US and around 1950 in Europe, maize cultivation has undergone a complete change. Numerous open-pollinated landraces adapted to specific regions were substituted by a limited number of hybrids bred from a large genetic basis. Today, the main hybrids cultivated in the world involve a restricted number of key inbred lines. Therefore, genetic diversity of those cultivars is almost certainly limited in comparison to the large genetic diversity available in gene banks (Le Clerc et al., 2005).

American breeders were already concerned by the genetic diversity among their maize hybrids after the Southern corn leaf blight of 1970 (Williams and Hallauer, 2000). Unfortunately, contemporary agriculture caused the cultivation of a very limited number of the most yielding hybrid varieties on very large areas. The series of studies carried out during the 1970s (Sprague, 1971; Zuber, 1975) pointed out to a broad use of a small number of public inbreds in the seed production of certified seeds. Maize breeders want to be assured that the genetic base of their hybrid varieties has not become too narrow to face unexpected environmental stresses. Therefore, in the process of maize breeding, great efforts have been made to broaden the genetic base of the material that has been offered to the market. Hallauer et al. (2010) have estimated that approximately 18.000 inbreds were tested in the USA during each year, i.e. 720,000 from 1939 to 1979. The number of self-pollinated, improved and selected inbreds to be tested probably amounts to a million. However, the majority of these inbreds are genetically related. These studies indicate that probably one of the 10.000 S_2 and S_3 tested inbreds finally ends up in the commercial production. So, although breeders are trying to develop and test a large number of inbreds (either in public or private sector), the frequency of the development of new, unique inbreds in the commercial sense is very low. A quite separate issue relates to recycling elite inbreds through pedigree selection.

It is estimated that approximately 3.000 inbreds in the hybrid combinations are annually tested at the Maize Research Institute, Zemun Polje. Thirty to forty out of the total number of hybrid combinations (i.e. 50-60 inbreds) are tested by the Variety Release Committee. Based on VCU tests, 10-20 hybrids, on average, are annually released (included those entered into the national list). Each year, only 1-2 out of this number of hybrids enter the commercial production after tests carried out in post-official production trials.

In the Europe, in the initial stage of the hybrids development, as a promising heterotic pattern, high yielding US dent lines were crossed with adapted European flint lines. The steady influx of dent germplasm from North America to Europe has continued over past 50 years. In contrast, the parental flint inbreds were developed by selfing from a few European open-pollinaded varieties such as Lacaune, Lizagaraute, Gelber Badischer Landmais and Rhentaler (Messmer et al., 1992). A total of 55 SSR markers was used by German researchers (Reif et al., 2005) when they observed five distinct Central European UPV varieties, 85 hybrids and their parental components. Genetic variation within and among varieties decreased significantly during the five decades. The five OPVs contain numerous unique alleles that were absent in the elite flint pool. Consequently, OPVs could present useful sources for broadening the genetic base of elite maize breeding germplasm. Therefore, it can be conjectured that 1) a bottleneck occurred in the flint pool during the transition from OPVs to hybrids and 2) OPVs, which did not serve as a germplasm source for the original flint inbreds, contained untapped allelic variation useful for future breeding progress.

The group of French scholars (Le Clerc et al., 2005) have quantified genetic diversity among modern and historical maize varieties (133 hybrids during the last five decades) with 51 SSR markers. The analysis of molecular variance showed that the variation among periods represented only 10% of the total molecular variation. However, the differentiation among periods, although low, was significant, except for the last two periods. Their results showed that the genetic diversity has been reduced by about 10% in the maize varieties bred before 1976 compared to those bred after 1985. The very low differentiation observed among varieties of the last two decades should alert French maize breeders to enlarge genetic basis in their variety breeding programmes.

A modern maize breeding programme based on the inbreeding-hybridization concept was established at the Maize Research Institute, Zemun Polje in 1953. Since then, the main objective in the breeding programme has been the development of maize hybrids with a superior potential for high yields and high quality of grain. Many generations of breeders, genetics, phytopathologists, entomologists, and physiologists have greatly contributed to achieved accomplishments (Drinic, et al., 2007). The development of the first ZP maize inbred lines from three local open-pollinated varieties (Vukovarski Dent, Rumski Golden Dent and Sidski Dent) started at the Maize Research Institute in 1953. Stojkovic, (1955) citing the original scientific paper written by Flajšman, has stated that all Ruma material, from which all varieties mentioned were derived, had originated from a parental ear (ear number 122 from the year 1909). What this fact may mean in terms of genetic diversity is a big question. Furthermore, the significant amounts of the following inbreds were imported in the former Yugoslavia in the 1953-1956 period: WF9, 38-11, Hy, Oh7, L317, W32, W22, W117, M14, N6, A374, A375, W153R, W37A, K148, K150, C103. Some of these inbreds considerably affected the future breeding programmes. The inbred C105, imported at the end of the 1950s, had a particular importance. It was used as a tester and a male component of the majority of single cross hybrids of the first cycle of selection (ZPSC 1, ZPSC 3, ZPSC 4, ZPSC 6 and NSSC 70). It has to be emphasised that due to this import, germplasm was significantly introduced from the US Corn Belt to the regions of the former Yugoslavia (Babic et al., 2012). Ivanovic et al. (2002) have singled out four inbreds originating from landraces that significantly affected breeding programmes in our institutes: NS796 - from Vukovarski Yellow Dent, R70Z from the Rumski Golden Dent variety; NS568 - developed by pedigree selection from the cross of one inbred of the BSSS origin to the adapted material from the Pannonian Plain and ZPPE25-10-1 - derived by pedigree selection from the cross of one inbred of the Lancaster origin and the inbred ZPPE25-10 developed from the Pecki Yellow Dent population. These inbreds were components of the leading commercial hybrids: NSSC 70, ZPSC 46a, NSSC 640 and ZPSC 677, while some of them are still present in the market because consumers want them even now. It is estimated that these hybrids together with another five hybrids (until 2002 when these data were published) were grown on approximately seven million hectares, which was the overall five-year maize production on the areas of the former Yugoslavia (Ivanovic et al., 2002).

Rumski Dent has been very popular in neighbouring Hungary. Hadi (2005a) states that this variety was grown on almost half the maize-growing area in Hungary for 30 years (1925-1955). The varieties derived from the parental plant Ruma 122 were known in Hungary under names "F" Early Yellow Dent and "F" Mczőhegyes Yellow Dent and latter was also popular in Yugoslavia under the name Novosadski Flajšman. Furthermore, these varieties had a significant role in the latter period of the development of self-pollinated inbreds and hybrid varieties in Hungary. The Caribbean Flints (introduced from Spain by the Turks in the 16th century) dominated maize production for nearly four hundred years. In the early 19th century these genetic stocks of the Caribbean origin were supplemented by Andean popping maize (Chutucuno Chico, Chutucuno Grande), introduced from Italy and to a lesser extent by Northern Flints (Pennsylvania 8-row). Under the influence of the American maize exhibition in the 19th century, Southern Dents (especially Gourdseed, but also Shoepeg, Hickory King and Tuxpan) and Corn Belt Dents (Queen of the Prairie, Iowa Goldmine, Leaming, Funk Yellow Dent) gained ground in Eastern Central Europe. These varieties were crossed to already grown flint varieties, and new varieties were developed, which then dominated maize production in the first half of the 20th century. As a result of hybrid maize breeding in Eastern Central Europe, two distinct gene pools were developed: Ruma and Mindszentpuszta (MYD) heterosis sources (Hadi, 2005b). Locally developed maize varieties, which have a background quite different from those developed in the North American Corn Belt, could, after suitable breeding, enrich the available sources of heterosis (Hadi, 2006). In their studies, Radović et al. (2000) and Vančetović et al. (2010) have pointed out to the existence of a new heterosis source in this material, which certainly has opened up the possibility for the improvement in contemporary breeding under conditions of temperate climate.

In Maize Research Institute, Zemun Polje, studies have been carried out to determine how much temporal cycles of selections differed in terms of genetics and what was happening with variability of materials originating from different cycles of selections. For the purposes of this research, 30 maize hybrids have been selected (six from each cycle) developed at the Maize Research Institute, Zemun Polje, which were typical representatives of the five periods of selection and widely grown. Genetic characterisation has been done by the use of RAPD markers. Although the tested hybrids were grouped into five clearly separated sub-clusters on the basis of the cluster analysis, the results of the discriminant analysis, indicate that the discrimination between groups is not strong, especially of the third and fourth clusters. The conclusion based on the results of three statistically analyses is that in genetic terms there are three different selection cycles. The distinction between the first and the second temporal cycles of selection is the move from four to two-line hybrids, while in the case of the third and the fourth temporal cycle of selection there is a change of parental pairs and no introduction of new genetic material. The fifth cycle is clearly separated, and this is in agreement with pedigree information, which indicates that at that time exotic germplasm and lines from new heterotic groups were introduced (Babic et al., 2009). Within the same studies, genetic variances of selection cycles were estimated on the basis of the RAPD analysis by parameterization of binary data. The comparison of genetic variances of different selection cycles shows that there were no differences in variability of hybrids over cycles, i.e. that the decrease/increase of variability did not occur over cycles of selection (Eric, 2004).

Conclusions

Monitoring the genetic diversity available to farmers is important, because the system that delivers the products of plant breeding and agricultural practices reduces the diversity of cultivated varieties leading to an increased genetic vulnerability. The assessment of the genetic diversity that exists in the available germplasm is fundamental in the improvement of agricultural plants. Recently, studies carried out at the Maize Research Institute, Zemun Polje, have been done on the following properties of collected germplasm: tolerance to drought, tolerance to herbicides, new sources of cytoplasmic male sterility (CMS) and new sources for increasing the content of available phosphorus in maize kernels (Babic et al., 2012; Vancetovic et al., 2009). A special attention is paid to former Yugoslav landraces, which could be a source of early maturity, ear length, a greater number of kernel rows, kernel length and kernel weight. Moreover, they can be a source of resistance to diseases, pests and abiotic stress or a source of different specific traits. The formation of a core collection tolerant to drought is in progress.

Although some efforts have been made, attention paid to the improvement of landraces has not been adequate, hence, they, in terms of modern breeding, eventually lost in importance. In what way and how much available variability will be used largely depends on work and funds to be invested in long-term breeding programmes.

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