5

Genetic Diversity of Maize Landraces as Sources of Favorable Traits

Snezana Mladenovic Drinic, Violeta Andjelkovic and Dragana Ignjatovic Micic
Maize Research Insitute, Belgrade
Serbia

1. Introduction

The Maize Research Institute Genebank conserves a sizeable collection of 2217 landraces and 3297 introduced accessions from 40 countries. Landraces are considered to be the most significant genotypes, since they represent the original biological material created by the process of natural selection and adapted to local growing conditions. The landrace collection is designated as national treasure and all the activities regarding the management, characterization and evaluation are given the highest priority in the Genebank. Moreover, accessions maintained in the collection are samples of the original populations which, through propagation, have lost a part of variability due to genetic drift and inbreeding. Genetic diversity of these landraces has never been comprehensively studied. Assessment of genetic diversity is an essential step helping to understand the relationship among and within the landraces, as well as their genetic structure. It is also valuable for setting priorities for genetic conservation, parental selection and as a source of favorable traits.

The search for superior genotypes regarding yielding ability, disease and pest resistance, stress tolerance or better nutritional quality is very hard, competitive and expensive. This is why breeders tend to concentrate to adapted and improved materials, avoiding wild parents, landraces and exotics, available in germplasm banks which would require long time, high financial support besides the difficulty to identify potentially useful genes. Evidently, there is a gap between available genetic resources and breeding program activities. While germplasm banks try to preserve as much as possible genetic variability to be used by breeders, breeding programs do not explore efficiently the available diversity, relying almost exclusively on their working collection. One of the consequences of the massive use of uniform commercial varieties in maize production is loss of its genetic variability. Only about 5% of maize germplasm is in commercial use.

Unlike high yielding varieties the landraces are endowed with tremendous genetic variability as they were not subjected to selection over a long period of time. This aids in the adaptation of landraces to wide agro-ecological niches making them important as genetic resources for useful traits.

With their reserve of ancestral genes, maize landraces are the real sources of genetic diversity and variability for maize improvement programs. Landraces can possess specific

characteristics: tolerance to abiotic and biotic stress, tolerance to herbicides, low antinutritional components content as phytate or a large grains content of proteins, oil or starch.

The objective of the chapter is to present the importance and usage of maize landraces genetic diversity as a source of favorable traits (resistance to abiotic and biotic stress, grain quality and other specific traits) for breeding purposes. Besides identification of target traits, activities designed to identify desirable characteristics encompass estimation of their genetic diversity using morphological and molecular markers.

2. Establishment of genetic diversity of maize landraces

Maize was introduced into Europe at the end of the 15th century from Central America and due to differences in climate needed to be adapted to cooler conditions. Dissemination from Spain, associated transmission over the old continent from different parts of America, and subsequent maize exploitation during the next five centuries contributed to the establishment of native maize genetic diversity in Europe. Alleles specific to European populations also emerged during adaptation to the local climate and environment (Rebourg et al., 2003).

The first introduction of maize from the west Indies, via southern Spain, Portugal and Mediterranean to the territory of the former Yugoslavia, (XVI century), consisted of the flint races, which were mostly maintained as exotic plants and garden speciemen (Radovic et al., 2000). The next introduction of new flint genotypes from Mexico increased the genetic diversity of the existing germplasm through crosses with the formely introduced flint types. Early and medium early flint types with shorter ear type, remained along Adriatic cost and its neighbouring continental area.

The new flint race from North America were brought in the thrid introduction wave (XVII century) and created local flints of unique genetic bases, better adapted to cooler conditions. Dent types from North America, especially Corn Belt Dent, spread rapidly after introduction at the end of 19th century and resulted in creation of orginal genotypes specific for European Corn Belt. Landraces specific for former Yugoslavia Corn Belt, involving Vojvodina, Slavonija and river valleys, were developed from crosses of flint and dent maize genotypes.

The first organized collecting of landraces from theritory of former Yugoslavia started in the 1960s. Nowdays, Maize Research Institute genebank maintains the collection of 2217 landraces collected from ex-Yugoslavian territories. Typical Corn Belt dents and denty flints of late maturity were collected in Yugoslavia Corn Belt region. Along the Adriatic cost flints of medium late maturity as well early maturity flints were predominant. At higher altitudes flints and denty flints were collected.

The most detailed study of Yugoslavian landraces was conducted by Pavlicic and Trifunovic (1966), when all the collected populations were aligned into 18 agro-ecological groups using the method of "natural classification" proposed by Anderson & Cutler (1942). The 18 agroecological groups are: Montenegrin flints, Bosnian early dents, Kosmet flinty dents, Macedonian flints, Eight rowed corn type of North eastern America, Derived flints, Mediterranean flint, Small-kernelled flints, Eight rowed soft dents, Romanian flints, Large-eared flints, White flinty dents Moravac, Dent type of USA corn belt dents, Derived dents, Dent type of southern east USA, Serbian dents, Flinty dents and Denty flints (Figure 1).

The utilization of genetic diversity of landraces in breeding programs started with development of the first inbred lines in Yugoslavia form Ruma Golden Dent, Vukovar Dent, Šidski, Beljski and Novi Sad Golden Dent populations. Crosses of self pollinated landraces with American inbred lines, which expressed a high level of heterosis, were the base for development of line-hybrids. First Yugoslav maize hybrids were created from inbred lines of local origin and foreign inbred lines genetically divergent from local germplasm. In modern maize breeding program landraces are used for creation of broad base synthetic populations, development of core collections and as potential sources of favorable traits.

Fig. 1. Agroecological groups of maize landraces from ex-Yugoslav territories

A core collection consists of a limited set of genotypes derived from the existing collection and has to represent the genetic diversity of that collection. The Maize Genebank adopted two approaches to develop core collections. Representatives of each eco-geographical group (20%) were chosen based on morphological and biological traits for development of eco-core collection. Core subsets are used as donors for traits typical for each ago-ecological group. On the other hand, the main criterion for selecting landraces for the elite-core was the combining ability. Combining abilities of 900 medium and late maturing landraces, from regions with temperate growing conditions, were tested with four commercial inbred lines, Mo17, B73, V395/31 and LD230 (Radovic et al., 2000). Four core subsets of high combining abilities with specific inbred lines were obtained for further use in breeding programs.

3. Genetic diversity assessment

Significant maize genetic diversity decrease in the last few decades is a consequence of development of modern hybrids and agricultural systems. The use of a limited number of elite lines and synthetics heightens the risk of genetic uniformity in commercial maize production fields (Hallauer et al., 1988). Thus, maize breeders became more aware of the need for both maintaining genetic diversity among hybrid varieties and improving the management of genetic resources through the conservation of landraces (Goodman, 1994).

Landraces are the cultivated maize material with the highest genetic variation as well as with the best adaptation to the natural and anthropological environment were they have evolved (Maxted et al., 1997). They contain locally adapted alleles and represent an irreplaceable bank of highly co-adapted genotypes (Quals et al., 1997). In this sense, genetic variability is a valuable natural source of beneficial traits providing plant adaptation to different stresses and undergoing climatic changes.

Different methods can be used to assess genetic variability in plant species, such as pedigree data, morphological and molecular markers. Data obtained by landrace description are further statistically processed. Multivariate analyses (e.g., cluster analysis, principal component analysis and discriminate analysis) for measuring the degree of divergence among populations have been useful in different fields of research, allowing obtaining a summary of the most relevant characteristics. Populations (landraces) can be grouped together based on informative data and be used directly in a breeding program.

A broad approach using phenotypic and molecular markers is required to analyze diversity and to support conservation, management, and development of plant genetic resources (Hammer et al., 1999). Both kinds of markers have their advantages and drawbacks and their combined utilization is recommended to increase the resolving power of genetic diversity analyses (Singh et al., 1991).

Considering the origins of the populations and the fact that trade and communications have always been very intensive on a relatively small ex-Yugoslav territory it can be assumed that genetic relationships between the 2217 landraces from Maize Research Institute gene bank could be inconsistent with the existing classifications. Moreover, accessions that are maintained in the collection are samples of the original populations which, through propagation, have lost a part of variability due to genetic drift and inbreeding. A new phenotypic analysis, as well as molecular analysis, is being performed on these landraces in order to assess their genetic variability and verify their relationships.

3.1 Phenotypic analysis

Phenotypic markers have been of great value in studies of maize landraces (Galarreta & Alvarez, 2001; Lucchin et al., 2003; Ortiz et al., 2008). The most commonly used descriptors are related to plant architecture traits, tassel traits, ear and kernel characteristics. Earliness is also frequently a part of the landrace description. Relationships between traits are statistically analyzed using graphs, correlation coefficient estimations and principal component analysis (PCA). Genetic similarity can be calculated from the morphologic data by unweighted pairgroup method (UPGMA) cluster analysis based on Euclidian distance coefficients. Cluster analysis is used to reveal the association between landraces.

A sample of 54 maize landraces was analyzed with three populations per agro-ecological group. Besides ex-Yugoslavian landraces, six introduced populations were included, used as

a check since they are expected to be genetically distant from local populations. These populations originate from France, Georgia and China.

All populations were sown at Zemun Polje in 2008, in two different sowing densities – 44640 and 64935 plants per ha. The experimental design was RCBD (*Randomized Complete Block Design*) with two replicas, four rows per replica and 20 plants per row. For morphological trait measurements two medium rows per population were used. We measured for each plot 15 morphological traits (plant height, ear height, leaf number, husk leaf length, tassel length, central spike/tassel length, number of tassel primary branches, branched part/tassel length, ear length, ear row number, ear kernel/row number, ear diameter, kernel length, kernel width and kernel thickness) taken from 20 competitive plants (40 plants per population) and from two kernels per ear.

Based on the average values of 15 morphological traits, as well as ASI (anthesis - silking interval) and their standard deviations cluster analysis was performed using square Euclidean distance and Ward complete linkage method. All the statistical analyses were performed using program NTSYSpc 2.1.

Cluster analysis based on phenotypic traits highlights distance between populations originating from ex-Yugoslav territories including two populations from France (clusters 1, 2 and cluster 3) and introduced populations from Georgia and China (cluster 4). Dendrogram can be divided into four clusters. The first cluster includes only two populations, one from Montenegrin flints and the other from Mediterranean flint agro-ecological groups. Cluster II encompasses only local flint populations. The third cluster can further be divided in two sub-clusters (Figure 2). Sub-cluster IIIA includes mostly local flint and intermediary (flinty-dents and denty-flints) populations, while sub-cluster IIIB includes mostly local dent and intermediary populations. Both French populations, with flint type of kernels, fall into cluster III. Introduced populations from Georgia and China form a distinctive cluster IV, which also includes two local populations (one dent and one flinty dent).

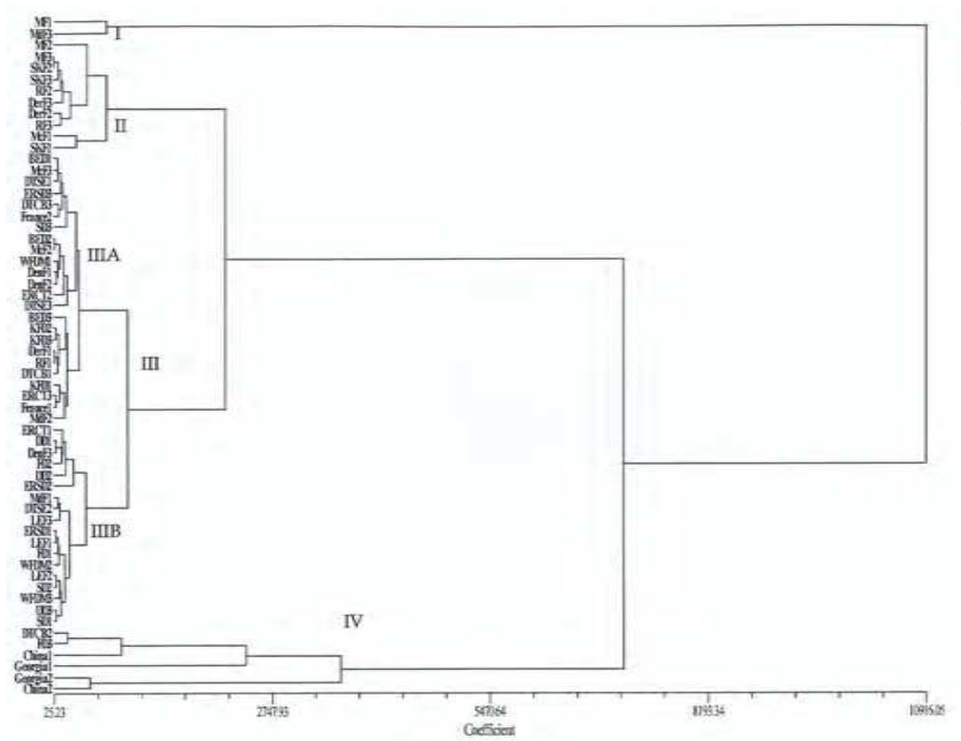
Phenotypic analysis revealed large heterogeneity among local populations indicating that populations are highly adapted to specific environmental conditions and uses. As such, they could be a valuable source of genetic variability. These results are in agreement with the findings that maize is the most diverse crop known, containing extensive diversity at both the phenotypic and molecular levels (Buckler et al., 2006). More ever, studies on European maize germplasm have already pointed out the presence of a great variability in morphological traits and large molecular diversity in traditional populations (Gauthier et al., 2002).

3.2 Molecular marker analysis

Development of molecular marker techniques, which enable estimation of genetic diversity directly at the DNA sequence level, are expected to supplement and refine the morphological-based classification. Molecular markers provide a direct measure of genetic diversity and go beyond indirect diversity measures, based on morphological traits or geographical origin.

RFLP (restriction fragment length polymorphisms), as well as PCR (polymerase chain reaction) based markers like RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphisms) and SSR (simple sequence repeats) have been successfully used in maize genetic diversity studies. RFLP have been extensively used in assessing genetic variability in maize (Dubreil & Charchosset, 1998; Gauthier et al., 2002; Rebourg et al., 2001; Rebourg et al., 2003) due to their high discriminative power. However,

RFLP technique is labor intensive and time consuming, needs large quantities of DNA and is difficult to automate. On the other hand, RAPD which overcome some of the RFLP drawbacks have shown some problems with reproducibility of amplification and scoring of error data (Demeke et al., 1997; Karp et al., 1997).



Abbreviations for the 18 agroecological groups are: MF - Montenegrin flints, BED -Bosnian early dents, KFD - Kosmet flinty dents, McF - Macedonian flints, ERCT - Eight rowed corn type of North eastern America, DerF - Derived flints, MdF - Mediterranean flint, SKF - Small-kernelled flints, ERSD - Eight rowed soft dents, RF - Romanian flints, LEF - Large-eared flints, WFDM - White flinty dents Moravac, DTCB - Dent type of USA corn belt dents, DD - Derived dents, DTSE - Dent type of southern east USA, SD - Serbian dents, FD - Flinty dents and DenF - Denty flints.

Fig. 2. Cluster analysis of the analyzed populations based on phenotypic traits using square Euclidean distance and Ward method

SSR and AFLP, the most informative among DNA-based markers, seem to be a better choice for genetic diversity studies (Smith et al., 1997; Pejic et al., 1998; Lubberstedt et al., 2000), since they overcome the problem of reproducibility and error data of RAPD markers, are generally much simpler to apply than RFLP, can be automated and yet provide results correlated with those from RFLP analysis (Pejic et al., 1998). They have been successfully utilized in maize genetic diversity studies (Lubberstedt et al., 2000; Barcaccia et al., 2003; Dubreil et al., 2006; van Eten et al., 2008; Eschholz et al., 2010; Hartings et al., 2010).

Although often used for the same scientific research AFLP and SSR present major distinctions. AFLP markers are genomic fragments detected after selective PCR amplification, while SSR markers consist of tandemly repeated units of short (1-6 bp) nucleotide motifs that show extensive length variation between individuals (Jarne & Lagoda, 1996). AFLP markers are dominant and biallelic, while SSR markers are co-

dominant and multiallelic. Pejic et al. (1998) suggested that SSR appear to be suited for the analysis of outcrossing heterozygous individuals and AFLP are advantageous when genome coverage is a major issue due to the presence of linkage disequilibrium, such as in inbred lines and breeding material. The choice among the markers to use depends on the material to be analyzed, the aim of the experiment and the facilities available.

Two studies with SSR and AFLP markers were conducted at MRI on the landraces from different agro-ecological groups. In the first study 21 landraces belonging to seven different agro ecological groups have been subjected to SSR analysis with aim to develop genetic fingerprint for characterization, identification and classification of the landraces, as well as for estimation of their genetic diversity (Ignjatovic-Micic et al., 2008). In the second study eighteen landraces from six different agro-ecological groups were assayed with AFLP and SSR markers (Ignjatovic-Micic et al., 2007). The main objectives of the work were to compare the level of information provided by SSR and AFLP and the genetic similarities (GS) obtained with the two marker systems. In both studies a DNA-pooling strategy that proved to be effective in RFLP analysis of maize populations (Dubreuil et al., 1999) was employed in order to assess if it could be applied with the same success for population analysis with SSR and AFLP markers.

SSR analysis was performed with 25 primer pairs covering all 10 maize chromosomes. The amplification reaction was carried out in 25µl reaction volume containing 1x enzyme buffer, 2.4 mM MgCl2, 200 µM dNTPs, 0.5 µM primers, 1 x BSA, 1U *Taq* polymerase and 200 ng of DNA. The amplification profiles followed were: an initial denaturation at 95°C/5min, followed by 15 cycles each of denaturation at 95°C/30sec, annealing at 63.5°C/1min (-0.5°C/cycle) and extension at 72°C/1min; another 22 cycles of 95°C/30sec, 56°C/1min and 72°C/1min were performed.

AFLP analysis was conducted using three primer combinations: EACA/M-CAG, E-AGA/M-CAG, E-ACA/M-CAT and E-AAA/CAG. *EcoRI* was used as a rare cutter and *MseI* as a frequent cutter. Pre-amplification was done by the following program: 1. 94°C/60sec, 2. 94°C/30sec, 3. 55°/30sec, 4. 72°C/60sec (30 cycles of steps 2-4). Amplification program consisted of: 1. 94°C/60sec, 2. 94°C/30sec, 3. 62°C/30sec, 4. 72°C/60sec (40 cycles of steps 2-4). Both SSR and AFLP amplified fragments were separated on 6% denaturated polyacrilamide gels and visualized by silver staining (Bassam et al., 1991). The gels were scanned and band profiles for each primer were scored visually.

The SSR probes used detected single loci and each detected band was assumed to be an allele. Allele frequency was scored as percentage of individual bands within the sample using peak height estimated by scanning. Genetic distances (GD) between populations were evaluated by the *Rogers' Distance* (Rogers, 1972). For the AFLP primer combinations bands were binary scored (1 and 0) with each band being considered a locus and the genetic similarities among tested populations were calculated by the *Jaccard coefficient* (Jaccard, 1908). Cluster analysis was performed with unweighted pair-group method (UPGMA). Statistical analyses were done by NTSYSpc2.1 program package.

Twenty five SSR markers generated a total of 224 polymorphic bands, while three AFLP primers combinations generated 188 bands with 79.25 being polymorphic. The average polymorphism content was higher for SSR and higher genetic similarity values were estimated for AFLPs. In our study ranking of the agro ecological groups (order from the highest to the lowest average GS) was congruent for both marker systems.

The cluster analysis did not group the populations precisely with expectations based upon their agro-ecological groups. Better agreement with expected results was accomplished with AFLP analysis (Figure 3). In the work of Reif et al. (2005) it was suggested that SSR analysis of bulked populations cannot detect alleles with a frequency below 0.2, so that a large number of alleles with low frequencies remains undetected. They also concluded that differences between populations are emphasized due to a concentration on alleles with high frequencies.

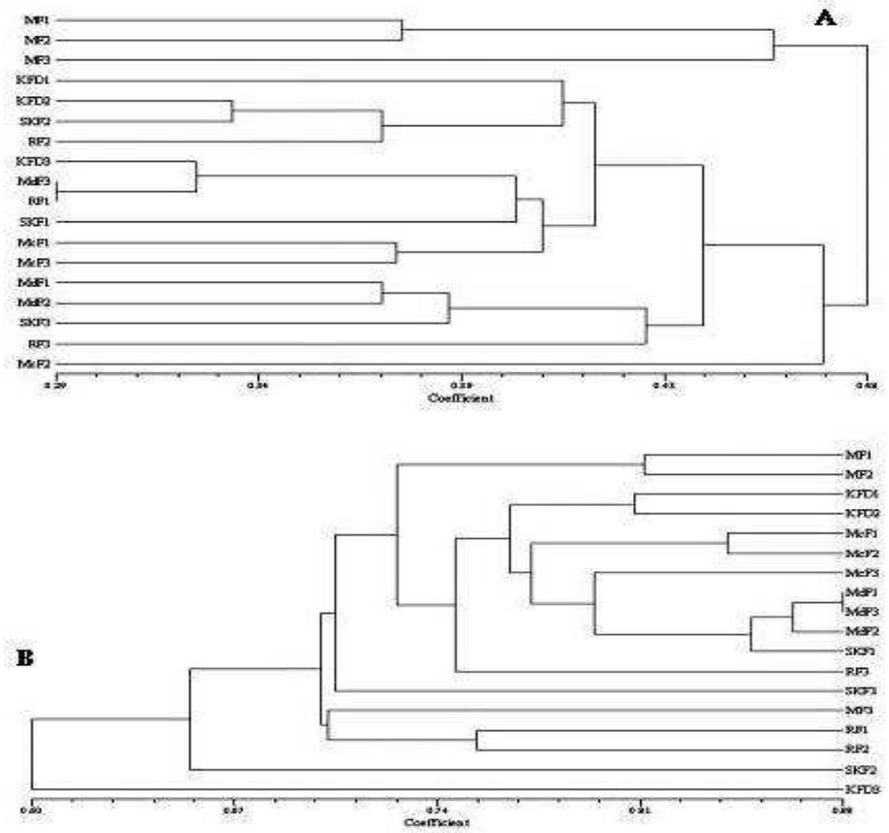


Fig. 3. Dendrograms of 18 maize populations (bulked population samples) constructed using UPGMA cluster analysis of A) Roger's distance values obtained by SSR analysis and B) Jaccard similarity values obtained by AFLP analysis. Abbreviations are the same as in Fig. 2.

The results in our work which indicate that SSRs are not a good choice for bulk analysis of populations could be explained by several reasons, besides the low sensitivity of bulk approach in detecting low frequency alleles. Use of di-nucleotide repeat SSR (excluded in the work of Reif et al., 2005) which tend to be more polymorphic than SSR with longer repeat motifs (Smith et al., 1997) could have caused higher scoring errors. The silver staining of the gels is probably not the right choice for bulk analysis due to its low sensitivity. It would be much better to use fluorescence as was done in the work of Reif et al. (2005), in which it was shown that the allele frequencies of a population could be estimated with high accuracy from the band intensities of SSR profiles of bulks. Populations cannot detect alleles with a frequency below 0.2, so that a large number of alleles with low frequencies remain

undetected. They also concluded that differences between populations are emphasized due to a concentration on alleles with high frequencies. However, in a simulated SSR analysis of bulks they showed that the correlation between genetic distance values of individuals and bulks is high, but it is still to be checked for final conclusions.

Assessing molecular marker diversity among heterogeneous populations using a DNA bulking strategy was shown to be successful in different plant species (Yu & Pauls, 1993; Sweeny & Dannenberger, 1997; Dubreuil et al., 1999; Mian et al., 2002). The question is what marker system to use in order to obtain the most accurate results with maximum efficiency. In our work we compared SSRs and AFLPs, the markers suggested as the best choice in genetic diversity studies. Based on results of this comparison it could be concluded that AFLPs are more reliable than SSRs in assessing diversity of maize populations when using a DNA bulking strategy.

4. Genetic diversity of landraces as source for cytoplasmic male sterility (CMS)

Absence of pollen and plant inability to produce functional pollen grains is known as male sterility. Male sterility can be determined by nuclear (genic male sterility) or cytoplasmic (cytoplasmic genetic male sterility – **CGMS**) genes. CGMS (onwards referred to as CMS) is successfully used in commercial production of hybrid seed, avoiding the drawbacks of hand or mechanical emasculation (Kaul, 1988). Identification of CMS types is important because commercial production of maize hybrid seed today stands upon utilization of C and S cytoplasmic types, as self-pollination in plants can severely jeopardize its production. Detasseling (removal of flowers from maize plants by hand) can be replaced by introducing male sterility in maize. This reduces possibility of self-pollination and also saves seed producers millions of dollars per year in labor costs.

Three main types of CMS were identified in maize: CMS-T, CMS-S and CMS-C. Male sterile cytoplasm is distinguished by specific nuclear genes (*Rf* genes) that restore pollen fertility. These genes, restorers of fertility, suppress the male-sterile effect of the cytoplasm, allowing the production of viable pollen.

The use of uniform materials can be a threat in crop production and genetic vulnerability must be a constant concern in plant breeding for all species. An example is the extensive use of CMS-T in 1950-ies and 1960-ies, which showed to be extremely susceptible to *Helminthosporium maydis* race T. Severe yield losses occurred in the United States in 1970-ies and the next year in other regions of the world, due to the *Helminthosporium maydis* pandemic. For new CMS sources landrace genotypes worldwide were searched and one source was found within Maize Research Institute gene bank collection.

Tester lines containing nuclear *Rf* genes are traditionally used for identification and classification of CMS types. However, test-crossing procedure is time consuming, labor intensive and not precise. Development and appliance of molecular methods revealed that mutations responsible for CMS are located in mitochondrial DNA (mtDNA) in many plant species (Shnable & Wise, 1998). It was confirmed that chimeric genes – DNA parts with open reading frames (ORF) that comprise sequences derived from different genes, are responsible for cytoplasmic male sterility. Himeric T-*urf13* gene was detected in mtDNA CMS-T (Dewey et al., 1986) chimeric *atp6-C* gene in mtDNA CMS-C (Dewey et al.,

1991) and a repeated DNA region "R" containing two ORFs in mtDNA CMS-S (Zabala et al., 1997).

The unique characteristics of himeric mtDNA regions were used for developing several molecular methods. RFLP analysis is time consuming to be applied routinely in breeding programs, while PCR-based markers are known to be rapid and definitive identifiers of the cytoplasm (Sato, 1998; Nakajima et al., 1999). Liu et al. (2002) developed a multiplex PCR assay combining three primer pairs specific for each type of CMS in a single reaction. This assay reveals 398, 440 and 799 bp specific DNA fragments identifying C, T and S cytoplasm, respectively. No DNA fragment is amplified from the N-type cytoplasm in this assay because an mtDNA fragment unique for the normal type does not exist. It was shown to be a quick and a reliable method, convenient for analysis of a huge number of samples.

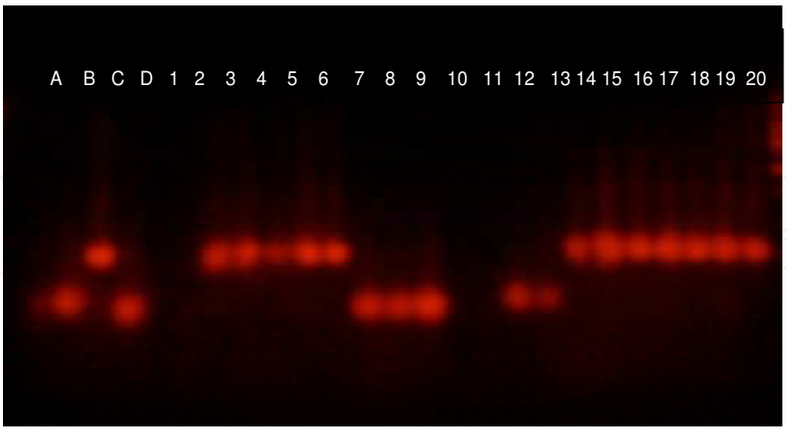
The multiplex PCR assay was successfully used for CMS identification of maize accessions from Maize Research Institute (MRI) Genebank collection. Over 100 sources (2% of the total accession numbers) of cytoplasmic male sterility (CMS) were found in field trials, distinguishable by their overwhelming frequency of male sterile plants in segregating test progenies. As the types of CMS involved were unknown, a screening experiment was performed using a multiplex PCR with specific primers for S, C and T cytoplasm (Liu et al., 2002).

For PCR analysis *e*ach source of CMS was tested in triplicate, i.e. three separate samples per genotype were analyzed. DNA extraction was done from ten seeds per sample with CTAB buffer by the modified method of (Saghai-Maroof et al., 1984). The PCR reaction mix contained 1× PCR buffer, 1.5 mM MgCl2, 0.8 mM dNTP, 50 pmol of CMSSF, CMSSR, CMSTF, CMSTR, CMSCF and CMSCR primers, 1U of *Taq* polymerase and 50 ng of DNA. The PCR amplification consisted of 40 cycles (steps 2, 3 and 4), as follows: 1) initial denaturation 94°C/2 min, 2) denaturation 94°C/1 min, 3) annealing 55°C/1 min, 4) elongation 72°C/1.30 min and 5) final elongation 72°C/5 min. The amplified fragments were separated on 1.5% agarose gels, stained with ethidium bromide and photographed. An illustration of the multiplex PCR is given in Figure 4.

The multiplex PCR method used in our research, which is recommended for large scale screening, gave conclusive results for most of the analyzed accessions. A small number of accessions could not be identified for cytoplasmic male sterility type as, for example, different PCR products were obtained. The structure of maize sterile cytoplasm types within a gene bank collection is given in Figure 5. The predominant CMS type within the analyzed accessions was S cytoplasm. Clear presence of some type of CMS was found in 4.56% of all the former Yugoslav landraces, which is a significant percentage and S type makes 84% of all CMS found. S type was also predominant (44%) within introduced populations. This significantly lesser presence of CMS-S within introduced populations could be the consequence of a smaller sample size analyzed. On the other hand, introduced populations and lines are of different provenience, coming from different parts of the world and it was shown that, for example, among populations from Argentina the dominant type was CMS-C and from Italy CMS-S (Vancetovic et al., 2010).

The multiplex PCR approach enabled a simple, fast and reliable large scale screening of maize cytoplasm within MRI gene bank accessions, reducing time for cytoplasm characterizations from several years to a few weeks. Indirectly, this also helps in pre-

breeding procedures when presence of specific sterile cytoplasm is required, with the final goal of commercial production of maize hybrids.



Lanes A – Cms-T (440bp), B – Cms-S (799bp), C – Cms-C (398bp), D – N type of cytoplasm; 1 – no band, 2 to 6 - Cms-S, 7 to 9 - Cms-C, 10 to 11 – no band, 12 to 13 - Cms-T, 14 to 20 - Cms-S.

Fig. 4. PCR amplification of CMS types with specific primers for C, T and S cytoplasm

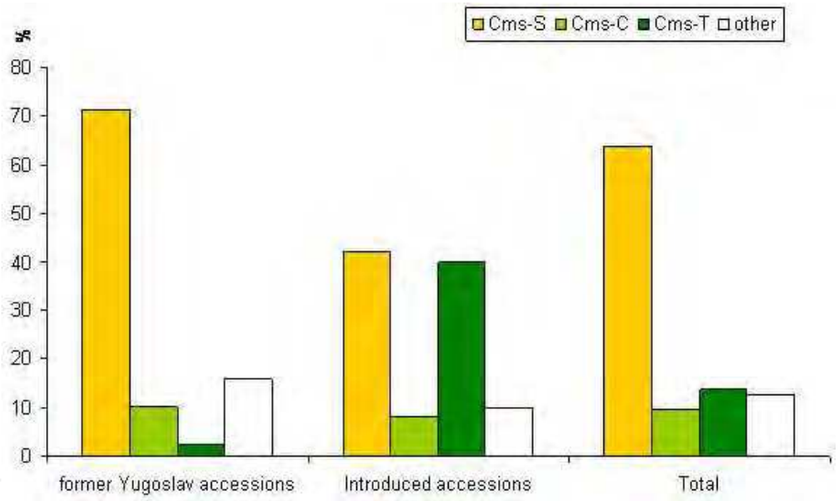


Fig. 5. The structure of maize sterile cytoplasm types within a gene bank collection

5. Genetic diversity as a source of drought tolerance

Drought can be defined as the absence of adequate moisture necessary for normal plant growth and to complete the life cycle (Zhu, 2002). It is a "single most common cause of severe food shortage" (FAO) in developing countries and predicted global warming in XXI century will increase drought impact on crop production. Estimations of Intergovernmental Panel on Climate Change (IPCC, www.ipcc.org) are that between 1990 and 2100, globall increase of temperature will be 1.4 to 5.8 °C. Although the effect of this warming will be regionally distributed, it is assumed that increase in temperature, reduction in rainfalls, together with increase of incidents of insects and pests will reduce crops grain yield, particularly in tropical and subtropical area (Ribaut, 2006).

In Southern Europe, drought followed by heat wave, reduces maize production for 20% (EC, 2004). The similar effect was in Serbia in 2007, when July the most critical period for grain yield formation was the hottest in last 50 years.

Drought severely affects maize grain yields by restricting season length and through unpredictable stress than can occur at any time during the vegetation season. Drought occurring at flowering has the greatest impact on yield losses compared to all the other developmental stages (Grant et al., 1989). Water deficit lasting one or two days during pollination may cause more than 20% reduction in yield (Hall et al., 1981).

However, drought tolerance is the most difficult task for maize breeders to be solved. It is polygenic and complex trait with high genotype x environment interaction. Moreover, this stress occurs randomly in timing and severity, making identification of drought tolerant genotypes more difficult. In agriculture, drought tolerance is generally evaluated at the end of growing season using grain yield (Dhanda et al., 2002). Maize, like other crops, can achieve drought tolerance through different approaches, involving escape, avoidance and tolerance of stress that influenced growth, development and grain yield formation (Cooper et al., 2004). Possible choice could be selection for earliness (drought avoidance or escape) but in years with adequate water supply it could bring lower yields. On the other hand, by given adequate rainfall, yield is usually correlated with late maturity, in maize, sorghum and sunflower (Edmeades et al., 1989). The most widely used strategy is to select for yield under optimal conditions, and latter on field testing under different moisture conditions. Assumptions of the approach are that genes for drought tolerance are present in high yielding material and the selection under optimal conditions can increase performance under sub-optimal conditions (Bolaños & Edmeades, 1996). However, in the absence of stress, drought tolerance in genotypes provides yield losses (Carena et al., 2009). Thus, many different strategies and traits have been proposed for using in maize breeding programs for improved drought tolerance and best improvement is achieved in long term processes.

Environmental conditions that caused drought and expression of genetic variation for drought tolerance are very important for successful breeding. The use of appropriate locations where drought stress can be controlled and applied at designated time and intensity (MSE- manage stress environment) has made significant improvement in maize breeding (Barker et al., 2005).

Moreover, because of difficulties in selecting for yield *per se* under drought conditions, it is recommended to use secondary traits to complement phenotypic selection. Measurement of secondary traits as well as grain yield is necessary in objective identification of drought tolerant genotypes. Secondary traits are "plant characteristics other than grain yield that give additional information about how yield will change under drought" (Fisher et al., 2003). Useful secondary trait is the trait that used together with yield makes expected progress from selection, greater than the progress made using grain yield alone. The use of secondary traits with grain yield, rather than selection for grain yield alone, has been shown to increase selection efficiency by about 20% in maize grown under stress induced by low nitrogen (Bänziger & Lafitte, 1997). Many physiological and morphological secondary traits related to survival or increased production in water limited environments have been proposed (Richards, 1996). Some of them have never been applied in breeding programs, because of their complexity or impossibility of routine application (Ribaut et al., 2006).

Ideally, secondary traits should be genetically associated with grain yield under drought, carry no yield penalty under favorable conditions, highly heritable, cheap and rapid to measure, stable over time and be able to be observed at or before flowering so that undesirable parents are not crossed (Edmeades et al., 1999).

A characteristic of maize under drought stress is a delay in silking resulting in an increase in the anthesis-silking interval (ASI), incomplete fertilization and decreased kernel development (Hall et al., 1984). The recommended secondary traits, routinely used by CIMMYT, for use in maize breeding programs are: flowering date (anthesis-silking interval, ASI), ears per plant (bareness), leaf rolling, tassel size and stay green (Monneveux et al., 2008).

Considering tremendous genetic diversity and importance of drought for maize production, long term project for improved drought tolerance has been started. All maize accessions, 6371, from Maize Research Institute Zemun Polje genebank were imposed to controlled water stress in Egypt, at the Sids Agricultural Research station (150 km south of Cairo). Among all accessions, 2217 landraces from former Yugoslavia were included into the experiment (Vancetovic et al., 2008).

Based on existing data, the material was divided into five groups, according to the duration of the vegetation season: extra early, early, medium, medium late and late. Groups were sown separately and have been irrigated until the appearance of the first tassels. After that, no irrigation was applied. The genotypes were estimated for ASI, staygreen (on the 1-5 scale, where 5 was the highest rate), total appearance of the plant (on the 1-8 scale, where 8 was the highest rate), barrenness, seed set and grain filing. A total of 167 landraces was selected according to reported criteria for further testing in Egypt and on two locations in temperate climate (Macedonia/Skopje and Serbia/Zemun Polje). According to the average of these traits for the three studied locations in two years, 14 most drought tolerant landraces were selected.

Besides field performance *per se*, development of good maize hybrids requires establishment of the best combination among heterotic groups (Barata & Carena, 2006). Therefore, chosen landraces were crossed to inbred lines from three heterotic groups (BSSS, Lancaster and independent source) in order to classify them into heterotic groups. The testcrosses for combining ability were studied in field trials at three locations in two years. The criterion for the selection of good testcrosses was the yield not statistically different from the check or performance index (based on grain yield and moisture at harvest) that was over 100% in comparison to the check (Andjelkovic et al., 2010).

5.1 Drought at seedling stage

Plant responses to environmental stresses include many adaptive mechanisms, changes in physiological and biochemical processes. Adaptation is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, betaines and proline (Yancey et al., 1982). It enables plants to maintain high rates of photosynthesis and can be advantageous in environments with large variation in water availability (Morgan, 1980). The experiments on maize genotypes (Moussa & Abdel-Aziz, 2008) pointed out that stress tolerance mechanism exists in seedlings stage and that early root and shoot development may provide useful information to enable the prediction of maize performance

under field conditions (Ruta, 2010). Usually, in laboratory conditions drought stress is investigated by withholding water. But, very often, different osmolytes (manitol, sorbitol, inorganic salts or polyethylene glycol-PEG) can be used to mimic water stress. Polymers of PEG with a molecular weight above 6000 Da do not penetrate cell wall and therefore mimic dry soil more closely (Verslues et al., 2006).

Maize landraces chosen within our project were analyzed in response to PEG treatment. Seeds were germinated for three days on moistened filter paper and then transferred into plastic pots containing Knopp solution with modified nitrogen content. For the next four days, plants were grown on ¼ strength nutrient solution supplemented with different forms of nitrogen. Nitrogen was supplied in the form of KNO_3, Ca $(NO_3)_2$ and $(NH_4)_2SO_4$. The initial pH of the solution was adjusted to 5.6. Plants were kept in a growth chamber under a 12-h photoperiod at 22/18°C, with the irradiance of 40Wm^{-2} and relative humidity of 70%. For the terminal 48 h of the growing period, one half of the plants (treatment) were grown on the fresh aerated nutrient solution supplemented with 4% polyethylene glycol (PEG, Mr 10 000), parallel to control plants grown on the nutrient solution without any addition. Root and shoot length, fresh and dry weight and prolin content were measured (Kravic et al., 2010).

Roots, responsible for water uptake, are the first plant organs sensing water stress. But roots and shoots usually respond differently to water deficit: in contrary to the shoot, relative or absolute size of the root might increase, depending on the root type and duration of stress (van der Weele et al., 2000). In our experiments drought stress at seedling stage affected shoot growth to a greater extent than root growth (Table 1).

In the experimental study of drought tolerant and sensitive maize seedlings (Ruta, 2010) the plant traits most severely affected by water shortage were shoot growth, (−75%) and leaf area (−68%), followed by root growth (−51%). It should be noted that at the same developmental stage, water stressed plants were two days older than the well-watered plants. This slower growth at water stress was much more pronounced at the shoot level than at the root level. Limited water availability retarded shoot development and promoted root development, consistent with results from plants stressed in solid substrates (Sharp & Davies, 1989; Weerathaworn et al., 1992a; 1992b). Genes for root development and proliferation during the seedling stage are also active at later growth stages, i.e. investigations of the genetic control of root traits at the seedling stage provide valuable information about its behavior at later stages of the plant development under the field conditions.

Although drought stress reduces plant water potential, it affects root and leaf growth differently (Frensch, 1997). It shifts the weight based shoot to root ratios in favor of the roots and it is a well known adaptive mechanism of preferring root over shoot development. The larger root-to-shoot ratio under severe stress is a further indicator that a moderate water stress had been achieved as it promotes a relatively better functioning of the roots (El Nadi et al., 1969; Sharp & Davies, 1979; Westgate & Boyer, 1985). Tendency of plants to increase the root to shoot ratio by allocating assimilates to the roots is typical response of plants in the field. In this experiment, and some others (Suharjo, 2007), plants were grown in solution, with mild (4% PEG) osmotic stress and root was not stimulated to grow deeper to extract more water (as in the soil). For this reason, root to shoot ratio does not differ too much compared to control conditions (Figure 6).

Landraces	Root lenght		% reduction	Shoot lenght		% reduction
	tretment	control		tretment	control	
L1	18.4	20.8	11.5	7.8	8.8	11.4
L2	9.7	11.1	12.6	6.5	6.6	1.5
L3	18.2	22.3	18.4	8.2	9.4	12.8
L4	17.1	20.0	**14.5**	7.4	9.2	**19.5**
L5	22.5	23.7	**5.1**	10.2	11.9	**14.3**
L6	17.0	18.0	5.5	8.3	8.5	2.4
L7	17.3	19.5	11.2	7.7	8.3	7.2
L8	17.1	17.2	**0.6**	8.4	9.4	**10.6**
L9	17.1	18.0	**5.0**	8.0	8.8	**9.1**
L10	14.7	16.3	9.8	7.5	8.2	8.5
L11	13.8	16.6	**16.8**	5.7	7.0	**18.6**
L12	16.4	17.7	**7.3**	9.3	10.2	**8.8**
L13	18.8	20.3	**7.4**	8.1	9.9	**18.2**
L14	20.4	22.4	**8.9**	8.4	10.1	**16.8**

Table 1. Root and shoot length and percent (%) of reduction under control and stress condition (PEG)

In the genetic study of quantitative trait loci (QTLs) in maize seedling some QTLs were specific to water stress, such as QTLs for root dry weight, shoot dry weight, and leaf area-to-root length ratio (Ruta, 2010). The QTLs for shoot-to-root ratio, were collocated and overlapped with QTLs for ear number in the field under intermediate and severe stresses (Ribaut et al., 1996) and for the anthesis-silking interval were collocated with QTLs for the numbers of crown roots and seminal roots. An early increase in growth of main roots seemed to be linked to a later decrease in ASI, while an early increase in lateral root length distribution on the main axile root may extend ASI. Considering that conventional breeding may take about ten years from crossing to the final release of new maize hybrids, it is important to find rapid screening technique under laboratory conditions. QTLs controlling the balance between early root and shoot development may provide useful information to enable the prediction of maize performance under field conditions.

6. Genetic diversity of landraces for grain quality

Characterization of genetic diversity of maize landraces aids efficient exploring of the allelic variation for genetic improvement of economically desirable traits, such as grain quality. Maize is a relevant food source, so the quantification of the nutritionally important grain constituents is important for the best exploitation of different genotypes. In this context, the landraces represent a good source of genetic variability and may help to identify the most suitable materials for the development of more nutritious foods. Many studies have documented genetic and phenotypic variability for grain composition traits in maize (Pollak & Scott, 2005; Reynolds et al., 2005; Berardo et al., 2009; Has et al., 2010, Mittelmann et al., 2011).

To determine genetic diversity for grain quality traits a set of 54 landraces was grown in a randomized complete block design (RCBD) with two replications at one location. Grain

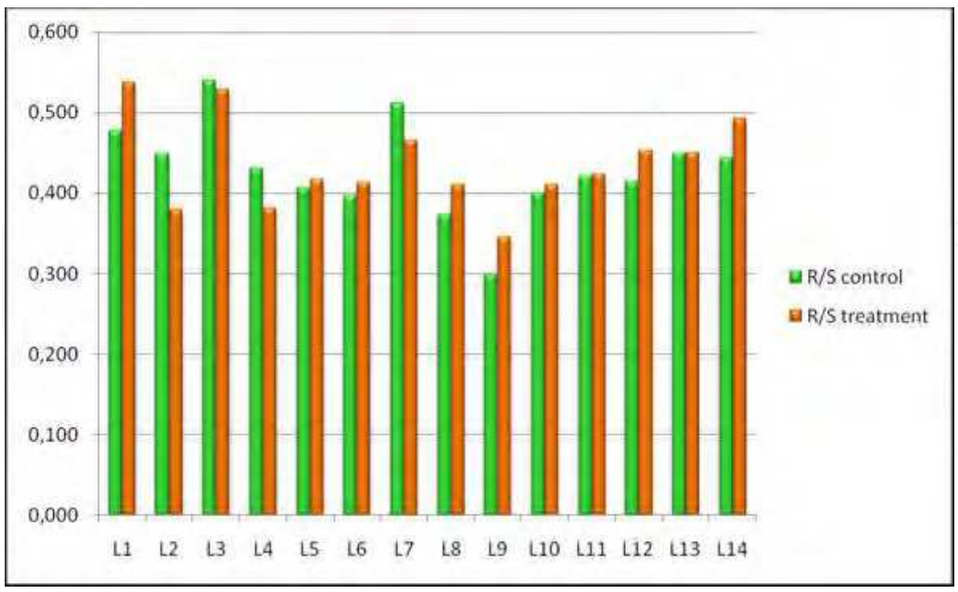


Fig. 6. Root to shoot ratio (dry weight) in maize landraces (L1-L14) at seedling stage

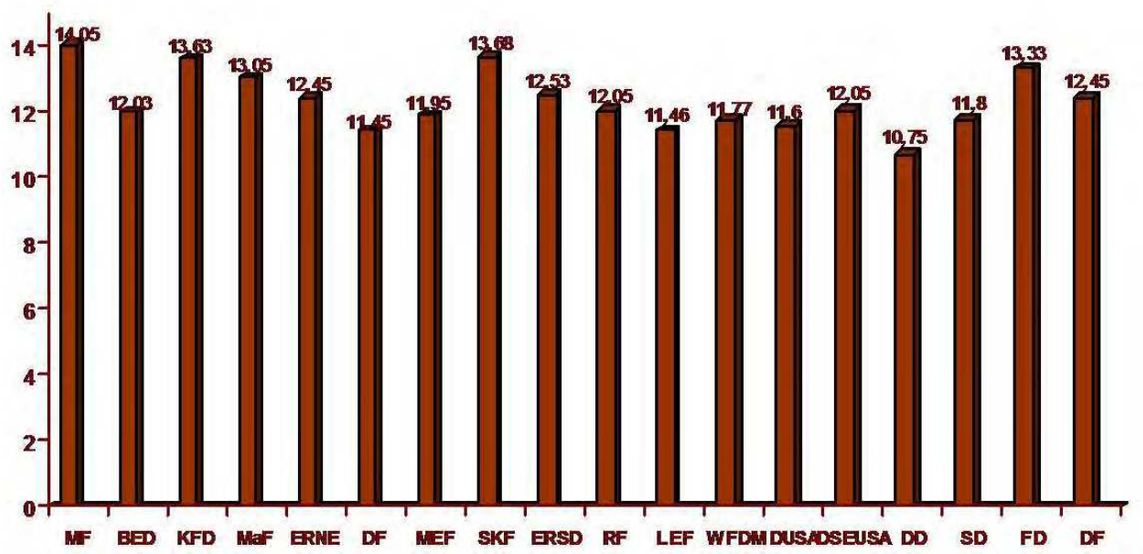
protein, oil and starch content were measured using Infratec 1241 Grain analyzer, (Foss Tecator, Sweden) and expressed in percents (%). Phytate and Pi were determined by the method of Latta & Eskin (1980), modified by Sredojević & Dragicevic (2009).

The genetic variability for protein, oil and starch content was recorded in the analyzed landraces (Andjelkovic et al., 2011). The protein content ranged from a low level of 10.10% (a population from Derivated dents) to a high level of 14.85% (a population from Small-kernelled flints) and averaged 12.34%. The highest average protein content of 14.05% had population from Montenegrin flints and the lowest of 10.75%, one population from Derived dents (Figure 7). Landraces belonging to flint types have higher protein content than dent type landraces.

Populations from Montenegrin flints and Macedonian flints had the highest average and the second highest average oil content, 4.8% and 4.75%, respectively (Figure 8). The lowest average oil content had populations from Large-eared flints, 3.63%. Landraces belonging to flint types had higher oil content than landraces of dents type.

The starch content of maize landraces ranged from 66.35% to 71.50% and averaged 68.61% (Figure 9). The highest average starch content had populations from Derived flints (70.43%), and the lowest populations from Montenegrin flints (67.53%). Among landraces some interesting forms with high level of starch content were identified: Dent flints pop. 709 (71.10%), Derived flints pop. 190 (71.50%) and pop. 186 (70.95%), Dent type of USA Corn Belt Dents pop. 268 (70.85%).

It could be concluded that many tested landraces show an exceptional kernel quality based on the oil, protein and starch content, and this makes them suitable to be used for futher breeding improvment programs.



Abbreviations: MF-Montenegrin flints; BED-Bosnian early dent; KFD-Kosmet flinty dents;MaF-Macedonian flints; ERME-Eight rawed corn type of NE America; DF-Derived flints; MEF-Mediterranean flint, SKF-Small kernel flint; ERSD-Eight rowed soft dents; RF-Romanian flints; LEF-large-earted flints, WFCM- White flinty dents Moravac; DUSA-Dent type of USA corn belt dents; DSEUSA-Dent type of southern east USA; DD-Derived dents; SD-Serbian dents; FD-Flinty dents; DF-Denty flints.

Fig. 7. Protein content of landraces of agro ecological groups

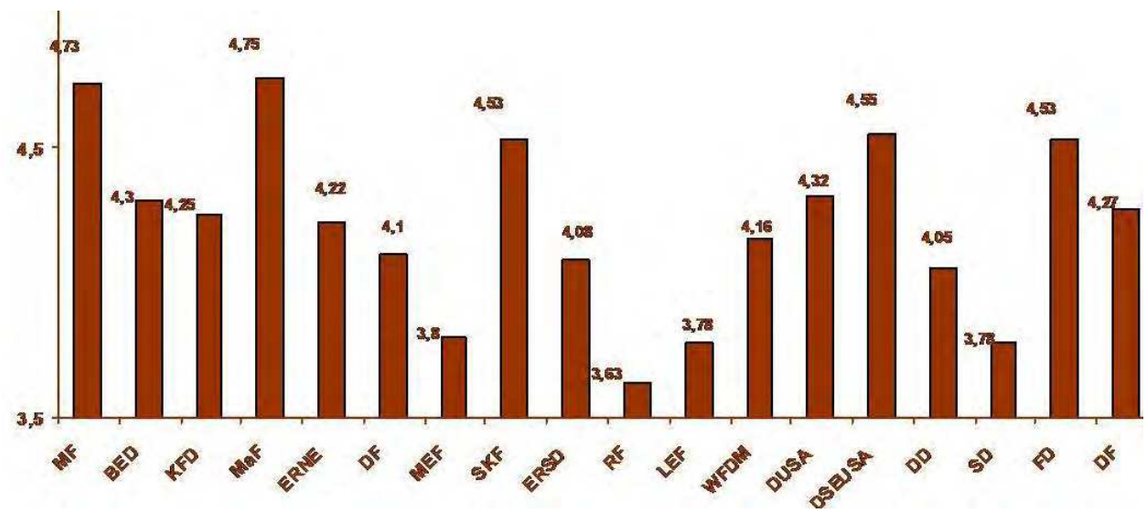


Fig. 8. Oil content of agroecological groups of landraces, abbreviations are same as on Fig. 7

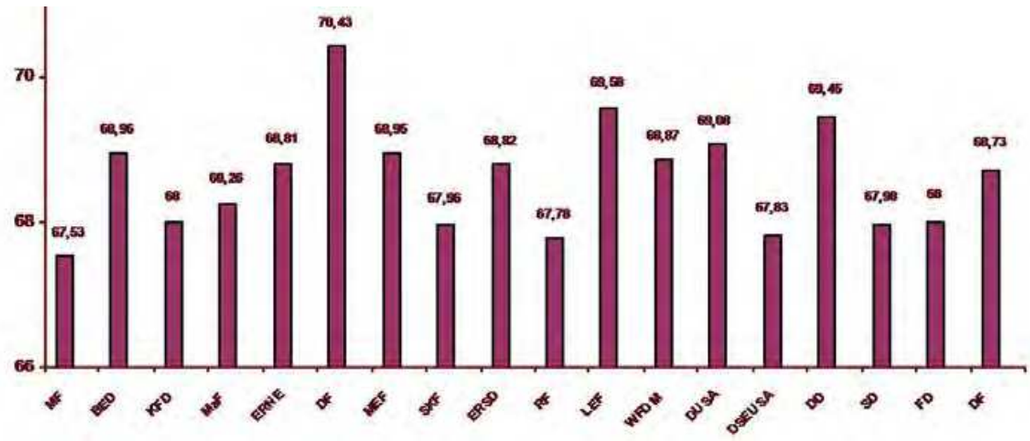


Fig. 9. Starch content of agroecological groups of landraces, abbreviations are same as on Fig.7

Many of the problems associated with P in maize grain are not due to concentration of the total P per se, but rather to the fact that most of the P is bound in phytate. Therefore, it would be desirable to increase the amount of available P and reduce the amount of phytate in maize grain. Approaches to resolve problems of the bad nutritive quality of grain phytate include engineering of crops to express high levels of *phytase* enzyme in seeds (Brinch-Pedersen et al, 2002), or through recurrent selection that uses the indigenous quantitative genetic variation (Raboy, 2009). Genetic variability in phytate contents of 54 landraces was observed, with values in the range from 1,147 to 4,13 and the average of 2.91 g kg-1 (Drinic et al, 2009). Twenty six population had high, twenty intermediate and eight low phytate content. Populations beloging to Derived flints had the lowest (1.95 g kg-1) while populations from Flinty dents had the highest (3.43 g kg-1) phytate content ,(Figure 10).

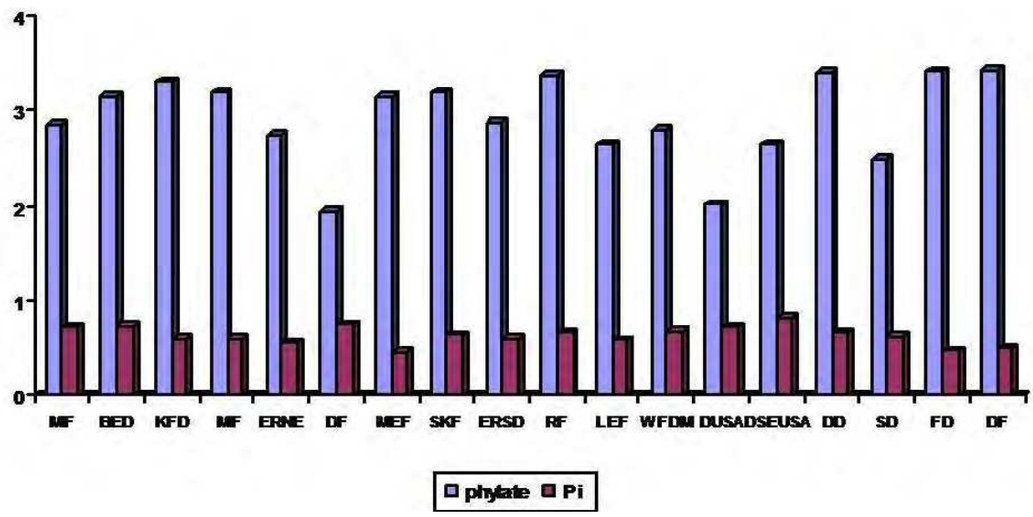


Fig. 10. Phytate and Pi contents of agroecological groups of landraces, abbreviations are same as on Fig.7

Populations from Mediterranean flints had the lowest (0.46 g kg-1) and populations from Dent type of southern east USA the highest (0.82 gkg-1) Pi content. One of the populations, i.e. population 216 was determined to have the lowest phytate concentration of 1.14 g kg-1,

a Pi concentration 30% greater than Pi mean and will be used for further breeding genotypes with low phytate content and good agronomic traits.

7. Conclusion

Landraces were developed through a complex adaptation process of different orginal genotypes to diverse climatic and soil conditions and therefore are the most accessible part of maize biodiversity. Genetic diversity of landraces have important role in maize breeding and could have a significant impact on the improvment of plants as a valuable source of useful traits. A considerable diversity among landraces from MRI genbank has been observed with morphological and molecular markers. The landraces are autochthonous source of potentially useful traits and alleles for improvement of the existing modern varieties. Fourteen most drought tolerant landraces were selected for further breeding programme aimed to broaden the genetic base of the elite breeding material. Presence of a wide genetic diversity of landraces based on their oil, protein, starch and phytate content, makes them more than suitable to be used for further maize breeding improvement programs.

8. References

Anderson, E.&Cutler, C. (1942). Races of Zea mays L.1.Their recognition and classification. *Ann.Missouri Bot.Gard*.29, pp.69-89.

Andjelkovic, V.; Kravic, N.; Vancetovic, J.; Dragicevic V. & Drinic Mladenovic S. (2011). Maize landraces as a natural source of beneficial traits. Proceeding of the 4th International scientific conference, Vukovar, Croatia, 1-3.06.2011, pp. 37-45, ISBN 978-953-7693-01-5

Andjelković, V.; Božinović, S.; Pavlov, M. & Vančetović, J. (2010). Combining abilities for grain yield of the most drought tolerant maize accessions from the MRI gene bank. In: *Book of Proceedings of 45th Croatian & 5th International Symposium on Agriculture*, February 15-19, 2010, Opatija, Croatia, pp. 368-371.

Banziger, M. & Lafitte, H.(1997). Breeding tropical maize for low N environments: II. The values of secondary traits for improving selection gains under low N. *Crop Science*, Vol.37, pp.1110- 1117, ISSN 0931-2250

Barata, C. & Carena, M. (2006). Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. *Euphytica*, Vol. 151, pp. 339-249. ISSN 0014-2336

Barcaccia,G.; Lucchin, M. & Parrini, P. (2003). Characterization of a flint maize (*Zea mays* var. *indurata*) Italian landrace, II. Genetic diversity and relatedness assessed by SSR and Inter-SSR molecular markers. *Genetic Resources and Crop Evolution,* Vol.50, pp. 253–271, ISSN 0925-9864

Barker, T.; Campos, H.; Cooper, M.; Donlan, D.; Edmeades, G.; Habben, J.; Schussler, J.; Wright, D. & Zinselmeier, C. (2005). Improving drought tolerance in maize. *Plant Breeding Reviews*, Vol. 25. Pp. 173-253. ISSN 0730-2207

Bassam, B.J.; Caetano -Anolles, G.& Gresshoff, P.M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, Vol. 196, pp. 80-83, ISSN 0003-2697

Berardo, N.; Mazzinelli, G.; Valoti, P.; Lagana, P. & Redaelli, R. (2009). Characterization of maize germplasm for the chemical composition of the grain. *J. Agric. Food Chem*., 57, pp.2378-2384, ISSN 0021-8561.

Bolaños, J. & Edmeades, G. (1996). The importance of the anthesissilking interval in breeding for drought tolerance in tropical maize. *Field Crops Research*, Vol, 48, pp.65–80. ISSN 0378-4290

Brinch-Pedersen, H.; Sørensen, L.D. & Holm P.B. (2002). Engineering crop plants: getting a handle on phosphate. *Trends Plant Sci*. 7, pp.118–125. ISSN 1360-1385

Buckler, E.S.; Gaut, B.S. & McMullen, M.D. (2006). Molecular and functional diversity of maize. *Current Opinion in Plant Biology*, Vol. 9, pp.172-176, ISSN 1369-5266

Carena; M., Bergman, G.; Riveland, N.; Eriksmoen, E. & Halvorson, M. (2009). Breeding maize for higher yield and quality under drought stress. *Maydica,*Vol. 54, pp.287-296. ISSN 0025-6153

Cooper, M.; Podlich, D. & Smith; O. (2004). Complex traits and gene-to-phenotype models. In: *Proceedings of the 4th International Crop Science Congress*, pp. 67, Brisbane, Australia.

Demeke, T.; Sasikumar, B.; Hucl, P. & Chibbar, R.N. (1997). Random amplified polymorphic DNA (RAPD) in cereal improvement. *Maydica*, Vol.42, pp. 133-142, ISSN 00256153

Dewey, R.E.; Levings, C.S.III; & Timothy, D.H. (1986). Novel recombinations in the maize mitochondrial genome produce a unique transcription unit in the Texas male sterile cytoplasm. *Cell*, Vol. 44, pp.439–449, ISSN 0092-8674

Dewey, R.E.; Timothy, D.H. & Levings, C.S.III. (1991). Chimeric mitochondrial genes expressed in the C male sterile cytoplasm of maize, *Current Genetics*, Vol. 20, pp. 475–482, ISSN 0172-8083

Dhanda, S. & Sethi, S. (2002). Tolerance to drought stress among selected Indian wheat cultivars. *Journal of Agricultural Science,* Vol. 139, pp. 319-326. ISSN 0021-8596

Drinic Mladenovic, S.; Ristic, D.; Sredojevic S.; Dragicevic V.; Ignjatovic Micic, D. & Delic N. (2009). Genetic variation of phytate and ionorganic phosphorus in maize population. *Genetika*, Vol. 41, No. 1, pp. 107 -115, ISSN 0534-0012.

Dubreil, P. & Charcosset, A. (1998). Genetic diversity within and among maize populations: a comparison between isozyme and nuclear RFLP loci. *Theoretical and Applied Genetics,* Vol. 96, pp. 577-587, ISSN 0040-5752

Dubreuil, P.; Rebourg, C.; Merlino, M. & Charcosset, A. (1999). The DNA-pooled sampling strategy for estimating the RFLP diversity of maize populations. *Plant Molecular Biology Reporter*, Vol. 17, pp. 123-138, ISSN 0735-9640

Dubreuil, P.; Warburton, M.; Chastanet, M.; Hoisington, D. & Charcosset, A. (2006). More on the introduction of temperate maize into Europe: large scale bulk SSR genotyping and new historical elements. *Maydica*, Vol. 51, pp. 281-291, ISSN 00256153

Edmeades, G.; Bolaños, J.; Lafitte, R.; Rajaram, S.; Pfeiffer, W. & Fischer, A. (1989). Traditional approaches to breeding for drought resistance in cereals, In: *Drought resistance in cereals,* F.W.G. Baker, (Ed.), 27–52, ICSU and CABI, ISBN 0 85198 641 2, Wallingford, UK.

El-Nadi, A. (1969). Efficiency of water use by irrigated wheat in Sudan. *Journal of Agricultural Science* (Cambridge). Vol. 73, pp. 2267-2266. ISSN 0021-8596

Eschholz, T. W.; Stamp, P.; Peter, R.; Leipner, J. & Hun d, A. (2010). Genetic structure and history of Swiss maize (Zea mays L. ssp. mays) landraces. *Genetic Resources and Crop Evolution,* Vol.57, pp. 71–84, ISSN 0925-9864

Fischer, K.; Lafitte, R.; Fukai, S.; Atlin, G. & Hardy, B. (2003). Breeding rice for drought prone environments. Los Baños (Philippines): International Rice Research Institute. 98 p. ISBN 971-22-0189-9

Frensch, J. (1997). Primary responses of root and leaf elongation to water deficits in the atmosphere and soil solution. *Journal of Experimental Botany*, Vol. 48, pp. 985–999, ISSN 0022-0957

Galarreta de Ruiz, J.I. & Alvarez, A. (2001). Morphological classification of maize landraces from northern Spain. *Genetic Resources and Crop Evolution,* Vol. 48, pp. 391–400, ISSN 0925-9864

Gauthier, P.; Gouesnard, B.; Dallard, J., Redaelli, R.; Rebourg, C.; Charcosset, A. & Boyat, A. (2002). RFLP diversity and relationships among traditional European maize populations. *Theoretical and Applied Genetics,* Vol.105, pp. 91-99, ISSN 0040-5752

Goodman, M.M. (1994). Maize. In: *Evolution of Crop Plants*, Simmonds N.W. (ed.), ISBN-10: 0470233729, Longman Group, Ltd., Essex, UK,

Grant, F.; Jackson, S.; Kiniry, R. & Arkin, F. (1989). Water deficit timing effects on yield components in maize. *Agronomy Journal,* Vol. 81, pp.61–65. ISSN 0002-1962

Hall, A.; Chimenti, N.;Trapani, F. & Cohen de Hunau, R. (1984). Yield in water-stressed maize genotypes: association with traits measured in seedling and in flowering plants. *Field Crops Research,* Vol. 9, pp. 41-46. ISSN 0378-4290

Hall, A.; Lemcroft, J. & Trapani, N. (1981). Water stress before and during flowering in maize and its effects on yield, its components and their determinants. *Maydica,*Vol. 26, pp.19-38. ISSN 0025-6153

Hallauer, A.R.; Russell, W.A. & Lamkey, K.R. (1988). Corn breeding. In: *Corn and Corn Improvemen,* Sprague G.F. and Dudley J.W. (eds), 463–564, ISBN 0-89054-029-2, Am. Soc. Agron., Inc., Madison, Wisconsin, USA,

Hammer, K.; Diederichsen, A. & Spahillari, M. (1999). Basic studies toward strategies for conservation of plant genetic resources. In: Serwinski J, Feberova´ I (eds) Proceedings of technical meeting on the methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, Prague

Hartings, H; Berardo, N.; Mazzinelli, G.F.; Valoti, P.; Verderio, A. & Motto, M. (2008). Assessment of genetic diversity and relationships among maize(Zea mays L.) Italian landraces by morphological traits and AFLP profiling. *Theoretical and Applied Genetics,* Vol. 117, pp. 831–842, ISSN 0040-5752

Has, V.; Has, I.; Pamfil, D.& Copandean A. (2010). Characterization of „turda" maize germplasm for the chemical composition of the grain. *Romanian Agricultural Research*, 27, pp.59-67, ISSN 1222 - 4227

Ignjatović-Micić, D.; Mladenović Drinić, S.; Nikolić, A. & Lazić-Jančić, V. (2007). Comparison of AFLP and SSR markers for genetic diversity studies in maize populations. *Maydica,* Vol. 52, pp. 399, Vol. -406, ISSN 00256153

Ignjatovic-Micic, D.; Mladenovic Drinic, S.; Nikolic, A. & Lazic-Jancic, V. (2008). SSR analysis for genetic structure and diversity determination of maize local populations from former Yugoslavia territories. *Russian Journal of Genetics,* Vol. 44, No. 11, pp. 1317–1324, ISSN 1022-7954

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Nat. 44: 223-270.

Jarne, P. & Lagoda, P.J.L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution*, Vol. 11, pp. 424-429, ISSN 0169-5347

Karp, A.; Edwards, K.; Bruford, M.; Vosman, B.; Morgante, M.; Seberg, O.; Kremer, A.; Boursot, P.; Arctander, P.; Tautz, D. & Hewitt, G. (1997). Newer molecular technologies for biodiversity evaluation: opportunities and challenges. *Nature Biotechnology*, Vol.15, pp. 625-628, ISSN 1087-0156

Kaul,M.L.H. (1988). *Male Sterility in Higher Plants.* Springer-Verlag Berlin, Germany.ISBN 0387179526

Kravić, N.; Vuletić, M. & Anđelković, V. (2010). Maize roots responses to osmotic stress. *Journal of Scientific Agricultural Research*, Vol.71 (255), pp. 57-65. ISSN 0354-5695

Latta, M. & Eskin M. (1980). A simple and rapid colorimetric method for phytine determination. *J.Agric. Food Chem.28*, pp.308-1311, ISSN 0021-8561.

Liu, Z.; Peter, S.O.; Long, M.; Weingartner, U.; Stamp, P. & Kaeser, O. (2002). A PCR assay for rapid discrimination of sterile cytoplasm types in maize, *Crop Science*, Vol. 42, pp. 566–569, ISSN 1835-2693

Lubberstedt, T.; Melchinger, A.E.; Dussle, C.; Vuylsteke, M. & Kuiper, M. (2000). Relationship among early European maize inbreds. IV.Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. *Crop Science*, Vol. 40, pp. 783–791, ISSN 1835-2693

Lucchin, M.; Barcaccia, G. & Parrini, P. (2003). Characterization of a flint maize (*Zea mays* L. convar. *mays*) Italian landrace: I. Morpho-phenological and agronomic traits. *Genetic Resources and Crop Evolution,* Vol. 50, pp. 315–327, ISSN 0925-9864

Maxted, N.; Ford-Lloyd, B. & Hawkes, J.G. (1997). Complementary conservation strategies. In: *Plant Genetic Conservation: The in situ Approach*, Maxted N., Ford-Lloyd B. and Hawkes J.G. (eds), 15–40, ISBN-10 0412637308, Chapman & Hall, London.

Mian, M.A.; Hopkins, A.A. & Zwonitzer, J.C. (2002). Determination of genetic diversity in tall fescue with AFLP markers. *Crop Science*, Vol. 42, pp. 944-950, ISSN 1835-2693

Mittelmann, A.; Filho, J; Nass,L; Lima,G.; Hara-Klein, C & da Silva R. (2011).Quantitative variation for grain quality in brazilian maize populations. *Sci. Agric. (Piracicaba, Braz.*), Vol.68, n.1, pp.50-56, ISSN 0103-9016

Monneveux, P.; Sanchez, C. & Tiessen, A. (2008). Future progress in drought tolerance in maize needs new secondary traits and combinations. *Journal of Agricultural Science*, Vol. 146, pp. 287–300. ISSN 0021-8596

Morgan, J. (1980). Osmotic adjustment in the spikelets and leaves of wheat. *Journal of Experimental Botany,* Vol. 31, pp. 655-665. ISSN 0022-0957

Moussa, H. & Abdel-Aziz, S. (2008). Comparative response of drought tolerante and drought sensitive maize genotypes to water stress. *Australian journal of Crop Science,* Vol.1, pp.31-36. ISSN 1835-2693

Nakajima, Y.; Yamamoto, T.; Muranaka, T. & Oeda, K. (1999). Genetic variation of petalord male-sterile cytoplasm of carrots revealed by sequence-tagged sites (STSs). *Theoretical and Applied Genetics,* Vol. 99, pp. 837-843, ISSN 0040-5752

Ortiz, R.; Sevilla, R.; Alvarado, G. & Crossa, J. (2008). Numerical classification of related Peruvian highland maize races using internal ear traits. *Genetic Resources and Crop Evolution*, Vol. 55, pp.1055–1064, ISSN 0925-9864

Pavlicic, J. & Trifunovic, V. (1966). A study of some important ecologic corn types grown in Yugoslavia and their classification. *Journal of Scientific Agricultural Research* 19, pp.44-62, ISSN 0354-5695

Pejić, I.; Ajmone-Marsan, P.; Morgante, M.; Kozumplick,V.; Castiglioni, P.; Taramino, G. & Motto, M. (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theoretical and Applied Genetics,* Vol. 97, pp. 1248-1255, ISSN 0040-5752

Pollak, L.M.& Scott, M.P. (2005). Breeding for grain quality traits. *Maydica*, 50, pp. 247-257, ISSN 0025-6153

Qualset, C.O.; Damania, A.B.; Zanatta, A.C.A. & Brush, S.B. (1997). Locally based crop plant conservation. In: *Plant Genetic Conservation: The in situ Approach,* Maxted N., Fordof Lloyd B. and Hawkes J.G. (eds), ISBN 0412637308,Chapman & Hall, London, pp. 160–175.

Raboy, V. (2009). Approaches and challenges to engineering seed phytate and total phosphorus. *Plant Sci*., Vol. 177, pp. 281–296, ISSN 0168-9452

Radovic, G.; Muminovic, J.&Jelovac D. (2000). Local maize germplasm-potentially valuable breeding material. *Genetika*, Vol.32, no.3, pp.221-234, ISSN 0534-0012.

Rebourg, C.; Gouesnard, B. & Charcosset, A. (2001). Large scale molecular analysis of traditional European maize populations. Relationships with morphological variation. *Heredity,* Vol.86, pp. 574-587, ISSN 0018-067X

Rebourg, C.; Gouesnard, B.; Welcker, C.; Dubreuil, P.; Chastanet, M. &, Charcosset, A. (2003) Maize introduction into Europe: the history reviewed in the light of molecular data. *Theor Appl Genet,* Vol. 106, pp.895–903, ISSN 0040-5752

Reif, J.C.; Hamrit, S.; Heckenberger, M.; Shipprack, W.; Maurer, H.P.; Bohn, M. & Melchinger, A.E. (2005). Genetic structure and diversity of European flint maize populations determined with SSR analysis of individuals and bulks. *Theoretical and Applied Genetics,* Vol. 111, pp. 906-913, ISSN 0040-5752

Reynolds, T. L.; Nemeth, M. A.; Glenn, K. C.; Ridley,W.P.& Astwood, J. D. (2005). Natural variability inmetabolites in maize grain: Difference due to genetic background. *J. Agric. Food Chem*., Vol. 53, pp.10061-10067, ISSN 0021-8561

Ribaut, J. (2006). *Drought adaptation in cereals.* Food Products Press, ISBN-13: 978-1-56022-27-4, New York, USA.

Ribaut, J.; Hoisington, D.; Deutsch, J.; Jiang, C.&Gonzales de Leon, D. (1996). Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. *Theoretical and Applied Genetics,* Vol. 92, pp. 905-914. ISSN 0040-5752

Richards,R.(1996). Defining selection criteria to improve yield under drought. *Plant Growth Regulatio*n, Vol. 20*,* pp. 57–166. ISSN 0167-6903

Rogers, J.S. (1972). Measures of similarities and genetic distances.pp. 145-153. In: M.R. Wheeler (Ed.), *Studies in Genetics*. VII. vol. 7213, Univ. of Texas Publications.

Ruta, N.; Liedgens, M.; Fracheboud, Y.; Stamp, P. & Hund A. (2010). QTLs for the elongation of axile and lateral roots of maize in response to low water potential. *Theoretical and Applied Genetics,* Vol. 120, pp. 621-631. ISSN 0040-5752

Saghai_Maroof, M.; Soliman, K.; Jorgensen, R. & Allard, R. (1984). Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of the National Academy of Sciences of the USA*, Vol. 91, pp. 8014–8018, ISSN 0027-8424

Sato, Y. (1998). PCR amplification of CMS-specific mitochondrial nucleotide sequences to identify cytoplasmic genotypes of onion (*Allium cepa* L.). *Theoretical and Applied Genetics,* Vol.96, pp. 367-370, ISSN 0040-5752

Schnable, P.S. & Wise, R.P. (1998). The Molecular Basis of cytoplasmic male sterility and fertility restoration. *Trends in Plant Science,* Vol. 3, pp. 175–180, ISSN 1360-1385

Sharp, R. & Davies, W. (1979). Solute regulation and growth by roots and shoots in water tressed maize plants. *Planta*, Vol. 147, pp. 43–49. ISSN 0032-0935

Sharp, R. & Davies, W. (1989). Regulation of growth and development of plants with a restricted supply of water. In: *Plants under stress*. H.G. Jone et al (Ed.), 71-93, Cambridge Univ. Press, ISBN 0521344239, Cambridge, England, UK.

Singh, S.P.; Gutierrez, J.J.A.; Molina, A.; Urrea, C. & Gepts, P. (1991). Genetic diversity in cultivated common bean. II. Marker-based analysis of morphological and agronomic traits. *Crop Science,* Vol. 31, pp. 23–29, ISSN 1835-2693

Smith, J.S.C.; Chin, E.C.L;. Shu, H.; Smith, O.S.; Wall, S.J.; Senior, M.L.; Mitchell, S.E.; Kresovich, S. & Ziegle, J. (1997). An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. *Theoretical and Applied Genetics,* Vol. 95, pp. 163-173, ISSN 0040-5752

Sredojevic, S. & Dragicevic V. (2009). The quantitative determination of antioxidative-protective and some storage substances of seeds and leaves-modification of methodological approach. *Journal of Scientific Agricultural Research*, 70, 249, pp. 13-20, ISSN 0354-5695.

Suharjo, U. (2007). Use of Polyethylene Glycol (PEG) 8000 for Rapid Screening of Potato (*Solanum tuberosum* L.) Genotypes for Water stress Tolerance: III: Root and shoot Growth. *Journal Akta Agrosia Edisi Khusas*, Vol. 1, pp. 11-18, ISSN 1410-3354.

Sweeny, P.M. & Danneberger, T.K. (1997). RAPD markers from perennial ryegrass DNA extracted from seeds. *HortScience,* Vol. 32, pp. 1212-1215, ISSN 0018-5345

van der Weele, C.; Spollen, W.; Sharp, R. & Baskin, T. (2000). Growth of *Arabidopsis thaliana* seedlings under water deficit studied by control of water potential in nutrient-agar media. *Journal of Experimental Botany*, Vol. 51, pp.1555-1562, ISSN 0022-0957

van Etten, J.; Lopez M.R.F.; Monterroso, L.G.M. & Samayoa, K.M.P.(2008).Genetic diversity of maize (Zea mays L. ssp. mays) in communities of the western highlands of Guatemala:geographical patterns and processes. *Genetic Resources and Crop Evolution,* Vol. 55, pp. 303–317, ISSN 0925-9864

Vancetovic, J.; Andjelkovic, V. & Shaboon, S. (2008). The identification of drought tolerant sources from the MRI gene bank. Modern variety breeding for present and future needs.In: *Proceedings of the 18th EUCARPIA General Congress*, 9-12.September 2008.Valencia-Spain, pp. 261.

Vancetovic, J.; Vidakovic, M.; Ignjatovic-Micic, D.; Nikolic, A.; Markovic, K. & Andjelkovic V. (2010). The structure of sterile cytoplasm types within a maize genebank collection. *Russian Journal of Genetics*, Vol.46, No.7, pp. 836–840, ISSN 1022-7954

Verslues, P.; Agarwal, M.; Katiyar-Agarwal, S.; Zhu, J. & Zhu, J. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant Journal,* Vol. 45, pp. 523–539, ISSN 0960-7412

Weerathaworn, P.; Soldati, A. & Stamp P. (1992b). Anatomy of seedling roots of tropical maize (*Zea mays* L.) cultivars at low water supply. *Journal of Experimental Botany,* Vol. 43, pp. 1015–1021, ISSN 0022-0957

Weerathaworn, P.; Soldati, A. & Stamp, P. (1992a). Shoot and root growth of tropical maize seedlings at different moisture regimes. *Maydica*, Vol. 37, pp.209-215, ISSN 0025-6153

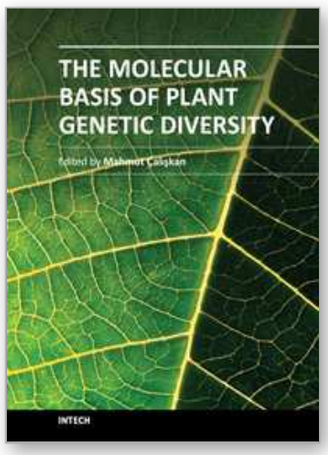
Westgate, M. & Boyer, J.(1985). Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. *Planta*, Vol. 164, pp. 540-549, ISSN 0032-0935

Yancey, P.; Clark, M.; Hand,S.; Bowlus,R. & Somero, G. (1982). Living with water stress: evolution of osmolyte systems. *Science*, Vol. 217, pp. 1214-1222, ISSN 0036-8075

Yu, K. & Pauls, K.P. (1993). Rapid estimation of genetic relatedness among heterogeneous populations of alfalfa by random amplification of genomic DNA samples. *Theoretical and Applied Genetics,* Vol. 86, pp. 788-794, ISSN 0040-5752

Zabala, G.; Gabay-Laughnan, S. & Laughnan, J.R. (1997). The nuclear gene *Rf3* affects the expression of the mitochondrial chimeric sequence R in S-type male sterility in maize. *Genetics*, Vol. 147, pp. 847–860, ISSN 0016-6731

Zhu, K. (2002). Salt and drought stress signal transduction in plants. *Annual Review in Plant Biology,* Vol.53, pp.247-273, ISSN 1543-5008



The Molecular Basis of Plant Genetic Diversity

Edited by Prof. Mahmut Caliskan

ISBN 978-953-51-0157-4
Hard cover, 374 pages
Publisher InTech
Published online 30, March, 2012
Published in print edition March, 2012

The Molecular Basis of Plant Genetic Diversity presents chapters revealing the magnitude of genetic variations existing in plant populations. Natural populations contain a considerable genetic variability which provides a genomic flexibility that can be used as a raw material for adaptation to changing environmental conditions. The analysis of genetic diversity provides information about allelic variation at a given locus. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen