ISSR markers as a tool to distinguish Idt and SSS populations of *Zea mays* L

Rozlíšenie Idt a SSS populácií kukurice siatej (*Zea mays*, L) ISSR markérmi

Jana ŽIAROVSKÁ[1]*, Slavomíra SENKOVÁ[1], Milan BEŽO[1] , Katarína RAŽNÁ[1], Miloslav MASNICA[2] and Mária LABAJOVÁ[1]

1) Department of Genetics and Plant Breeding, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976, Nitra; correspondence e-mail: jana.ziarovska@uniag.sk

2) ZEAINVENT TRNAVA s.r.o.; ŠM Farský Mlyn 2/6913 Trnava 917 01 Slovakia

Abstract

Maintaining genetic diversity for crop improvement and improving the quality of genetic resource management is both an inevitable part of nowadays maize breeding. ISSR markers brings the potential of finding a marker system to discriminate Iowa Stiff Stalk Synthetic and Iodent Reid populations of *Zea mays*, L. and on the base on coefficients of genetic distance and UPGMA grouping appropriate maize genotypes for the best potential for hybridization can be selected. The work presents the potential of ISSR markers in analysis of length polymorphism when a specific ISSR pattern can be used for fast screening of differences among maize genotypes and based of these differences lines can be used in breeding programms in a very specific mode.

Key words:
breeding, ISSR, Idt population, maize, SSS population

Abstrakt

Zachovanie genetickej rozmanitosti v šľachtení rastlín a zvyšovanie kvality genetických zdrojov sú nevyhnutnou súčasťou aj súčasného šľachtenia kukurice. ISSR markéry umožňujú realizovať takúto požiadavku ako markérovací systém schopný generovať odlišné dĺžkové profily pre Iowa Stiff Stalk Synthetic a Iodent Reid populácie kukurice a na základe koeficientov genetickej vzdialenosti a UPGMA zoskupení vybrať genotypy kukurice poskytujúce najlepší potenciál vo vzájomnom krížení. V práci je prezentovaná využiteľnosť ISSR markérov pri rýchlom skríningu odlišností jednotlivých línií kukurice a zapojiteľnosť takýchto výsledkov do praktických otázok šľachtenia.

Kľúčové slová:
ISSR, Idt populácie, kukurica siata, SSS populácie, šľachtenie

Detailed abstract

V práci je analyzovaný potenciál náhodne sa naväzujúcich mikrosatelitných markérov odlíšiť ISS profil línií kukurice siatej patriacich k Iowa Stif Stalk Synthetic a Iodent Reid líniám. Biologický materiál vstupujúci do analýz bol tvorený desiatimi líniami tak, aby boli zastúpené línie s potvrdeným typom, línie v procese šľachtenia a spolu s nimi línia s otáznym typom, ktorú bolo cieľom na základe molekulárnych analýz priradiť vo vetvovom členení k jednej zo skupín. Línia s otáznym genotypom, o ktorej sa predpokladalo, že je Idt x SSS poskytovala v rozpore s predpokladom jej pôvodu veľmi dobrý heterózny efekt s SSS líniami. Biologický materiál bol získaný zo Zeainvent Trnava s.r.o., SR. Predpokladaný pôvod, resp. príslušnosť línii do skupín, bol odhadnutý na základe mnohoročných pozorovaní pri výbere šľachtiteľského materiálu, vychádzajúc predovšetkým z rodokmeňov línií. Celková genomická DNA bola izolovaná z čerstvých, zelených listov podľa Protokolu Rogers a Bendich. Analýzy ISSR markérmi boli uskutočnené s celkovo desiatimi prajmermi, z ktorých dva, $(CA)_6GT$ a $GT(CA)_4$, boli vyhodnotené ako markéry s potenciálom odlíšiť SSS a Idt línie a priradiť spornú líniu k jednej zo skupín. Molekulárne analýzy prebehli v dvoch samostatných cykloch. V prvom boli uskutočnené testy schopnosti markérov odlišovať SSS a Idt línie a ich vzájomné zoskupenie vo vetvovom členení na základe Jaccardovho koeficientu genetickej príbuznosti. V týchto analýzach boli zaradené iba línie s potvrdeným typom, jedinú výnimku tvorila línia s otáznym typom tak, aby v následných analýzach bolo možné potvrdiť stabilitu zoskupovania vo vetvovom členení. Vo vetvovom členení bola línia otázneho pôvodu zaradená do zhluku Idt genotypov. Veľkosť framentov zmnožených v ISSR sa pohybovala v rozpätí 230 - 1400 bp a jednotlivé použité prajmery dosahovali úroveň polymorfizmu od 77 % do 73 %. V druhom cykle analýz boli do reakcií s prajmermi vyselektovanými v prvom cykle všetky analyzované línie. V prípade zoskupovania testovaných línií a línie so sporným pôvodom zostalo zoskupovanie známych línií nezmenené a línia s otáznym genotypom bola opätovne priradená k Idt líniám. Idt založenie jej pôvodu namiesto predpokladaného Idt x SSS bolo následne potvrdené aj v poľných podmienkach.

Introduction

Maize breeding programs depend on the understanding and knowledge of genetic diversity and relationship between inbred lines and breeding material. That is especially fundamental in assigning inbreeds to heterotic groups and planning outstanding hybrid crosses (Srdic, et al., 2007). For exploiting the potential of hybrid breeding in maize, many maize inbreeds have been developed from a limited number of elite lines and elite line synthetics, a practice that heightens the risk of decreased genetic diversity in commercial maize production fields (Hallauer, et al., 1988). The identification of superior hybrid is important for the success of a hybrid breeding program. However, field evaluation of all possible crosses between inbred lines require extremely large resources (Schrag, et al., 2010).
Developing and selecting inbreeds in classical breeding programs and evaluating hybrid performance from extensive yield trials is easy, but also costly and time consuming. The high level of dominance for the grains yield makes impossible to predict hybrid performance based on the performance of inbred parent (Srdic, et al., 2007). Moreover, the large number of possible hybrids produced from relatively small

number of inbred parents does not allow the evaluation of all hybrids (Hallauer, et al., 1990).

The prediction of a combinative potential of inbred lines entering hybridization is a fundamental step of efficient maize hybrid production. A combinative potential from genetic polymorphism and genetic diversity among inbred lines can be determined. Heterosis is a phenomena of commercial maize breeding and the result of combinative potential (Bežo, et al., 2007). Breeders knowledge about population and genetic diversity of lines is the keystone in breeding and germplasm preservation. The classification of elite germplasm into heterotic groups and assignment of inbred lines to established heterotic groups are major decisions in any breeding program for hybrid maize (Hallauer, et al., 1988). In the past five decades, many of maize inbreeds have been developed from a limited number of elite lines and elite line synthetics. This engenders the danger of a loss of genetic diversity and restricts the possibility of crosses between genetically divergent parents. Knowledge of the genetic relationship among breeding materials could help to avoid the great risk for an increasing uniformity in the elite germplasm and could ensure long-term utilization gains (Messmer, et al., 1993).

Using of genetic markers based on PCR are nowadays useful in a wide range of different applications in plant, animal and food science for systems of genotyping, identification and authentification of samples (Prasad, et al., 2000; Štefúnová and Bežo, 2002; Ansari-Mahyari, et al., 2008; Bojinov, et al., 2009; Zeleňáková, et al., 2009). The use of genetic markers in plant breeding to assess the genetic divergence among pairs of inbred lines has been suggested as a means to overcome these drawbacks, allowing the prediction of single-cross hybrid performance (Lanza, et al., 1997).

The maize genome is a source of tremendous phenotypic and molecular diversity. Whether measured by allozymes, microsatellites (or Simple Sequence Repeats – SSRs) or DNA sequences, maize has long been known to be genetically diverse. On the DNA sequence level, exotic and elite maize genotypes contain more diversity than humans (Buckler, et al., 2006). Better understanding on the genetic diversity ensures the breeder in planning crosses for hybrid and line development, in assigning lines to heterotic groups, and in plant variety protection (Pejic et al., 1998). In this paper, we report an usefulness of ISSRs as genetic markers to discriminate, and to show associations among inbred lines of maize. Among the microsatellites there is the potential of finding a marker system to discriminate Iowa Stiff Stalk Synthetic and Iodent Reid populations of *Zea mays*, L. and on the base of genetic distance coefficients and UPGMA grouping select the appropriate maize genotypes for the best combinative potential.

Materials and Methods

Tested biological material was kindly provided as private inbred material from Zeainvent Trnava, Slovakia and the publicly available line B84 (SSS- Stiff Stalk Synthetic) originally from the USA was used in analysis. In total ten maize inbred lines were analyzed. The line A which comes from Iodent Reid (Idt) population was represented by one tested sample A. Lines X and Z come from the Iowa Stiff Stalk Synthetic. The other analyzed lines (C, B) originated from an experimental crossing between populations Iodent Reid, Iowa Stiff Stalk Synthetic and Lancaster Sure

Crop. The tested accessions include lines (I1-2, J1-2) (table 1) that are by pedigree not related to the previous groups (table 1).

Table 1. Characteristics of tested accessions

Tabuľka1. Charakteristika analyzovaného biologického materiálu

Synthetic population	Inbred line
Idt	A
SSS	X*
	Z
Idt x Lsc	C
IDt x Lsc x SSS	B
? - (Idt x SSS)	Y
Lsc - C103	I1
Lsc - C123	I2
UN - Cargil 588	J1
UN - type F2	J2

Idt – population Iodent Reid, SSS – population Iowa Stiff Stalk Synthetic, X* - syn. for B84 line that origins in Reid Yellow Dent, what is a variation of Idt, Lsc – population Lancaster Sure Crop, C - 103 + C - 123 - breeding cycle of Lsc, UN - unrelated lines, ? - expected synthetic population
Idt – Iodent Reid populácia, SSS – Iowa Stiff Stalk Synthetic populácia, X* - syn. pre B84 líniu, ktorá má pôvod v Reid Yellow Dent, čo je variant Idt línií, Lsc –Lancaster Sure Crop populácia, C - 103 + C - 123 - cyklus šľachtenia Lsc, UN - necharakterizované línie, ? - populácia sporného pozadia

Leaf samples for DNA isolation was collected from 5 days-old *in vitro* seedlings and the extraction and purification of the genomic DNA from each accession was carried out following the Rogers and Bendich (Rogers and Bendich, 1994). DNA quality and quantity of each genotype was assessed by electrophoresis in the 0.8 (w/v) agarose gel comparing the DNA to the known standards. All the DNA samples were diluted to have a final concentration of 10 ng/µl.

A total of 12 ISSR primer pairs [$(GT)_6CC$, $(GA)_6AA$, $(GAG)_3GC$, $(GA)_6GG$, $(CTG)_3GC$, $(GA)_9C$, $GT(GA)_4$, $(CA)_6GG$, $(CTC)_2AA$, (CAG)3AA, $(CA)_6GT$ and $GT(CA)_4$], synthesized from MICROSYNTH were used for PCR amplification of repeat sequences from the genomic DNA of each sample. The primer pairs used for final analyses are $(CA)_6GT$ and $GT(CA)_4$. PCR reactions were performed using Biorad MJ Mini™ Gradient Thermal Cycle in 20 µl volume containing 30 ng DNA, 2.9 - 4.1 pmol of each primer, 10x PCR buffer, 2.25 mM dNTPs and 5 units of Taq polymerase. Amplifications were done following the conditions 94°C for 2 minutes followed by 45 cycles of 94°C for 1 minute, 55 °C for 1 minute, 72°C for 3 minutes followed by final extension at 72°C for 10 minutes. PCR products were loaded on 2 % agarose gels and electrophoresis was performed in 1x TBE buffer (pH 8.3) at constant voltage (60V) and amperage (30mA) for 5 hours. The amplified DNA fragments size was identified by comparison to DNA marker (250 bp; INVITROGEN) and evaluated by picture analytical system KODAK 1D.

Only clear and unambiguous bands of ISSR markers were scored. All the markers´ bands were treated as a single locus and across the accessions were scored for their presence or absence of the band. The individual fragment of a given length was recorded in binary code. By comparing the banding patterns of genotypes for a

specific primer, genotype specific bands were identified and faint or unclear bands were not considered. The binary data generated this way was used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Data were evaluated using POPGENE software version 1.32 [16] for the Shannon index (H'j) [17] as defined for multilocus markers as H'j = -∑pi log pi, where pi is the frequency of the *ith* fragment in the sample. Polymorphic information content (PIC) was calculated for each marker using the formula PICi = 1 - ∑P2ij, where Pij is the frequency of the *jth* allele in clone (i) according to Smith et al. (1997). PIC values were used to calculate the Primer index (Rajwade, et al., 2010) generated by summing up the PIC values of all the loci amplified by the same primer. Pair-wise dissimilarity matrices were generated by 1- Jaccard´s coefficient of similarity [18] by using the SYNTAX software format. A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the HIERCLUS module of SYNTAX to show a phenetic representation of genetic relationships as revealed by the dissimilarity coefficient. The ability of each primer individually and also all their combinations to distinguish the SSS and Idt populations was analyzed in cluster analysis and finally based on these results (data not shown) only the markers presented in the study was used because of grouping SSS and Idt maize lines.

Results

Two separate analysis were done. The first, marker discrimination ability was tested to find a marker for SSS and Idt populations grouping in the dendrogram. This analysis was performed with only known lines with clear synthetic population characteristics. The only exception was the line Y. In this line the Idt x SSS was only an assumption, because of in the crosses witd SSS lines a very good heterosis effect was observed (Masnica, personal communication) what was in contrary to the assumption of being this line the Idt x SSS (Figure 1).
Zea mays, L. accessions were analyzed using microsatellite markers of which all produced reproducible polymorphic banding patterns. A total of 22 band levels were obtained of which 16 (72,73 %) were polymorphic. All the polymorphic bands produced by the used set of primers were 160.
The size of the amplified products varied from 230 to 1400 bp. Non of the used marker profiles give the 100% polymorphism, but the individually used primers gives the polymorphism of 77 % and 73 %.
Using the PIC value as a measure of variability at a specific locus gives the information about the probality that polymorphism will exists between two randomly selected genotypes at that locus. The mean polymorphic information content calculated from the frequency of polymorphic bands across all lines was 0,47 (table 2) and the summing up primer values PIC index was 10,3.

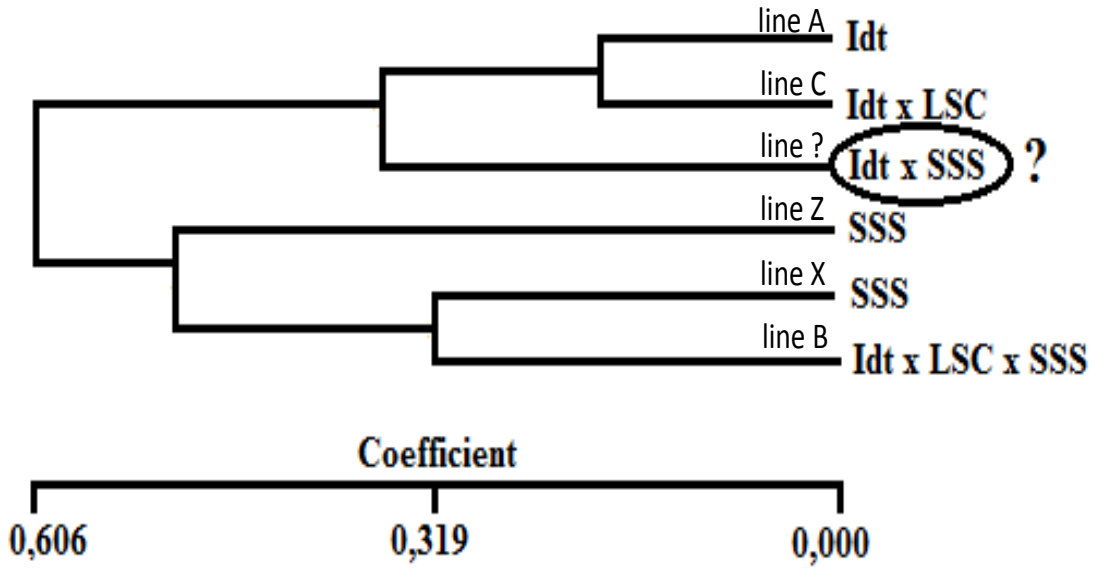

Figure 1. Dendrogram of testing the marker discrimination ability of maize accesssions based on Jaccard´s similarity coefficient for $(CA)_6GT$ and $GT(CA)_4$ primers

Obrázok 1. Dendrogram analýzy schopnosti markéra odlíšiť analyzované populácie kukurice zostrojený na základe Jaccardovho koeficientu genetickej príbuznosti pre prajmery $(CA)_6GT$ and $GT(CA)_4$

The second analysis, when the marker system for SSS and Idt lines was set out, the unrelated and breeding cycles accessions was added into analysis (Figure 2). For the grouping of tested unknown genotype, the primary groups of Idt and SSS maize populations remained unchanged and the inclusion of questionable Idt x SSS genotype among Idt populations was confirmed again. The origin of these hybrids are the subject of corporate know-how and patents and is a subject of testing on the known pedigree crosses contrast testers aimed to the classification of new breeding material and finding of a significant class of origin manifested itself as the dominant representative. Such classified genotype is suitable for further breeding work. Once the genetic relationships are set, the search for crosses of high genetic diversity of a favorable breeding material is done for the purposes of heterosis prediction on important economic traits and characteristics. At this breeding phase it can be very useful to compare dendrograms of molecular markers of all the potential breeding material.

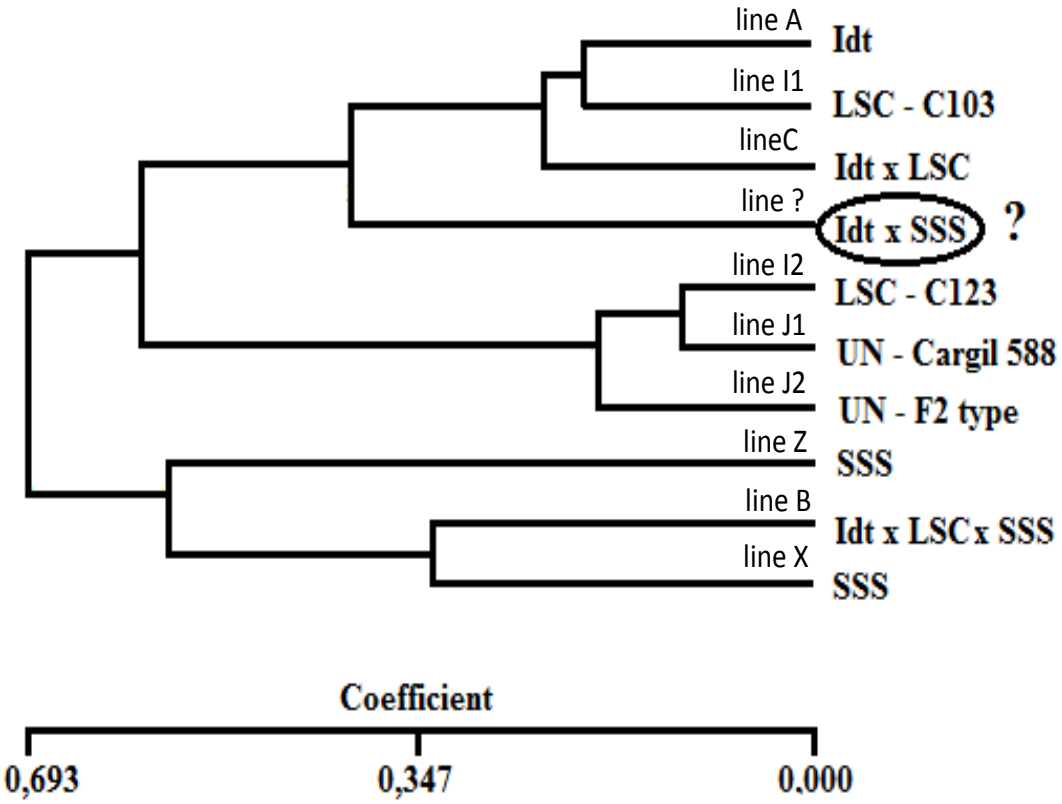

Figure 2. Dendrogram of tested lines in groupes localization of maize accesssions based on Jaccard´s similarity coefficient for $(CA)_6GT$ and $GT(CA)_4$ primers

Obrázok 2. Dendrogram analyzovaných línií kukurice zostojený na základe Jaccardovko koeficientu genetickej príbuznosti pre prajmery $(CA)_6GT$ and $GT(CA)_4$

Table 2. Jaccard´s distance matrix of *Zea mays*, L. accessions

Tabuľka 2. Matica genetických vzdialeností analyzovaných línií kukurice

	A	X	I1	C	Y	Z	B	I2	J1	J2
A	***									
X	0.61	***								
I1	0.15	0.53	***							
C	0.15	0.38	0.20	***						
Y	0.61	0.32	0.53	0.53	***					
Z	0.38	0.53	0.32	0.45	0.26	***				
B	0.38	0.53	0.32	0.20	0.53	0.61	***			
I2	0.32	0.45	0.53	0.38	0.45	0.53	0.38	****		
J1	0.32	0.45	0.53	0.38	0.45	0.53	0.53	0.10	***	
J2	0.45	0.61	0.53	0.53	0.61	0.69	0.38	0.20	0.10	***

In both of dendrograms (figuer 1, figure 2), grouping pattern for two SSS lines is the same, but the closer genetic relationship is observed for populations X and B as for those of X and Z. Here, the wider genetic backround is assumed for ISSR pattern. Similar, LSC-C103 and LSC-C123 are not in the same cluster but in different ones. Both of the possible reasons are discussed later in the text.

The analyzed data and the genetic background of the questioned Y inbred line was confirmed in the field tests (Masnica, personal communication) as to be Idt line. The weak heterosis effect was observed in the crosses with Idt and Lsc population and good heterosis was observed in the crosses with SSS breeding material.

Discussion

Maize is the third important cereal crop in the world next to rice and wheat (Ranatunga, et al., 2009). There exists an urgent need to promote maize breeding to meet the increasing demands for maize grain and its products. In this context, maize hybrid breeding remains the choice of methods considering its success over years. Better understanding on the genetic diversity ensures the breeder in planning crosses for hybrid and line development, in assigning lines to heterotic groups, and in plant variety protection (Rogers and Bendich, 1994).
The number of polymorphic bands generated per primer was very balanced from 12 to 15 what provides a readable and easy scoring markers for maize lines genotyping. The results obtained in the present study is in accordance with the results of Buckler, et al. (2006) where in 6.8 markers per primer were noticed using 27 microsatellite primer pairs. Warburton et al. (Warburton, et al., 2001) reported an average of 6.3 markers per primer using 85 SSR loci and Yu et al. (Yu, et al., 2007) have reported an average of 5.34 markers per primer pair using 49 SSR primer pairs.
The level of polymorphism detected in this study ranged from 73 % to 77 % as for intergenic space markers is comparable by other authors (Srdic, et al., 2007; Smith, et al., 1997) in contrary to (Ranatunga, et al., 2009) - the study summarized the SSR polymorphism among 45 maize genotypes as 100 %. The aim of presented analyse was not to discriminate individually lines among themselves, but grouped lines with the same synthetic population characteristics.
Polymorphism between individuals mainly result from sequence differences in one or both of the primer binding sites and are visible as the presence or absence of a particular amplification product. These polymorphism behave, therefore, as dominant genetic markers (Sperisen, et al., 1998). As the PIC provides a measure that is influenced by both the number and frequency of alleles, the maximum PIC value for markers for markers where two alleles per locus are assumed is 0,5 (Henry et a., 1997; Lee, et al., 2001). The value of 0,47 calculated in this study refers to the high discriminatory capacity for the used markers where the number of 22 loci ensure effective percentages of their frequencies.
Microsatellites as an effective molecular tool in maize breeding is reported by Nagy, et al. (2009) when SSRs are summarized as proved to be extremely efficient in detecting genetic polymorphism between the maize lines, and could be useful tools for characterizing traits such as starch content.
As written in results, in both of dendrograms, grouping pattern for two SSS lines is the same, but the closer genetic relationship is observed for populations X and B as for those of X and Z. ISSR profile as a type of marker system connected mainly to

the intergenic space can display wider genetic backround when comparing to the markers related directly to the coding regions. Some basic lines were developed from SSS - B14A, B64, B68, B73 and B84 (Troyer, 2000). For them, 16 lines were used at the beginning of the breeding process. 10 of them originated in Reid Yellow Dent as a variant of Idt and the rest 6 lines were of unknown origin. The X line used in this study is B84 line, that originates in Reid Yellow Dent and that is why its ISSR profile is more closer to the B line as to the Z line, that originates in unknown lines used in the first breedings. Similar, LSC-C103 and LSC-C123 are not in the same cluster but in different ones. Assumption of this can be thinking in the connection toward the fact, that the lines of the LSC originated as „out crosses“ from Idt lines (Troyer, 2000) and the closer genetic ralationship of the ISSR profile exists for the LSC-C103 as for LSC-C123 as is displayed in the dendrogram.

The use of molecular markers offer significant time saving in breeding process. First, it is not necessary to phenotypic screen the progeny for the desired character, prventing the need to wait until plants reach a specific maturity stage. Instead, plants can be screened at an extremely young age for those containing the correct parental mix of chromosomal regions. Then only those plants that contain the desired mix are then propagated for subsequent rounds of back crossing. Thus both the numbers of plants that can be screened may be increased, and the length of time necessary for identification of positives may be dramatically decreased, especially for plants that take a long time to reach maturity. Second, in terms of back crossing to elite parental lines, the number of back crosses necessary can be significantly decreased using molecular markers. The reason for this comes down to the numbers of plants that can be screened. The more plants that can be screened, the greater the likelihood of identifying one with the appropriate genetic combinations (Brady, et al., 2007).

Conclusion

The relationship among maize inbred lines has been tested using the ISSR markers and the estimation of the lines origin has been confirmed in the filed tests. ISSR provide a discrimination of Idt and SSS lines and is suitable for determining intra population relationships based on the genetic distance coefficients, too. The results of the method can be readily interpreted in terms of loci across a range of maize germplasm. Based on these results, is possible to state that the ISSR markers are suitable for the determination of genetic polymorphism among maize inbred lines and discriminating SSS lines and the technique represents the effective for the identification and pedigree validation of maize. The practical importance of using such an analysis of genetic diversity is in the time savings when identifying the origin of maize lines bred from commercial hybrids characterized by high accumulation of favorable genes conditioning important economic traits and characteristics affecting mainly the performance and stability or performance or adaptability to different environmental conditions. Finally, the use of the identified lines related to hybrid components results into the creation of hybrids with seed productivity increase without any reduction of final hybrid seeds heterosis.

References

Srdic, J., Mladenovic Drinic, S., Pajic, Z., Filipovic, M. (2007) Characterization of maize inbred lines based on molecular markers, heterosis and pedigree data. Genetika. 39, 355 -363.

Hallauer, A.R., Russell, W.A., Lamkey, K.R. (1988) Corn breeding. In: Sprague GF, Dudley JW (eds.) Corn and corn improvement, 3rd edn. Agron. Monogr. 18. ASA, CSSA, and SSSA, Madison, Wisconsin, USA, pp. 463-564.

Schrag, T.A., Mohring, J., Melchinger, A.E., Kusterer, B., Dhillon, B.S., Piepho, H.P., Frisch, M. (2010) Prediction of hybrid performance in maize using molecular markers and joint analyses of hybrids and parental inbreds. Theor Appl Genet. 120, 451–461.

Hallauer, A.R. (1990) Methods used in developing maize inbreds. Maydica. 35, 1-16

Bežo, M., Masnicová, S., Hrubíková, K., Bežo, M. jr. (2007) Genetic polymorphism analysis of selected maize lines by PCR RAPD markers for prediction of combinative potential of inbred lines for hybridization with high level of heterose. Agriculture (Poľnohospodárstvo). 53,161-168.

Messmer, M.M., Melchinger, A.E., Herrmann, R., Boppenmaier, J. (1993) Relationships among early European maize inbreds:II. Comparison of pedigree and RFLP data. Crop Sci. 33, 944–950.

Bojinov, B.M., Danailov, Z.H.P. (2009) Applicability of ISSRs for genotype identification in a tomato breeding collection. Acta Hort. (ISHS). 830,63-70.

Zeleňáková, L., Šesták, M., Židek, R. (2009) Monitoring of sheep milk and milk products adulteration on common European food market. Potravinárstvo. 3, 69-74.

Ansari-Mahyari, S., Sørensen, A.C., Lund, M.S., Thomsen, H., Berg, P. (2008) Across-family marker-assisted selection using selective genotyping strategies in dairy cattle breeding schemes. J Dairy Sci., 91,1628-39.

Stefunova, V., Bezo, M. (2002) Genetic diversity analysis of amaranth (*Amaranthus cruentus*) germplasm collection by RAPD. Biologia,. 58, 53-57.

Prasad, M., Varshney, R.K., Roy, J.K., Balyan, H.S., Gupta, P.K. (2000). The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet. 100,584–592.

Lanza, L.L.B., Souza, C.L. jr., Ottoboni, L.M.M., Vieira, M.L.C., Souza, A.P. (1997) Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. Theor Appl Gen. 94,1023-1030.

Buckler, E.S., Gaut, B.S., McMullen, M.D. (2006) Molecular and functional diversity of maize. Current Opinion in Plant Biology. 9,172–176.

Pejic, I.P., Ajmone-Marsan, M., Morgante, V., Kozumplic, P., Cati-Glio, N.I., Taramino, G., Motto, M. (1998) Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theor. Appl. Genet. 97, 1248-1255.

Rogers, S.O., Bendich, A.J. (1994) Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin, S.B., Schilperoort, R.A. Plant Molecular Biology Manual D1. Dordrecht, (The Netherlands), Kluwer Academic Publishers, 1994, p. D1/ 1–D1/8. ISBN 0-7923-2858-2

Yeh, F.C., Boyle, T.J.B. (1997) Population genetic analysis of codominant and dominant markers and quantitative traits. Belg J Bot. 129, 157.

Shannon, C.E., Weaver, W. (1949) The mathematical theory of communication. University of Illinois Press, Urbana

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

Ranatunga, M.A.B., Meenakshisundaram, P., Arumugachamy, S., Maheswaran, M. (2009). Genetic diversity analysis of maize (*Zea mays* L.) inbreds determined with morphometric traits and simple sequence repeat markers. Maydica. 54, 113-123.

Warburton, M.L., Xianchun, X., Crossa, J., Franco, J., Mel Chin-Ger, A.E., Frisch, M., Bohn, M., Hoisington, D. (2002) Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. Crop Sci. 42, 1832-1840.

Yu, Y., Wang, R., Shi, Y., Song, Y., Wang, T., Li, Y. (2007) Genetic diversity and structure of the core collection for maize inbred lines in china. Maydica 52, 181-194.

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. Theor Appl Genet. 95,163-173,

Sperisen, C., Bücher, U. (1998) Cloning of random amplified polymorphic DNA (RAPD) to generate codominant genetic marker. Karp, A., Issac, P.G., Ingram, D.S.(eds) Molecular Tools for Screening Biodiversity, Chapman & Hall. London, pp: 217-222.

Henry, R.J. (1997) Practical application of plant molecular biology. Chapman and Hall. London. pp 59-98

Li, Z., Nelson, R.L. (2001) Genetic diversity among soybean accessions from three countries measured by RAPDs. Crop Science. 41,1337–1347.

Nagy, E., Timár, I.,Hegyi, Z., Spitkó, T., Marton, L.C. (2009) SSR markers as a tools in maize breeding for high starch content. Maydica. 54, 253-257.

Brady, M.S., Provart, N.J. (2007) Extreme breeding: Leveraging genomics for crop improvement. Journal of the Science of Food and Agriculture. 87, 925–929.

Rajwade, A.E. et al. (2010) Relatedness of Indian Flax Genotypes (*Linum usitatissimum* L.): An Inter-Simple Sequence Repeat (ISSR) Primer Assay. Mol Biotechnol. 45,161–170.

Troyer, A. F. (2000). Temperate Corn. Background, Behaviour, and Breeding. London: CRC Press, LLC, 2001, p. 393-466 ISBN 0-8493-2377-0