Genetic divergence of elite maize inbred lines comparing to Illinois high oil source

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Genetische Verschiedenheit von Elitemaisinzuchtlinien im Vergleich zu Illinois Hochölzuchtmaterial

1. Introduction

Single cross hybrids are dominant types of hybrids in maize production in USA, Europe, as well as in Croatia. Maize is the most important energy source in animal feeding. Increased oil concentration in maize grain can increase animal feeding efficiency since oil contains 2.25 times more calories per gram of dry matter than starch or protein (SUGHROUE and ROCHEFORD, 1994). Approximately 60 % of the maize grain produced in the USA each year is used for livestock feed (BERKE and ROCHEFORD, 1995), and in Croatia probably more than 80 %.

Commercial high oil corn hybrids have not been widely used by growers because their grain yield potential is lower than normal dent hybrids (LAMBERT, 1994). Recently there has been interest in producing high oil corn using the TopCross" grain production system which has overcome most of the major problems associated with the use of high oil hybrids in the past – significantly lower grain yields, greater stalk lodging and disease susceptibility (THOMISON et al., 1997). But further work on breeding for this trait is certainly worth of doing, because according to the prediction of Purdue scientists, over the next several decades plant oil (corn, soybean, etc.) will become just as

Zusammenfassung

Für die Einführung von Hochölinzuchtlinien können polymorphe SSR Marker (Mikrosatelliten) verwendet werden. Die Ziele der Untersuchung waren (1) die Bestimmung des Polymorphieniveaus auf 39 SSR Markern unter dem Illinois Hochölzuchtmaterial (IHO) und den Eliteinzuchtlinien und (2) eine Schätzung der genetischen Ähnlichkeit von Eliteinzuchtlinien und IHO sowie der Vergleich der gewonnenen Resultate mit den Abstammungsdaten. Mit 39 SSR Markern wurde ein relativ geringer Polymorphismus gefunden. Verglichen mit den anderen Inzuchtlinien zeigte die IHO-Linie keine extreme Verschiedenheit. Jedenfalls zeigte die Clusteranalyse für IHO eine eigene Gruppe im Dendrogramm. Die anderen Inzuchtlinien wurden zumeist entsprechend den auf Abstammungsdaten gestützten Erwartungen geclustert.

Schlagworte: Mais, SSR Marker, Hochölgehalt, Polymorphismusanalyse, genetische Ähnlichkeit.

Summary

For introduction of the high oil trait into elite inbred lines polymorphic SSR loci can be used. The objectives of the study were to 1) determine the level of polymorphism at 39 SSR loci among Illinois High Oil (IHO) high oil source and elite maize inbred lines, and 2) estimate genetic similarity of elite maize inbred lines and IHO and compare the obtained results with the pedigree data. For 39 SSR loci relatively low polymorphism was found. The IHO did not show extreme divergence comparing to the other inbred lines. Anyway, cluster analysis positioned IHO on the separate branch of the dendrogram. The other inbred lines were mostly clustered according to expectations based on pedigree data.

Key words: maize, SSR markers, high oil source, analysis of polymorphism, genetic similarity.

essential to everyday life as fossil fuels are today (TALLY, 2001).

That is why scientists want to map genes responsible for high oil concentration to enable quicker and more precise introduction of the high oil trait into elite inbred lines by using Marker Assisted Selection (MAS). BERKE and ROCHEFORD (1995) found using a cross of Illinois High Oil by Illinois Low Oil (Early Maturity) thirty-one RFLP loci located in 11 regions were significantly associated with oil concentration. Beside RFLP, mapping can be successfully conducted with a help of different molecular markers. Especially favorable for MAS are co-dominant markers based on PCR, such as SSR markers, which are very abundant in corn genome (WANG et al., 1994). Also, there are a lot of publicly available primer sequences (> 1700; which on average is more than 170 markers per chromosome), which is very good basis for mapping and MAS.

SMITH et al. (1997) concluded that SSR technology presents potential advantages of reliability, reproducibility, discrimination, standardization and cost effectiveness over RFLPs. They also added that SSRs represent the optimum approach for the identification and pedigree validation of maize genotypes compared to other currently available methods. SENIOR et al. (1998) found that SSRs for measuring genetic diversity, for assigning lines to heterotic groups and for genetic fingerprinting equals or exceeds RFLP markers. PEJIĆ et al. (1998) reported that SSR (and AFLP) technologies could replace RFLP markers for genetic similarity studies. BERNARDO et al. (2000) concluded that SSR markers are superior to RFLP markers for estimating genetic relationships.

Earlier studies show that the level of genetic divergence between two maize inbred lines is sometimes correlated with heterosis level of their F1 offspring (STUBER, 1992; KOZUMPLIK et al., 1996). Polymorphic SSR loci can be used for the projects of mapping the loci for high oil content, as well as, for the prediction the level of heterosis between commercial inbred lines and those with the high oil content.

The objectives of the study were to 1) determine the level of polymorphism at SSR loci among Illinois High Oil (IHO) high oil source and elite maize inbred lines, and 2) estimate genetic similarity of elite maize inbred lines with regular oil content and the high oil source IHO and compare it with the pedigree data.

2. Material and methods

2.1 Plant material

The Illinois High Oil (IHO) source, result of 90 cycles of selection for high oil concentration (19,3%) – (DUDLEY and LAMBERT, 1992) and eleven inbred lines from Bc Institute Zagreb, Croatia were used for the study (Table 1). In the following text for both IHO and inbred lines we will use the term genotype.

Table 1: Illinois High Oil (IHO) high oil source and Bc inbred lines* included in calculating of parameters and their oil content
Tabelle 1: Illinois Hochölmaterial (IHO) und in die Berechnung der Parameter einbezogene Rückkreuzungsinzuchtlinien sowie deren Ölgehalte

IHO and Inbred lines	Related to	Oil content (%)
IHO	open-pollinated cultivar "Burr's White"	19.3
Bc1	OH 43	2.7
Bc2	IODENT	1.9
Bc3	IODENT	1.4
Bc4	IODENT	3.2
Bc5	OH 43	3.7
Bc6	OUTCROSS	0.5
Bc7	IODENT	4.3
Bc8	LANCASTER	3.0
Bc9	IODENT	1.8
Bc10	W 153 R	2.1
Bc12	BELJE YELLOW DENT	3.4

* Rough estimates of oil content for Bc inbred lines performed by automated NIR analysis

Fifteen to twenty kernels per genotype were germinated in the growth chamber in pots containing a mixture of soil and Sunshine LB2 Basic Mix (product made by Sungro Horticulture Company, 211 Glen Cairn Road, Winnipeg, Canada.). Conditions in the growth chamber were as follows: temperature was set at 25 °C, 12 hours of light, and 12 hours of darkness, and relative humidity 80 %. Approximately 10–15 plantlets per genotype, 40–50 cm tall, were harvested and bulked. Immediately after harvesting, plantlets were put into the freeze drier where samples of all cultures were dried for one week starting at – 25 °C and finishing at room temperature for 24 hours. Dried tissue was ground, and DNA extracted according to the CTAB procedure (SAGHAI-MAROOF et al., 1984).

2.2 SSR assay

Thirty-nine SSR markers (out of 73) that were screened as reliable and polymorphic (Table 2) were used for genotyping according to following protocol. The PCR reactions were performed in 0.2 ml 8-strip tubes using Perkin Elmer – Gene Amp – 9600 PCR Thermal Cycler. PCR reactions were performed in volume of 29.2 µL containing 1 µL of each primer (20 µM), 0.2 µL of Taq DNA Polimerase (5 u/µL) – Promega, 2 µL of genomic DNA (30 ng/µL), and 25 µL of PCR master mix. PCR master mix was from Promega Life Science. At the beginning of each PCR reaction there was a denaturation step lasting 7 min at 95 °C. After that 35 cycles of amplification followed. Each cycle consisted of 30 sec at 94 °C, 1 min at 53 °C, and 1 min at 72 °C. The last cycle was followed by 10 min at 72 °C, and the PCR reaction was terminated with a continuous cycle at 4 °C. Products were separated by electrophoresis in a 20.5 x 24 cm horizontal gel system using 1x TBE on a 4 % agarose gel (1/4 of Ultra Pure^{TM}, Gibco BRL agarose mixed with 3/4 of NuSieve® GTG® agarose – BMA, low melting temperature agarose; certified for the recovery of nucleic acids ≤ 1 kb) containing 5 µL ethidium bromide (20 mg/ml) per 250 ml of gel. Gels were run ~ 15 min at 90 V and after that ~ 4 hours and 15 min at 120 V, visualized with UV light and documented with BIO RAD Gel Doc 2000 Gel Documentation System.

Because of the horizontal electrophoresis in agarose gel, there was no estimation of fragment length. To all the alleles on each locus, on the basis of their relative length, letters A, B, C, etc. were assigned, depending on the number of alleles per locus in population of studied genotypes, where A stands for the longest fragment (allele), and the last letter in row for particular locus represents the shortest polymorphic fragment (allele). Alleles were than binary coded by 1 or 0 for their presence or absence in each genotype.

2.3 Data analysis

The data of alleles from 39 polymorphic SSR loci of 11 Bc inbred lines and the high oil source IHO were used for estimate of genetic variability through the following parameters: the average (A) and the effective (A_e) number of alleles per locus, the allele frequency (p), and the expected heterozygosity per locus (He).

The average number of alleles per locus: $A= \Sigma n(j)/P$, where n is the number of alleles per locus j, and P the total number of loci (primers). The effective number of alleles per locus (MORGANTE et al., 1994) was calculated as: $A_{e(j)}= (\Sigma p^2_{(ij)})^{-1}$, where $p_{(ij)}$ is the frequency of allele i on the locus j. The allele frequency: p=n(j)/N, where N is the total number of analyzed genotypes, and n(j) is the number of genotypes that carry particular allele on the locus j. For the purpose of calculating N each inbred line or IHO were represented with the number 1 when they were homozygous (in most cases) or with the number 2 when they were heterozygous on particular locus. The expected heterozygosity of locus was calculated according to FALCONER (1981) as: $He_{(j)}=1- \Sigma p^2_{(ij)}$, where p(ij) is the frequency of each single allele i on the locus j. The average heterozygosity was calculated as an average of all the single values for P loci assayed with SSRs. On the basis of SSR data the genetic similarities (GSs) were calculated among all possible pairs of genotypes using the Dice similarity index (NEI and LI, 1979): $GS_{(ij)}=2N_{(ij)}/[N_{(i)} + N_{(j)}]$, where $GS_{(ij)}$ is the measure of relative genetic similarity between genotypes i and j, $N_{(ij)}$ is the total number of shared bands for genotype i and j, and $N_{(i)}$ i $N_{(j)}$ are the total number of bands for genotype i and j, respectively. The relations of divergence among genotypes are visualized by cluster analysis on the basis of GS values. For the hierarchical grouping of genotypes the UPGMA (unweighted pair-group method, arithmetic average) algorithm was performed with the help of SAHN subprogram from package NTSYS-pc (ROHLF, 1990). Obtained clusters are shown in the form of a dendrogram.

3. Results

3.1 SSR polymorphism

At 39 SSR polymorphic loci occurred successful amplification for the majority of tested lines and IHO high oil source. The number of alleles per locus ranged from 2 to 5, and the average number of alleles per locus was 2.6 (Table 2). The majority of loci had 2 alleles while only Phi 080 had 5 alleles. The average effective number of alleles per locus was 2.0 (Table 2). The expected heterozygosity varied from 14.7 % for the locus phi 015, to 72.1% on the locus bnlg 105 (Table 2). The average expected heterozygosity of all loci was 46.0 %. The number of loci at which IHO had different allele comparing to the other inbred lines varied from 10 for Bc1 to 20 for Bc6 and Bc8 (Table 3). At 4 SSR loci (Bnlg 180, Phi 036, Phi 116, and Phi 080), out of 39 assayed, IHO showed unique alleles.

Table 2: Thirty-nine public SSR loci used for analysis and their corresponding values for the exact (n) and the effective (A_e) number of alleles per locus, the allele frequency and the expected heterozigosity (H_e)

Tabelle 2: 39 für die Analyse verwendete öffentliche SSR Marker und deren entsprechende Werte für die genaue (n) und die effektive (A_e) Zahl von Allelen pro Locus, die Allelhäufigkeit und die erwartete Heterozygotie (H_e)

BIN	Locus	n	Allele frequency					A_e	He
			A	B	C	D	E		
1.09	Phi 055	2	0.63	0.37	-	-	-	1.9	46.6
1.09	Bnlg 100	3	0.17	0.33	0.50	-	-	2.6	61.2
1.11	Bnlg 131	3	0.50	0.10	0.40	-	-	2.4	58.0
1.11	Phi 064	3	0.55	0.27	0.18	-	-	2.5	59.2
2.05	Bnlg 180	3	0.17	0.33	0.50	-	-	2.6	61.2
2.09	Bnlg 1520	3	0.31	0.38	0.31	-	-	3.0	66.3
3.04	Phi 036	3	0.08	0.08	0.83	-	-	1.4	29.8
3.08	Phi 046	2	0.58	0.42	-	-	-	2.0	48.7
3.09	Phi 047	2	0.47	0.53	-	-	-	2.0	49.8
4.00	Phi 072	2	0.17	0.83	-	-	-	1.4	28.2
4.03	Phi 021	4	0.17	0.08	0.33	0.42	-	3.1	67.9
4.04	Phi 096	3	0.27	0.33	0.40	-	-	2.9	65.8
4.08	Phi 093	3	0.08	0.75	0.17	-	-	1.7	40.2
5.01	Bnlg 143	2	0.91	0.09	-	-	-	1.2	16.4
5.02	Bnlg 105	4	0.13	0.37	0.25	0.25	-	3.6	72.1
5.03	Bnlg 557	3	0.33	0.33	0.33	-	-	3.0	67.3
5.07	Bnlg 118	2	0.17	0.83	-	-	-	1.4	28.2
5.09	Bnlg 389	3	0.36	0.18	0.45	-	-	2.7	63.6
6.07	Phi 070	3	0.18	0.36	0.45	-	-	2.7	63.6
6.08	Phi 089	2	0.27	0.73	-	-	-	1.7	39.4
7.01	Phi 057	2	0.75	0.25	-	-	-	1.6	37.5
7.01	Phi 112	3	0.08	0.50	0.42	-	-	2.3	56.7
7.02	Bnlg 398	2	0.20	0.80	-	-	-	1.5	32.0
7.05	Phi 082	4	0.08	0.08	0.75	0.08	-	1.7	41.8
7.06	Phi 116	3	0.83	0.08	0.08	-	-	1.4	29.8
8.03	Phi 115	2	0.69	0.31	-	-	-	1.7	42.8
8.05	Bnlg 162	2	0.80	0.20	-	-	-	1.5	32.0
8.08	Phi 015	2	0.92	0.08	-	-	-	1.2	14.7
8.08	Phi 080	5	0.67	0.08	0.08	0.08	0.08	2.1	52.6
8.09	Dup ssr 14	2	0.18	0.82	-	-	-	1.4	29.5
9.04	Phi 032	2	0.83	0.17	-	-	-	1.4	28.2
9.07	Bnlg 128	3	0.25	0.08	0.67	-	-	1.9	48.2
9.07	Bnlg 619	2	0.33	0.67	-	-	-	1.8	44.2
9.07	Bnlg 279	2	0.25	0.75	-	-	-	1.6	37.5
10.00	Phi 041	2	0.09	0.91	-	-	-	1.2	16.4
10.00	Phi 117	2	0.64	0.36	-	-	-	1.9	46.1
10.02	Phi 059	2	0.44	0.56	-	-	-	2.0	49.3
10.02	Phi 063	3	0.50	0.17	0.33	-	-	2.6	61.2
10.03	Bnlg 210	3	0.15	0.31	0.54	-	-	2.4	59.0
avg		2.6						2.0	46.0

3.2 Genetic similarity

The estimate of genetic similarity for 11 Bc maize inbred lines and the high oil source IHO as well as average divergence for each of them (GS_{avg}) is shown in table 3. The most divergent pairs based on GS were Bc7 and Bc12 (40.0), Bc9 and Bc10 (42.4) and Bc6 and Bc12 (42.7), while the most similar pairs were Bc1 and Bc5 (80.0), Bc7 and Bc9 (80.0), and Bc2 and Bc9 (82.9). The high oil source IHO was found to be the most divergent from inbred lines Bc6 (45.5) and Bc8 (44.7), while it was the most similar to inbred line Bc1 (68.3). The highest average divergence (GS_{avg}) in comparison to the other materials had inbred lines Bc8 (49.5) and Bc12 (49.9), while IHO was on the fourth place (53.7). The mean value of the GS estimate was, as expected, lower for Lancaster (LSC) x Iodent (Iod) pairs than within the Iod group itself. However, the LSC group itself had unexpectedly lower mean value than LSC x Iod pairs (Table 3).

Table 3: Dice GS indices among 11 Bc maize inbred lines and the high oil source IHO calculated from SSR data (below the diagonal) and the number of expected heterozygous loci (N_{HE}) at which IHO had different alleles in comparison to the other inbred lines (above the diagonal)

Tabelle 3: Aus SSR Daten errechnete GS Indizes unter den 11 Rückkreuzungsmaisinzuchtlinien und dem Hochölmaterial IHO (unterhalb der Diagonale) und die Anzahl der erwarteten heterozygoten Loci (N_{HE}), auf denen IHO im Vergleich zu den anderen Inzuchtlinien abweichende Allele hatte (oberhalb der Diagonale)

	IHO	Bc4	Bc5	Bc6	Bc8	Bc12	Bc1	Bc2	Bc3	Bc7	Bc9	Bc10	avg
N_{HE} with IHO		16	14	20	20	15	10	15	15	15	16	16	15.6
Bc4	60.9												
Bc5	62.9	66.7											
Bc6	45.5	55.8	51.8										
Bc8	44.7	50.6	50.6	51.3									
Bc12	54.3	45.6	50.0	42.7	45.9								
Bc1	68.3	66.7	80.0	70.9	46.6	55.1							
Bc2	54.8	70.7	69.1	70.0	51.4	47.9	71.6						
Bc3	51.9	66.7	67.6	68.5	52.2	57.6	76.7	78.4					
Bc7	47.8	75.8	62.5	63.5	50.0	40.0	68.8	73.8	66.7				
Bc9	49.3	74.3	73.2	61.8	51.6	50.8	66.7	82.9	73.0	80.0			
Bc10	50.0	44.7	53.3	54.1	50.0	58.5	53.3	52.6	58.0	54.2	42.4		
GS avg	53.7	61.7	62.5	57.8	49.5	49.9	65.9	65.7	65.2	62.1	64.2	51.9	

GS avg (Iod x Iod) = 74.2
GS avg (LSC x LSC) = 59.1
GS avg (Iod x LSC) = 63.0

The genetic similarity tree, formed of 11 Bc maize inbred lines and the high oil source IHO based on SSR data, is presented in Figure 1. It can be seen that four Iodent-related lines (Bc2, Bc9, Bc7, and Bc4) are clustered together, as well as two Oh43-related lines (Bc5 and Bc1). Lancaster-related line (Bc8), Belje Yellow Dent-related line (Bc12) and the high oil source IHO are positioned on the separate branches of the genetic similarity tree. It might be expected that though together Oh43-related inbred lines Bc1 and Bc5 are closer to Lancaster-related inbred line, Bc8 or that maybe Belje Yellow Dent-related inbred line, Bc12 is not clustered together with W153R-related inbred line, Bc10 (figure 1).

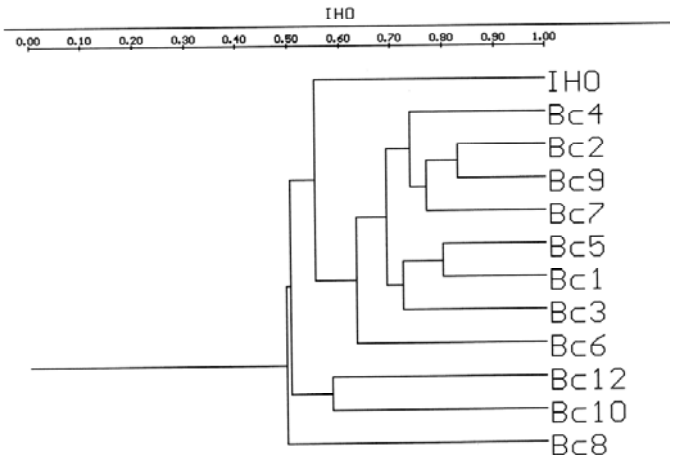

Figure 1: Resulting dendrogram of cluster analysis of 11 Bc maize inbred lines and the high oil source IHO based on SSR data

Abbildung 1: Dendrogramm der Clusteranalyse über 11 Rückkreuzungsinzuchtlinien und IHO (gestützt auf SSR Daten)

4. Discussion

An average of 2.6 alleles per locus found in this study seems to be rather low polymorphism in comparison to other studies. PEJIC et al. (1998) found with 27 polymorphic SSR markers in 33 maize inbred lines 6.8 alleles per locus, while SENIOR et al. (1998), using 70 pairs of primers in 94 inbred lines detected 5.2 alleles per locus. In practical breeding programs for planning size of breeding population the number of marker alleles per locus might be valuable information but it is not always objective parameter, because some alleles are present in very low frequency. This was the reason while MORGANTE et al. (1994) suggested calculation of so cold the effective number of alleles per locus, which takes into account contribution of rare alleles. In our investigation we found only 2.0 effective alleles per locus (Table 2). This could be the consequence of using elite germplasm, that already passed through several cycles of breeding under the same selection criteria, but also the consequence of using the low-discriminating system of electrophoresis, that does not allow reliable differentiation of polymorphism below 4 bp. So, these values should be taken with prudence.

PEJIĆ et al. (1998) found higher value for the average heterozygosity than it was found in our investigation, 72 %. This was in correlation with a respectively higher average number of alleles per locus. The average expected heterozygosity per locus in this study was substantially lower. It should be stressed that the real heterozygosity per locus would be even lower, because the expected heterozygosity is based on ideal mating conditions. It is interesting to stress that the most polymorphic locus with five alleles (phi 080), because of low frequency of four alleles, had the expected heterozygosity only slightly higher than an average.

The estimated parameters suggest that analyzed material in this paper has shared clusters of desired genes, that are transferred by the targeted crosses and consistent selection criteria through small breeding populations from generation to generation. And while this is maybe acceptable from the breeder's point of view, it can be a limitation to efficient MAS. Obtained values in Table 3 show us that for mapping of high oil genes on the basis of material and assay technology used in our investigation mapping population should be quite large and have large number of marker loci.

The fact that the highest average divergence (GS avg) in comparison to the other material had inbred line Bc8 (49.5), while the high oil source IHO was on the fourth place (53.7), suggest that genetic background of IHO is similar to genetic background that is used in commercial breeding programs and that possible introduction of high oil genes should not lead to significant introgression of unwanted genes. Even though the high oil source IHO is not extremely divergent comparing to the other lines, it was clustered separately (Figure 1).

In this study some relationships were unexpected. The problem of biased estimate of GS on the basis of molecular markers data, especially among unrelated inbred lines was reported by BERNARDO (1993). On the other side, expected relatedness on the basis of pedigree data can be influenced by circumstances specified by MELCHINGER et al. (1991). PEJIĆ et al. (1998) show that molecular markers offer more realistic picture of genetic similarity comparing to pedigree data. Anyway, in general it can be concluded that cluster analysis, based on SSR data, clustered inbred lines and the high oil source IHO correctly, though with some exceptions, according to expectation on the basis of pedigree data. Good agreement between SSR data and pedigree data were also found by SMITH et al. (1997), PEJIC et al. (1998), and SENIOR et al. (1998).

Reliability of estimate of genetic similarity, beside on the type of marker and the way of calculation (algorithm), depends on the number of analyzed marker loci, too. Studies conducted by PEJIĆ et al. (1998), VUYLSTEKE et al. (2000) and BERNARDO (1993) indicate that using 39 SSR markers should yield a reliable estimate, but further increase in loci number would result in even more reliable estimate of genetic similarity and grouping of breeding material more accurately according to heterotic pool. These findings might make easier and less expensive the process of testing inbred lines.

5. Conclusions

For the 39 polymorphic SSR loci relatively low level of polymorphism was found. Comparing to the 11 inbred lines the high oil source IHO had different alleles on the 15.6 loci and at 4 loci IHO had unique alleles.

On the basis of values for GS index, IHO did not show extreme divergence comparing to the other inbred lines.

Cluster analysis, based on SSR data, showed that IHO, though not extremely divergent, is still divergent enough in comparison to 11 inbred lines to be placed on the separate branch of the genetic similarity tree.

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