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Genetic variability within accessions of the B73 maize inbred line

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Abstract

The maize inbred line B73 has been extensively studied at the molecular level. Researchers now have access to the genome sequence of B73 as well as to databases of biallelic and multiallelic markers where functional polymorphisms between B73 and a public inbred line population can be compared to relate genotypes to phenotypes. This indicates the importance to determine the genetic fidelity of the germplasm during preservation and propagation processes, in particular, when seeds of reference inbred lines such as B73 are maintained. The aim of this study was to assess the genetic uniformity among three different sources of the B73 inbred line by means of 75 Simple Sequence Repeats (SSRs). The three B73 sources showed homozygosis; however, some accessions differed greatly from the expected locus size predicted at the reference B73 genomic sequence. A particular haplotype was prevalent in the USDA accession PI550473. The error rate of the allele size determination was estimated. The genotyping technique used in this work allowed the separation of alleles of ± 2 bp range difference within the same electrophoresis run, whereas allele size estimations between experiments, within the laboratory, differed in ± 4 bp range difference. Besides experimental errors in genotyping, the putative cause of differences among accessions could be attributed to seed contamination and genetic drift. The B73 accessions evaluated in our work can be shared among laboratories to precise genotyping and phenotyping of maize inbred lines.

Keywords: B73, fidelity, SSRs, fingerprinting

Introduction

Maize is a cross-pollinated species, thus a maize population or variety includes a unique mixture of genotypes. However, the complexity of genotypes of individual maize plants has been reduced by plant breeding to pure genotypes in the form of inbred lines (Jones, 1939), which are the basic germplasms used as parental stock for the production of hybrids (Shull, 1908; Crow, 1998). Inbreeding and the consequent homozygosis is important in the modern concept of maize breeding (Hallauer and Miranda, 1988). Besides selfing, alternative inbreeding systems, such as half-sibbing, full-sibbing, and backcrossing, can be used to approach homozygosity. Sooner or later, these systems finally lead to certain amount of inbreeding depression by deleterious mutations (Charlesworth and Willis, 2009).

Modern-day maize production is based on the heterosis phenomenon, which accounts for the vigor of the hybrid progeny relative to the homozygous parents (Shull, 1909). Heterosis is larger when crossing genetically divergent lines than when crossing genetically close or related lines. Both heterosis and the genetic divergence among inbred lines is the basis to define «heterotic groups» and the term «heterotic pattern» as a specific pair of two heterotic groups, which express high heterosis in their cross (Melchinger and Gumber, 1998). In the 1960's, commercial single crosses started with inbreds B14, B37, and B73 developed at the Iowa State University from the Iowa Stiff stalk synthetic (BSSS) and their derivate which were called Stiff Stalk (SS) and used as female parent. The BSSS is an important breeding population from Ames, Iowa, US, which is extensively used in selection programs for yield improvement and resistance to maize pests (Hallauer and Miranda, 1988). Inbreds that combined well with BSSS were designated as Non-Stiff Stalk (NSS) and placed in the male pool (Duvick et al, 2004; Tracy and Chandler, 2004). Several germplasm sources were used to establish the male pool in the public sector such as the Lancaster-derived lines C103, C123, MO17, and OH43 (Reif et al, 2005). Specifically, Inbred line B73 (BSSS origin) and Mo17 (Lancaster origin), and their improved versions, are the inbreds from contrasting heterotic patterns most frequently used to develop hybrids of FAO maturity groups 600-700 (Zuber and Darrah, 1980; Kauffmann et al, 1982; Stojakovic et al, 2007). In Argentina, the Argentine flint and the US dent (B73 and B73-derived line) heterotic groups show contrasting heterotic patterns for various agronomic characters (Delucchi et al, 2012). In agreement, this divergence was seen at the molecular level by means of clustering-based and model-based methods. Thus, the Argentine flint collection was split into two subpopulations that were separated from the BSSS-BS13-related lines (Olmos et al, 2014).

Because their representativeness of the BSSS group, line B73 has been extensively studied at the molecular level. Researchers now have access to the genome sequence of inbred line B73 (Schnable

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et al, 2009), with the last Zea Mays AGPv3 release at the Gramene database (Monaco et al, 2013). The sequencing project of B73 has shown that the maize genome has a great number of retroelements that have evolved to differentially occupy and exploit this genomic diversity (Baucom et al, 2009). In addition, insertion-deletion (INDEL) and single nucleotide polymorphism (SNP) markers from HapMap 1 (Gore et al, 2009) and HapMap 2 (Chia et al, 2012) databases, can be mapped on gene sequences of B73 to predict the variation effects on genes functionality. In addition, several recombinant inbred lines (RIL) populations by the cross of B73 with Mo17 were created for quantitative trait loci (QTL) identification. The intermated B73xMo17 (IBM) population is a widely used resource for maize mapping (Lee et al, 2002). The Maize Genetics and Genomics Database (MaizeGDB) contains data from outlining experiments in maize that have statistically determined the approximate location of a set of 2,281 QTLs at once (as of May 2014) comprising maps that describe the distances between the loci numerically (Schaeffer et al, 2011). More than 1,700 genetic recombination maps are available online via MaizeGDB (Andorf et al, 2010) which allow the identification of associations between the phenotype and the corresponding marker genotype in segregating biparental populations. Moreover, the inbred line B73 was the common parent to cross with each of the 25 diverse inbred lines in order to develop RIL populations from the F_2 progenies of the crosses (Zhao et al, 2006). These populations were called nested association mapping (NAM) and have become a powerful tool to perform a joint QTL and association analysis (Glaubitz et al, 2007). More than 5,000 F_6 RIL families were produced by single seed descent with selfing, genotyped with 1,100 SNPs (Bernardo, 2002) and phenotyped in several locations (Garcia-Zavala, 2008). In addition, MaizeGDB currently provides 2,034 maize simple sequence repeat (SSR) markers with genome-wide distribution for mapping purposes. Thus, when a SSR is genotyped, the precise band of local inbred lines can be estimated by comparing the recovered SSR alleles with that recovered from a B73 DNA local source line and that expected at the corresponding SSR locus based on the AGI's B73 RefGen_v2 reference sequence (Olmos et al, 2014).

All the above indicates the importance to determine the genetic fidelity of germplasm during the preservation and propagation processes, in particular, when seeds of reference inbred lines such as B73 are maintained. The maintenance and preservation of inbred lines and populations from maize breeding projects suffer limitations in labor and facilities required to prevent genetic variability (Paterniani and Goodman, 1977). All these factors might attempt to multiply inbred lines genetically uniform. SSR analysis has been shown to be a valuable tool to assess identity and the parental contribution among maize inbreds (Romero-Severson et al, 2001; Bernardo and Kahler, 2001; Kahler et al, 2010). The aim of this study was to assess with SSRs the genetic uniformity within and among different sources of the B73 inbred line currently available in Argentina.

Materials and Methods

Plant material

Three different sources of inbred line B73 were analyzed. Sources were selected from i) two B73 accessions (locally called B73-11/12-2095 and B73-05-6081) introduced in Argentina from the Iowa State University in 1995 and propagated at the Pergamino station of the National Institute of Agricultural Technology of Argentina (INTA Pergamino) by Dr Guillermo Eyhérabide; ii) a B73 accession from the US Department of Agriculture (USDA) propagated at the Universidad Nacional de Rosario (UNR), Zavalla, Santa Fe, Argentina, by Dr Lucas Borrás; and iii) a B73 accession PI550473 (lot 08ncai02), original seeds, imported from the USDA, US in 2011 by INTA Pergamino.

SSR genotyping

DNA was extracted according to Kleinhofs et al (1993). For the B73 sources one and three, DNA was extracted from 6-day-old seedlings. At least one DNA bulk comprising five individual seedlings from each accession was prepared. Thus, for accession B73-11/12-2095, and for accessions B73-05-6081 and PI550473, six and one DNA bulks were analyzed, respectively. For the B73 source two, two DNA bulks comprising leaf samples from five field-grown individual plants collected at the vegetative stage were analyzed.

For the accession B73-11/12-2095, we used 13 SSRs from chromosomes 1, 2, 3, 5, and 8. The remaining samples were genotyped with 75 SSR loci that were distributed almost evenly throughout the maize genome. No prior information about the genomic location of loci in coding or noncoding regions or about locus proximity to genes was used for the selection of loci. Primer sequences are available at MaizeGDB (http://www.maizegdb.org/). PCR reaction mixtures contained approximately 30 ng of DNA, 250 nM each primer, 200 µM each dNTP, 1.5 mM Mg^{2+}, 0.5 unit Taq DNA polymerase (Invitrogen), 1 x PCR buffer and sterile double-distilled water to a final volume of 13 µl. PCR reactions from samples that were genotyped with all the 75 SSRs were repeated twice. A touchdown cycling profile (annealing temperature 65-55°C) was used and the PCR products were separated on a 6% denaturing polyacrylamide gel (8 M urea) following standard procedures. Gels were silver-stained. The band size of B73 alleles was visually estimated with a 25-bp DNA ladder (Invitrogen) by interpolation. SSR alleles of the B73 bulks analyzed were compared with the corresponding expected SSR size predicted in the AGI's B73 RefGen_v2 reference sequence, for all cases in which the SSR were physically mapped. A total of 69 out

of 75 loci were physically mapped except bnlg1429, bnlg504, bnlg420, umc1918, phi034, and bnlg1161. In the case of SSRs that produced stuttered or multiple and diffused bands, the match of the amplified allele with the amplicon size predicted in the AGI's B73 RefGen_v2 served to score the precise the B73 allele.

Results

In all cases, DNA bulks were homogeneous and comprised plants in homozygosis (Table 1). All loci fingerprinted in the two B73 sources from INTA Pergamino (accessions B73-11/12-2095 and B73-05-6081) were identical in size, except for SSR phi053, whose allele had a similar size to that of source three (accession B73-PI550473).

Nineteen loci were monomorphic among sources one (B73-05-6081) and sources two and three. On the other hand, only five loci had alleles that matched the exact expected allele size from the AGI's B73 Ref-Gen_v2 sequence reference (i.e. umc1734, phi053, phi072, umc1917, and umc2093). Besides, 84% and 65% of loci physically mapped had allele size estimates that differed in ± 3 bp and ≥ 4 bp, respectively, from the expected primer amplicon based on the AGI's B73 RefGen_v2 reference sequence.

Most loci resulted in allele sizes that were either identical or differed in ± 3 bp from the predicted size in the AGI's B73 RefGen_v2, except for the SSRs umc1792, phi420701 and bnlg1834, which had alleles that varied greater in size. Alleles that differed ≥ 4 bp from the AGI's B73 RefGen_v2 reference were more frequent in the B73-PI550473 (source number 3) accession and varied from 4 to 87 bp (bnlg1045).

When SSR amplified fragments were run in the same gel electrophoresis, allele sizes below 175 bp were well separated, which allowed detecting slight differences among accession sizes (Figure 1). Such was the case of phi084 (3 bp), umc2318 (2 bp), umc1752 (3 bp), and umc1299 (2 bp). Thus, within the same electrophoresis run, the minimum detectable difference among alleles was 2 bp.

The rate of experimental error in the allele size estimation among experiments within the laboratory was assessed by comparing common SSRs run in the B73 accession B73-05-6081 with those mapped in Olmos et al (2014) which used an independent B73 bulk sample from accession B73-05-6081. Thus, 43 common SSR loci were compared. The molecular weight differences of 37 out of the 43 loci compared (86%) varied from 0 to 4 bp, whereas six loci (phi128, bnlg1070, phi053, bnlg1217, umc1078 and bnlg1270) produced greater allele differences. This difference indicates a putative allele switching within the B73 accession sample used in Olmos et al (2014).

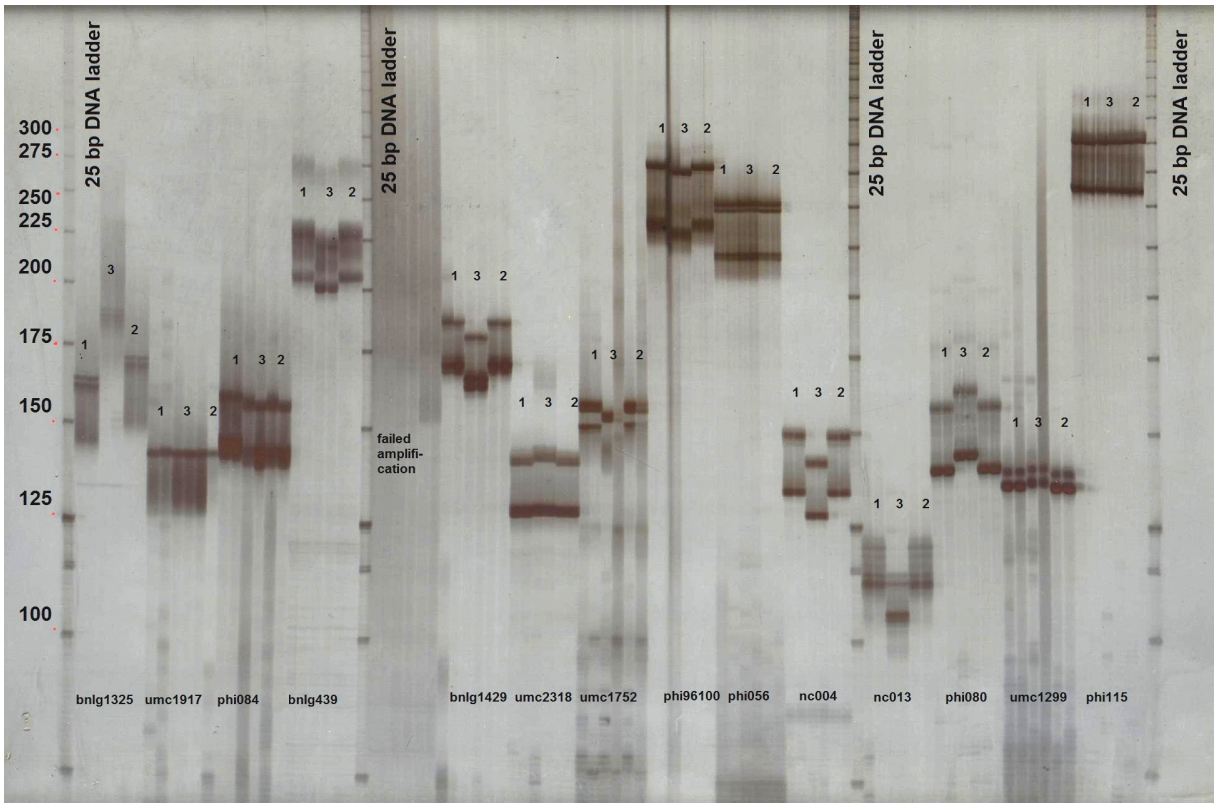

Figure 1 - Gel electrophoresis run example of several SSR amplified alleles in three different sources of the B73 inbred line. The presence of a different allele in the source 3 (accession PI550473) can be seen for mostly SSRs. Within run, the genotyping technique allowed to detect at least 2 bp allele differences for umc2318 and umc1299 which amplified fragments in the range of 140 bp size.

Table 1 - List of SSR mapped in three different sources of the B73 inbred line: source 1 - local B73 from INTA Pergamino, Argentina, called B73-11/12-2095 and B73-05-6081; source 2 - local B73 from Universidad Nacional de Rosario (UNR), Argentina, and source 3 - original seeds from the B73 accession PI550473 introduced in Argentina from the USDA, US. Previous B73 band size estimation of the B73 accession B73-05-6081 from Olmos et al (2014), is also presented.

		Source 1							Source 2		Source 3		
SSR	Chr. Bin	Bulk 1. B73-11/12-2095	Bulk 2. B73-11/12-2095	Bulk 3. B73-11/12-2095	Bulk 4. B73-11/12-2095	Bulk 5. B73-11/12-2095	Bulk 6. B73-11/12-2095	Bulk 1. B73-05-6081	Bulk 1. B73-UNR	Bulk 2. B73-UNR	Bulk 1. B73-PI550473-USDA	Estimated allele size for B73[1]	B73 allele size from Olmos et al (2014)[6]
phi056	1.01	247[2]	247	247	247	247	247	247	247	247	247	249	247
bnlg1429	1.02	184	184	184	184	184	184	184	184	184	177	*gap*[5]	182
bnlg439	1.03	225	225	225	225	225	225	225	225	225	213[3,4]	228	225
umc1917	1.04	143	143	143	143	143	143	143	143	143	143	140	
umc2025	1.05	126	126	126	126	126	126	126	126	126	147	129	126
umc1734	1.05							116	116	116	116	116	
bnlg2238	1.07							188	188	188	239	189	
umc2116	1.08							134	134	134	134	135	
phi011	1.09							220	229	229	220	228	
umc1774	1.10							157	157	157	160	158	
bnlg504	1.11	176	176	176	176	176	176	176	176	176	169	*gap*	173
umc2246	2.00							147	147	147	123	148	147
phi96100	2.01	280	280	280	280	280	280	280	280	280	273	279	281
bnlg1297	2.02							198	198	198	154	199	
bnlg1064	2.03							196	196	196	184	197	
phi083	2.04							128	128	128	134	129	129
umc1749	2.06							147	147	147	179	148	143
dupssr21	2.05	111	111	111	111	111	111	111	111	111	108	112	113
bnlg1045	2.07							203	203	203	114	201	
phi127	2.08	129	129	129	129	129	129	129	129	129	127	125	128
umc2101	3.00							153	153	153	162	154	
phi104127	3.01							154	154	154	154	156	159
bnlg1325	3.03							163	170	170	187	171	
phi029	3.04							161	150	150	150	149	
bnlg420	3.05							86	83	83	76	*gap*	82
phi053	3.05	171	171	171	171	171	171	191	171	171	191	171	168[7]
bnlg127	3.06							246	225	225	246	224	
phi047	3.09	145	145	145	145	145	145	145	145	145	142	142	144
phi072	4.01							142	142	142	149	142	141
umc1276	4.01							103	103	103	112	102	
nc004	4.03							150	150	150	142	149	150
bnlg1217	4.05							214	204	204	218	205	208
umc1299	4.06							140	140	140	142	138	137
bnlg1137	4.06							248	248	248	241	246	246
phi093	4.08							291	291	291	281	292	
umc1917	4.10							142	142	142	142	142	
phi019	4.11							98	98	98	100	99	99
umc1240	5.00							122	122	122	122	119	120
bnlg1006	5.00							229	229	229	234	231	
phi113	5.03							115	115	115		118	119
umc1752	5.06							159	159	159	155	156	159
phi128	5.07							110	103	103	103	105	105
umc1792	5.08	120	120	120	120	120	120	120	120	120	122	116	118
phi075	6.00							240	240	240	216	237	239
phi077	6.01							150	150	150	139	149	
umc1083	6.02							110	110	110	104	112	
umc1918	6.04							131	131	131	131	*gap*	
umc1979	6.04							141	141	141	151	140	139

Table 1 - cont.

umc2317	6.04							156	156	156	150	155	
umc2055	6.05							86	86	86	82	83	
umc2318	6.05							142	142	142	144	141	143
nc013	6.05							120	120	120	113	122	
umc2059	6.08							144	144	144	124	143	141
phi057	7.01							153	153	153	153	154	155
phi034	7.02							123	123	123	123	*gap*	123
bnlg1070	7.03							223	210	210	264	213	214
bnlg1161	7.04							196	196	196	206	*gap*	
phi082	7.05							122	122	122	122	123	
umc2190	7.06							154	154	154	151	158	158
phi420701	8.00	295	295	295	295	295	295	295	295	295	295	300	
phi115	8.03							305	305	305	305	303	302
bnlg1834	8.03							205	205	205	205	209	
phi014	8.04							161	161	161	165	160	162
phi080	8.08							159	159	159	164	157	154
bnlg1131	8.09							101	101	101	110	103	
umc2093	9.01							111	111	111	111	111	111
phi065	9.03							151	151	151	131	154	
umc1078	9.05							122	128	128	110	129	130
bnlg1270	9.06							156	145	145	173	148	148
umc1129	9.08							196	196	196	199	197	
umc1380	10.00							146	146	146	152	148	149
phi041	10.00							202	202	202	202	203	203
umc1576	10.02							104	104	104	104	102	
umc1938	10.03							152	152	152	152	154	154
phi084	10.04							158	155	155	155	156	156

[1] AGI's B73 RefGen_v2 sequence length expected for the SSR primer amplicon.
[2] Orange letter: indicates ± 3 bp difference from the AGI's B73 RefGen_v2 sequence allele size
[3] Red letters: indicates ± 4 bp difference from the AGI's B73 RefGen_v2 sequence allele size
[4] Filled gray squares: indicates the putative occurrence of a different allele among accessions
[5] Gap indicates that the SSR locus was not found in the AGI's B73 RefGen_v2 sequence
[5] Molecular weight of B73 estimated in Olmos et al, (2014) by using the same genotyping technique but independent experiments
[6] Yellow filled squares indicates a putative allele switching of the B73 local accession used in Olmos et al, (2014)

Discussion

Inbred line B73 was selected from an advanced recurrent selection population (C5) of Iowa Stiff Stalk Synthetic (BSSS) and released in 1972 (Russell, 1972). Since then, seeds for breeders have been produced by self-pollination in ear-to-row progenies in the Iowa Experiment Station, USA. The molecular base to define the identity of maize inbred lines was discussed by Romero-Severson et al (2001), who addressed the importance of permitting some degree of polymorphic SSRs among different sources of the same inbreds. In addition, they explained how such polymorphic SSRs might arise from seed contamination, experimental errors in the genotyping and genetic drift promoted by small population size during the increased of the inbred seed stock. In a fingerprinting of a maize inbred collection with SSRs with genome distribution (Olmos et al, 2014), the five microsatellites mapped on chromosome 1 showed high gene diversity and provided unique genotypes for 99 out of the 103 inbred lines, showing that the discriminatory power is upon the SSR loci. In our experiment, only 26% of the monomorphic loci were found among the B73 sources. Shared alleles and haplotypes were more frequent between the local B73 accessions (source 1: B73-11/12-2095 and B73-05-6081) and the B73-UNR (source 2) than that introduced B73 source from USDA (source 3: B73-PI550473). The most discriminatory power was obtained with chromosome 2, which showed an arrangement of all nine loci that formed a distinct haplotype that completely distinguished the source three, B73-PI550473, from the Argentine accessions B73-05-6081 and B73-UNR. Haplotype sharing arisen from the transmission of unrecombined DNA through the gametes of a recent common ancestor and particularly rare haplotypes provides evidence of essential derivation (Romero-Severson et al, 2001). In maize, a threshold of 0.90 has been proposed for essential derivation. Thus, if an inbred has had a parental contribution greater than the threshold, the inbred is declared to be essentially derived from the parental inbred (Smith et al, 1995). The parental contribution of alleles and haplotypes from the B73 accession B73-PI550473

is unknown because it was imported in 2011 from US and plantlets were analyzed prior multiplication. It is known that seed stocks of inbreds may have changed genetically over time by mutation, contamination by stray pollen, or physical mixing with seeds from another inbred (Bernardo et al, 2000). However, there is not enough information on the amount of variation assessed at the molecular level that can be tolerable among different seed stocks of reference inbred germplasms.

Furthermore, the procedure used for SSR genotyping had limitations for SSR scoring and readability, in particular when multi-allelic SSRs is used. Thus, within electrophoresis, the genotyping technique used in the present work allowed the separation of allele size within the ± 2 bp range difference. To evaluate the error rate of the B73 allele estimation between experiments within our laboratory, we compared the results obtained from genotyping the B73-05-6081 accession with common 43 SSRs previously assessed in Olmos et al (2014). Results showed that 86% of loci were scored with a ± 4 bp range difference, which indicates the likely general error rate that can occur between experiments in our laboratory. Greater differences in allele size estimation can be attributed to a misleading estimation when stutter band patterns of certain loci, for instance when phi128 and bnlg1070 SSRs, are scored. In contrast, in the case of loci phi053, bnlg1217, umc1078, and bnlg1270, greater allele differences are more likely to have occurred due to allele switching between the two B73-05-6081 accessions analyzed by genetic drift. The use of a system of allele nomenclature based on allele size estimation other than coding allows the inclusion of new alleles without a frameshift of the allele database of loci. Row error rates of SSR allele size estimation ranging from 5.8% to 9.7% have been obtained between laboratories as a consequence of frameshift errors, wrong allele size, failed amplification, and the scoring of extra alleles (George et al, 2004). However, in the automatic sizing of DNA fragments, biases in allele size differences can be found due to the nucleotide composition of the microsatellite loci (Haberl and Tautz, 1999). In our experiments, stuttering of SSR alleles was neither corrected by optimizing PCR conditions, nor discarded as recommended Guichoux et al (2011). However, the www.maizegdb.org database provides 2,034 maize SSRs with genome-wide distribution (Schaeffer et al, 2011), to avoid stuttering problems easily scorable SSRs can be chosen for mapping purposes. Delmotte et al (2001) suggested that reference standard DNA genotypes should be shared between collaborating laboratories to avoid scoring errors in SSR allele size and the consequent misleading conclusions. Thus, the B73 accessions evaluated in our work can be shared among laboratories to precise the manual technique genotyping of maize inbred lines and for phenotyping trial purposes.

Conclusion

The multi-allelic nature of SSRs is useful because SSRs provide higher discrimination power with fewer markers (Galbusera et al, 2000). As a result of this work it was demonstrated the ability of SSRs to assess the fingerprinting of the B73 inbred line. The accession showed genetic uniformity; however, some accessions in particular de USDA B73-PI550473 differed greatly from the expected locus size predicted at the reference B73 genomic sequence. The genetic variability among B73 sources might comprises reproducibility of genotyping and phenotyping experiments when used as a control inbred line. On the other hand, the use of highly polymorphic SSRs would bias the assessment of an inbred line fingerprinting and would compromise the genetic relationship when diversity of a population is assessed. This highlights the importance of following standardized protocols for maize SSR fingerprinting and the need to include reference B73 accessions and standard alleles with a system of allele nomenclature based on allele size estimation to be compared during the fingerprinting of a diverse collection of maize inbred lines.

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