Analysis of genetic diversity in Argentinian heterotic maize populations using molecular markers

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Abstract


Over the past three decades, traditional Argentinean Orange Flint maize cultivars have been replaced by the higher yielding U.S. Yellow Dent germplasms. However, flint cultivars are potentially resistant to biotic and/or abiotic stress. Thus, knowledge of genetic diversity and relationships among flint inbred lines would help reduce genetic vulnerability and broaden the genetic base of crops in national improvement programs. In this study, we report the analysis of 25 inbred Orange Flint germplasms and one dent using 21 microsatellite markers or Simple Sequence Repeats (SSR). The aim was to assess genetic diversity among these accessions and evaluate the usefulness of SSR markers for defining heterotic groups in temperate germplasm. Genetic diversity values for flint germplasm (25 inbreeds) was relatively high. The number of alleles per locus was 5.14 and expected heterozygosis ($H_e$) was 0.68. When testing for genetic differentiation among the four heterotic populations established by topcross, twelve loci from a total of twenty-one displayed significant $P$-values. Even though we cannot observe a significant agreement between groupings based on topcross and clustering based on molecular data. On the other hand, Bayesian grouping (STRUCTURE software) performed better when compared to the clustering based on genetic distance (UPGMA-Modified Roger’s Distance).

Key words: Cluster analysis, microsatellite, Zea mays.

Introduction

The strategies used in maize breeding programs (Zea mays L.) are frequently characterized by a decrease of genetic diversity in the pool of germplasms and an increase in the genetic evenness in cereal production (Lee, 1998). This might cause important problems, particularly sensitivity to new diseases and/or a decreased tolerance to high temperatures or drought (Duvick, 1989).

Argentina is fifth as maize produce country, second as maize export country. A strategy frequently used in Argentinean improvement programs is to take advantage of the hybrid vigor of crossings between the national Cristalino Colorado material and the U.S. yellow dent material (Eyhérabide et al., 2006). Dent hybrids, developed and/or introduced in Argentina, follow mainly the Reid Yellow Dent (RYD) vs. Lancaster Sure Crop (LSC) pattern; they present better behavior with respect to grain yield,
especially in favorable environments, and are appreciated for their dry milling quality. On the other hand, although orange flint hybrids have lower yields than flint × dent and dent × dent crosses, they are appreciated for the hardness of their endosperm (Robutti et al., 2000), their biological value (Eyérabide et al., 2006) and their resistance to local diseases such as Mal de Río Cuarto (Morata et al., 2003). Recent research in the United States has also shown that Argentinean germplasm presents resistance to Gibberella and Fusarium ear rots (Presello et al., 2004) and has lower aflatoxin concentrations than flint hybrids (Ochs, 2005). Consequently, knowing the constitution of Cristalino Colorado germplasm and understanding the relations between the lines would help to reduce the genetic vulnerability and increase the genetic base of national programs, allowing the assignment of new lines of heterotic patterns previously determined (Hallauer and Miranda, 1988).

Variations in the DNA sequence have been used as molecular markers in plants and animals during the last two decades (Korzun, 2003). Moreover, they have been used as a tool to determine new heterotic groups and/or assign new materials to pre-existing heterotic groups (Melchinger, 1999; Reif et al., 2003). It has been reported that microsatellites or Simple Sequence Repeats (SSR) present the advantages of reproducibility, discrimination and low cost/benefit ratio with respect to other markers (Pejic et al., 1998; Smith et al., 1997). They have consequently been proposed for the characterization of genetic resources (Pejic et al., 1998; Smith et al., 1997). The objectives of the present study were to determine the levels of genetic diversity and relationships between lines of the Cristalino Colorado germplasm and to evaluate the usefulness of microsatellites to define heterotic groups in a temperate climate germplasm.

Materials and methods

This research involved 26 lines selected out of 48, previously arranged in four heterotic groups by test cross with four synthetic populations (Nestares et al., 1999; Eyérabide et al., 2006). The four synthetic populations used as testers were: sB73 and sMo17 from the Reid × Lancaster pattern and HP3 and P5L2 from the local flint pattern (Nestares et al., 1999). All lines, except B73, evaluated in this work (Iowa Stiff Stalk Synthetic) were developed by the Argentinean INTA (Instituto Nacional de Tecnología Agropecuaria) from different origins of Cristalino Colorado maize, mainly local races (Table 1). The election of lines was based on seed availability and the degree to which the four groups represent the entire population. For each line, the DNA from young and fresh leaves was extracted in “bulk” from five plants by the CTAB method reported by Hoisington et al. (1994). Although the lines used in this study are homozygotes, several plants were used in the extraction to avoid a possible contaminating seed. The DNA quality and amount was verified by electrophoresis in 0.8 % agarose gels. The quantification of each extraction was made by comparing to DNA samples of uncut lambda phage of known amount and by fluorescence with ethidium bromide.

The primer sequences used for PCR amplification were selected from the MaizeGDB database (http://www.agron.missouri.edu). The microsatellite loci selected were chosen based on the size of the repetitions and their location, to obtain a representative sampling of the whole genome (Table 2). The PCR reaction was made in a final volume of 11 µl containing 10-20 ng of mould DNA, 0.1 mM dNTPs, 0.25 mM of primers (forward and reverse), 0.75 mM MgCl₂, 0.025 U Taq DNA polymerase, and reaction buffer 1X (10 mM Tris-HCl pH 8.8, 50 mM KCl, and 1.5 mM MgCl₂). Negative controls without DNA template in the reaction mixture were included in each PCR run. Amplifications were carried out in a PTC-100 MJ thermocycler (MJ Research, Watertown, MA) with the following conditions: an initial denaturalization cycle at 94°C for 2 min; five touch down cycles: 60 s at 94°C, 60 s at 65°C (decreasing 1°C per cycle) and 2 min at 72°C; 30 conventional cycles of 60 s at 94°C, 60 s at 60°C and 2 min at 72°C and, finally, an elongation cycle at 72°C for 5 min. The amplification products were solved by electrophoresis in denaturing gels (6M urea) of 6% (w/v) acrylamide/bis-acrylamide solution (29:1) and detected by silver nitrate staining.
Table 1. Maize inbreed lines used in this study, source and heterotic group established by topcross (Eyhéribide et al., 2006; Nestares et al., 1999).

<table>
<thead>
<tr>
<th>Line</th>
<th>Population of origin</th>
<th>Origin abreviation</th>
<th>Heterotic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>B73</td>
<td>BSSS(C8)</td>
<td>BSSS</td>
<td>I</td>
</tr>
<tr>
<td>LP117</td>
<td>Argentino Caribe</td>
<td>CAC</td>
<td>I</td>
</tr>
<tr>
<td>LP32</td>
<td>Sintética Colorada Dura</td>
<td>SCD</td>
<td>I</td>
</tr>
<tr>
<td>LP521</td>
<td>Sintética Colorada Dura</td>
<td>SCD</td>
<td>I</td>
</tr>
<tr>
<td>LP122</td>
<td>Argentino Caribe</td>
<td>CAC</td>
<td>I</td>
</tr>
<tr>
<td>LP123</td>
<td>Argentino Caribe</td>
<td>CAC</td>
<td>II</td>
</tr>
<tr>
<td>LP153</td>
<td>Cross A1×LP70</td>
<td>A1×LP70</td>
<td>II</td>
</tr>
<tr>
<td>LP22</td>
<td>Sintética Colorada Dura</td>
<td>SCD</td>
<td>II</td>
</tr>
<tr>
<td>LP44</td>
<td>Poblaciones Coloradas Argentinas</td>
<td>PCA</td>
<td>II</td>
</tr>
<tr>
<td>LP662</td>
<td>Single Cross A×252</td>
<td>A×252</td>
<td>II</td>
</tr>
<tr>
<td>LP70</td>
<td>Sintética A</td>
<td>SA</td>
<td>II</td>
</tr>
<tr>
<td>P1338</td>
<td>Argentino×Exótico</td>
<td>Arg×Exot</td>
<td>II</td>
</tr>
<tr>
<td>LP13</td>
<td>Sintética Colorada Dura</td>
<td>SCD</td>
<td>III</td>
</tr>
<tr>
<td>LP146</td>
<td>Resistente Paraná</td>
<td>CRP</td>
<td>III</td>
</tr>
<tr>
<td>LP147</td>
<td>Resistente Paraná</td>
<td>CRP</td>
<td>III</td>
</tr>
<tr>
<td>LP19</td>
<td>Sintética Colorada Dura</td>
<td>SCD</td>
<td>III</td>
</tr>
<tr>
<td>LP199</td>
<td>Compuesto II</td>
<td>CII</td>
<td>III</td>
</tr>
<tr>
<td>ZN6</td>
<td>Población Local</td>
<td>LocPop</td>
<td>III</td>
</tr>
<tr>
<td>LP38</td>
<td>Poblaciones Coloradas Argentinas</td>
<td>PCA</td>
<td>IV</td>
</tr>
<tr>
<td>LP62</td>
<td>Sintética A</td>
<td>SA</td>
<td>IV</td>
</tr>
<tr>
<td>LP103</td>
<td>Selección Masal</td>
<td>SM</td>
<td>IV</td>
</tr>
<tr>
<td>LP109</td>
<td>Selección Masal</td>
<td>SM</td>
<td>IV</td>
</tr>
<tr>
<td>LP110</td>
<td>Selección Masal</td>
<td>SM</td>
<td>IV</td>
</tr>
<tr>
<td>LP138</td>
<td>Colección Exótico</td>
<td>Exot</td>
<td>IV</td>
</tr>
<tr>
<td>LP140</td>
<td>Resistente Paraná</td>
<td>CRP</td>
<td>IV</td>
</tr>
<tr>
<td>LP152</td>
<td>Cross de P578</td>
<td>P578</td>
<td>IV</td>
</tr>
</tbody>
</table>

(Silver sequence Promega Biotech, Madison, WI). The different bands obtained were evaluated by visual inspection, a 25 bp DNA ladder (Life Technologies-Gibco BRL) was used as a molecular weight marker. For the same primer, the products of different size were considered different alleles. The information obtained was coded in a worksheet for further analyses.

The number of alleles and the genetic diversity (expected heterozigocity) were estimated in each locus for a cluster of 25 orange flint lines;
line B73 was not included in this analysis. The expected heterozygosis ($H_e$), sometimes known as PIC or polymorphic information content (Smith et al., 1997), was estimated according to Nei (1978):

$$H_e = 1 - \sum_{i=1}^{N} p_i^2$$

where $p_i$ is the frequency of the i-th allele. The $H_e$ value is defined as the probability that two alleles chosen at random within the same sample are different, and it shows the reach of the marker’s discriminatory power in considering not only the number of alleles but also their relative frequencies (Kostova et al., 2006). The analysis was implemented by PowerMarker v3.25 (Liu and Muse, 2005). The level of genic differentiation among the four heterotic populations previously determined by the topcross method (Eyherabide et al., 2006) was estimated with the program GENEPOP v.3.4, using the pre-set parameters and under the null hypothesis: “the allelic distribution is identical through all the populations” (Raymond and Rousset, 2004). This program allows us to obtain an unbiased $P$-value for each locus using an exact test (Raymond and Rousset, 2004).

We used cluster analysis was for the whole group of 26 characterized lines based on the Unweighted Pair Group Method using Arithmetic Averages (UPGMA). The cluster analysis was implemented on the modified Roger’s distance (Reif et al., 2005):

$$MRD = \frac{1}{\sqrt{2m}} \left( \sum_{i=1}^{m} \sum_{j=1}^{m} (p_{ij} - q_{ij})^2 \right)^{1/2}$$

where $p_{ij}$ and $q_{ij}$ are the frequencies of the i-th allele at the j-th locus in the two lines considered, $a_j$ indicates the number of alleles for the j-th marker and $m$ indicates the total number of analyzed loci. The calculations of genetic distance and the cluster analysis were made with the TPFGA software v1.3 (Miller, 1997). The cluster analysis was also carried out by helus and stats packs of the R environment (http://www.r-project.org/), to estimate the cophenetic correlation (correlation between the distance values estimated during the tree construction and the values of initial distances) and identify the potential heterotic groups.

The program STRUCTURE was used as a second approach to determine the possible heterotic groups from molecular data (Pritchard et al., 2000). STRUCTURE uses a Bayesian algorithm to infer the individual membership, maize lines in this case, to the different populations. The number of populations (K) was previously determined to equal 4. The main parameters of the program (and number of replications) were both determined in 1,000,000. A script in R language was implemented to determine the best agreement level between the clusters based on molecular data obtained in this work and the cluster based on the top crosses made by Nestares et al. (1999). The program allowed us to compare the number of individuals coinciding in the four groups determined on the basis of the molecular information and in the four groups determined based on the topcross method. The degree of association (coincidence) was estimated by Cohen’s Kappa coefficient, provided in the psy pack (R project).

**Results and discussion**

The 21 polymorphic SSR markers used to estimate the genetic diversity of the population of 25 lines of Argentinian Cristalino Colorado maize allowed for the detection of 108 total alleles. The number of alleles per loci varied from 2 to 14 with a mean of 5.14; the 108 alleles were sufficient to completely discriminate the 25 lines (Figure 1). The values of genetic diversity for each locus varied from 0.36 to 0.90 with a mean of 0.68 (Table 2).
These results are similar to the results obtained in previous studies made in maize, for example: Kostova et al. (2006) analyzed 41 Bulgarian lines with 18 microsatellites and obtained a mean of 9.1 allelic variants, Pejic et al. (1998) observed a mean of 6.8 alleles per locus in 33 American characterized lines with 27 SSR, while Bantte and Prasanna (2003), characterizing 23 tropical lines with 36 SSR, determined a mean of 3.25 alleles per locus.

The average value of He obtained in this work was also in agreement with the values obtained in the works mentioned, for example: Kostova et al. (2006) found a mean He of 0.71, while Pejic et al. (1998) reported a value of 0.72, and

Table 2. Information about the 21 SSR loci used in this study, including names, bin location, repetition size, number of alleles, genetic diversity value (He) and P-value of the genetic differentiation test (Raymond and Rousset, 2004) for the four heterotic groups in the 25 Orange Flint lines set.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Bins</th>
<th>Repetition</th>
<th>No. of Alleles</th>
<th>He</th>
<th>P-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>phi001</td>
<td>1.03</td>
<td>AG</td>
<td>5.00</td>
<td>0.77</td>
<td>0.0002</td>
</tr>
<tr>
<td>Bnlg400</td>
<td>1.09</td>
<td>-</td>
<td>7.00</td>
<td>0.83</td>
<td>0.0582</td>
</tr>
<tr>
<td>umc1065</td>
<td>2.05</td>
<td>(ACA)17</td>
<td>7.00</td>
<td>0.72</td>
<td>0.0260</td>
</tr>
<tr>
<td>bnlg1169</td>
<td>2.08</td>
<td>(AG)14</td>
<td>5.00</td>
<td>0.75</td>
<td>0.3483</td>
</tr>
<tr>
<td>Bnlg602</td>
<td>3.04</td>
<td>-</td>
<td>7.00</td>
<td>0.74</td>
<td>0.5331</td>
</tr>
<tr>
<td>Bnlg197</td>
<td>3.07</td>
<td>-</td>
<td>5.00</td>
<td>0.75</td>
<td>0.7520</td>
</tr>
<tr>
<td>phi026</td>
<td>4.05</td>
<td>CT</td>
<td>6.00</td>
<td>0.78</td>
<td>0.0009</td>
</tr>
<tr>
<td>phi093</td>
<td>4.08</td>
<td>CTAG</td>
<td>3.00</td>
<td>0.60</td>
<td>0.0310</td>
</tr>
<tr>
<td>phi113</td>
<td>5.03</td>
<td>GTCT</td>
<td>5.00</td>
<td>0.69</td>
<td>0.0494</td>
</tr>
<tr>
<td>Bnlg609</td>
<td>5.06</td>
<td>-</td>
<td>10.00</td>
<td>0.86</td>
<td>0.0949</td>
</tr>
<tr>
<td>nc013</td>
<td>6.05</td>
<td>AG</td>
<td>4.00</td>
<td>0.67</td>
<td>0.0040</td>
</tr>
<tr>
<td>phi089</td>
<td>6.08</td>
<td>ATGC</td>
<td>2.00</td>
<td>0.48</td>
<td>0.8848</td>
</tr>
<tr>
<td>phi057</td>
<td>7.01</td>
<td>GCC</td>
<td>5.00</td>
<td>0.69</td>
<td>0.0218</td>
</tr>
<tr>
<td>phi116</td>
<td>7.06</td>
<td>TGAC-GAC</td>
<td>2.00</td>
<td>0.48</td>
<td>0.0125</td>
</tr>
<tr>
<td>phi119</td>
<td>8.02</td>
<td>AG</td>
<td>4.00</td>
<td>0.72</td>
<td>0.4014</td>
</tr>
<tr>
<td>phi015</td>
<td>8.08</td>
<td>TTTG</td>
<td>3.00</td>
<td>0.64</td>
<td>0.1585</td>
</tr>
<tr>
<td>phi068</td>
<td>9.01</td>
<td>AT</td>
<td>3.00</td>
<td>0.59</td>
<td>0.0132</td>
</tr>
<tr>
<td>Bnlg127</td>
<td>9.03</td>
<td>-</td>
<td>14.00</td>
<td>0.90</td>
<td>0.0004</td>
</tr>
<tr>
<td>phi041</td>
<td>10.00</td>
<td>AGCC</td>
<td>4.00</td>
<td>0.67</td>
<td>0.2024</td>
</tr>
<tr>
<td>bnl1451</td>
<td>10.02</td>
<td>(AG)24</td>
<td>3.00</td>
<td>0.36</td>
<td>0.0253</td>
</tr>
<tr>
<td>bnlg1839</td>
<td>10.07</td>
<td>(AG)24</td>
<td>4.00</td>
<td>0.51</td>
<td>0.0210</td>
</tr>
<tr>
<td>Media</td>
<td>-</td>
<td>-</td>
<td>5.14</td>
<td>0.68</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Genetic differentiation test were performed on the complete set of 26 lines and as described in Raymond and Rousset (2004). Significant P-values (5%) are indicated in bold. - Missing data.
Bantte and Prasanna (2003) a value of 0.54. He gives an idea of the information available from the SSR loci and their potential to detect differences between lines based on their genetic relation. The differences among these studies may be attributed mainly to differences in sample size and the genetic base of the populations analyzed. We also considered the fact that microsatellites with repetitions of two nucleotides show a higher number of allelic variants; however, a heterozygosis value of 0.67 was obtained when the results of microsatellites with this number of replications were excluded from the analysis (phi001, phi026, nc013, phi119 and phi068). The number of alleles as well as the diversity values confirm the wide genetic base of the population analyzed in this work (Table 1) (Eyherabide et al., 2006).

Finally, the level of genic differentiation among the four heterotic populations previously determined by the topcross method was evaluated; 12 loci showed statistically significant values (p < 0.05) (Table 2).

The identification of heterotic groups is essential in modern programs for genetic maize improvement, as it allows for selection of only those crosses expressing the maximum heterosis potential, which permits a more efficient use of germplasm (Hallauer and Miranda, 1988). The most used methods for establishment of heterotic patterns are top cross tests (de Azevedo Duarte et al., 2003; Nestares et al., 1999) and the diallelic analysis, not implemented very often due to the high number of crossings required (Pinto et al., 2001). It has been stated that microsatellite markers might complement or allow for the replacement of top cross tests in establishing new heterotic patterns. According to Reif et al. (2003), if the program has generated a large number of lines and the heterotic patterns have not been determined yet, then the genetically divergent germplasm may be identified by molecular markers. Based on this information, field tests may be planned more efficiently and economically.

UPGMA clustering was applied on the modified Roger’s distance or MRD based on the microsatellite data. MRD values between the lines varied between 0.52 and 0.96 (with a mean of 0.79), while the value of cophenetic tree correlation was 0.65. In general, the cluster coincided with the germplasm origin (i.e., related lines grouped together, Figure 1); the rect.hclust function (stats package of R project) allowed us to determine possible heterotic groups in the dendogram 4 (Figure 1).

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The program STRUCTURE was used as a second alternative to classify the lines according to the molecular data. As stated by Pritchard
et al. (2000), this program presents advantages with respect to the methods based on genetic distance mainly because the inference of the parameters corresponding to each group is made along with the inference of the membership degree of each individual to the groups. The groups determined by the molecular information (Figure 1 and Table 3) were compared with the four groups determined previously by topcross (Table 1) using a program implemented in R language (http://www.r-project.org/) and According to quantitative genetics, hybrid vigor is partly attributed to loci presenting a heterozygote condition (Falconer and MacKay, 1996). Consequently, the alleles whose frequencies present significant differences between two diverging heterotic groups are the best candidates for involvement in the heterotic response. Therefore, a second cluster was made based on genetic distance, but using this time only those loci selected in the test of genic differentiation (Table 2). The 12 loci were sufficient to discrim-

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**Table 3.** Grouping of lines according to the STRUCTURE software (Pritchard et al., 2000) using the complete set of 21 loci.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>lp103 (SM), lp122 (CAC), lp123(CAC), lp22(SCD), lp32 (SCD), lp38(PCA), lp44(PCA)</td>
</tr>
<tr>
<td>B</td>
<td>B73 (BSSS), lp110(SM), lp138(Exot), lp140(CRP), lp19(SCD), lp62(SA), lp662(A×252)</td>
</tr>
<tr>
<td>C</td>
<td>lp117(CAC), lp152(P578), lp199(CHI), lp521(SCD), p1338(Arg×Exot), ZN6(LocPop)</td>
</tr>
<tr>
<td>D</td>
<td>lp109(SM), lp13(SCD), lp146(CRP), lp147(CRP), lp153 (A1×LP70), lp70(SA)</td>
</tr>
</tbody>
</table>

1 The denomination of the groups is arbitrary.
2 The origin of the germplasm is indicated between parentheses.

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Cohen’s Kappa coefficient. Cohen’s Kappa coefficient allows us to determine the degree of agreement between two methods or evaluators, taking into account the agreement expected only by chance (Cohen, 1960). In general, most reports use the cluster methods based on genetic distance (Reif et al., 2005). However, it was observed in this work that the cluster obtained by STRUCTURE showed a better degree of agreement than the UPGMA-MRD clustering when they were compared with the cluster based on topcross (κ = 0.33 vs. κ = 0.16). We can attribute this outcome to: i) the low value of cophenetic correlation (0.65), which indicates the degree of fit between the distances observed in the tree to the matrix of genetic distances, and/or ii) the best performance of STRUCTURE per se (Pritchard et al., 2000).
The assumption used to establish the heterotic groups based on molecular maker data is that the loci analyzed contributed in a similar fashion to heterosis, thus lines clustered together present a similar heterotic behavior independently of the crossing evaluated (Reif et al., 2005). We suspect that this could be the main cause of the low level of agreement between the cluster based on molecular data and heterotic groups based on topcross tests. Consequently, not only must those markers associated with the heterosis be selected for the cluster, but more refined cluster algorithms considering the situation previously mentioned must be also designed.

In conclusion, the relatively high genetic diversity values (i.e., expected number of alleles per locus and heterocigosity) confirm the wide genetic base of the material of origin. From the 21 loci analyzed, 12 showed significant p-values, with respect to the test of genic differentiation among the four heterotic populations previously determined by Nestares et al. (1999). Although the maximum likelihood clustering (Program STRUCTURE) showed a better behavior than traditional methods based on genetic distance (UPGMA- modified Roger’s distance), in general a significant agreement was not observed between the molecular data and the cluster based on the topcross method. Results obtained, along with the bibliographic reports, show the need for designing more refined clustering algorithms, thus the molecular marker information may replace the field tests for determining heterotic groups.

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Resumen

M. Morales, V. Decker y L. Ornella. 2010. Análisis de diversidad genética de poblaciones heteróticas de maíz argentino utilizando marcadores moleculares. Cien. Inv. Agr. 37(1): 151 – 160. Desde las tres últimas décadas, las variedades tradicionales argentinas de maíz Cristalino Colorado han sido reemplazadas por germoplasma más competitivo de origen norteamericano. Sin embargo, los cultivares flint son una fuente potencial de resistencia a estrés biótico y abiótico. En consecuencia, el conocimiento de la diversidad genética y relación entre las líneas ayudaría a reducir la vulnerabilidad genética y aumentan la base genética del cereal en los programas de mejoramiento nacionales. En este trabajo se reporta el análisis de 25 líneas de germoplasma Cristalino Colorado y 1 línea de maíz dentada utilizando 21 marcadores microsatélite o SSR (Simple Sequence Repeats). El objetivo fue evaluar la diversidad genética entre dichas entradas y la utilidad de los marcadores SSR para definir grupos heteróticos en germoplasma de clima templado. La población de 25 líneas de maíz Cristalino Colorado presentó valores relativamente altos de diversidad genética: Número de alelos/locus = 5.14 y $He = 0.68$. El test de diferenciación génica, aplicado sobre las cuatro poblaciones heteróticas establecidas por topcross, reveló 12 loci, de un total de 21, con valores de $P$, significativos. Aunque no se observó un acuerdo importante entre los agrupamientos basados en información molecular y los grupos heteróticos establecidos por topcross, el agrupamiento bayesiano (programa STRUCTURE) presentó un mejor comportamiento respecto al agrupamiento basado en distancia genética (UPGMA-Modified Roger’s Distance).

Palabras clave: Análisis de conglomerados, microsatélite, Zea mays.

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