# SNP and SSR MARKER ANALYSIS AND MAPPING
# OF A MAIZE POPULATION

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Although highly polymorphic SSRs are currently the marker of choice worldwide in maize breeding, single nucleotide polymorphisms (SNPs) as a newer marker system are recently used more extensively. The objective of this study was investigate the utility of SSR and SNP markers for mapping of a maize population adapted to conditions of Southeast Europe. Total of 294 $F_{2:3}$ lines derived from a biparental mapping population were genotyped using 121 polymorphic SNP and SSR markers.

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The SNP markers were analyzed using the SNPlex technology. 56 of the 142 tested SNPs (39%) were polymorphic between the parents of the mapping population and were successfully mapped. The remaining markers were either not functional (5 = 3.5%) or not polymorphic (81 = 57%). No mapped SNP marker showed more than 10% missing data. On average, the level of missing data for SNPs (1.5%) was considerably lower than that for SSRs (3.4%). For the mapping procedure, the SNP data were combined SSR data. A comparison of the mapping data with the publicly available mapping data on SSR markers and the proprietary mapping data indicates that the map is of good quality and that the map position of almost all markers agrees with their published map position. Thus, information obtained from both marker systems is utilizable for further QTL analysis.

*Key words:* maize population, mapping, SNP markers, SSR makers

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## INTRODUCTION

Single nucleotide polymorphisms (SNPs) are increasingly becoming the marker of choice in genetic analyses due to rapid development of high-throughput methods for their detection. Recently, they are used routinely as markers in crop breeding programs (GUPTA *et al*., 2001) including constructions of genetic maps, linkage disequilibrium–based association mapping, studies of genetic diversity, cultivar identification, phylogenetic analysis and characterization of genetic resources (RAFALSKI, 2002). According to TENAILLON *et al*., (2001) and CHING *et al*. (2002) maize has a relatively high frequency of SNPs. A set of 640 SNPs, discovered in sequences of 60 public inbreds from the USA and Europe, that can be utilized in genetic studies and breeding applications were selected (JONES *et al* , 2009).

In maize breeding, highly polymorphic SSRs are currently the marker of choice worldwide. The same is true for Southeast Europe – European Corn Belt (e.g. BRKIĆ *et al*., 2003; JAMBROVIĆ *et al,* 2008, IGNJATOVIĆ MICIĆ *et al,* 2007, IGNJATOVIĆ MICIĆ *et al.,* 2008, DRINIĆ *et al,* 2002). However, JONES *et al*. (2007) stressed that there have been problems in maize in accurately sizing SSR markers, unequal allele amplifications, null alleles and size homoplasy (alleles of the same size may not necessarily be identical in sequence). Compared to other marker systems such as RAPDs, RFLPs, AFLPs, CAPS and microsatellites (SSRs – simple sequence repeats), SNPs are less labor intensive and less time-consuming, and the associated costs allow performing high-throughput genotyping. SNPs markers are biallelic, have lower information content than polyallelic SSR markers, but they occur at much higher density in genome, and have lower genotyping error rate (RAFALSKI, 2002; KENNEDY *et al*., 2003; MORIN *et al*., 2004). HAMBLIN *et al* (2007) compare 89 SSRs and 847 SNPs for characterization of 259 maize inbred lines and found that SSRs performed better at clustering germplasm into population than SNPs. They suggested that large number of SNP loci will be required to replace highly polymorphic SSRs in study of diversity and relatedness.

The objective of this study is to investigate the utility of SSR and SNP markers for mapping of a maize population adapted to conditions of Southeast Europe.

## MATERIALS AND METHODS

Two parents – maize inbred lines (B84 and Os6-2) belonging to opposite gene pools of U.S. Corn Belt germplasm were crossed in order to develop a mapping population. The line B84 is well known BSSS line, while OS6-2 is related to the line C103 of Lancaster origin. LIU *et al.* (2003) gave detailed background of B84 and C103 and their relation. Development of the biparental population B84xOs6-2 was described by ŠIMIĆ *et al*., (2009) in detail. 294 $F_{2:3}$ lines of the population were used for the DNA analysis.

All steps of the DNA analysis were conducted by TraitGenetics GmbH (D-06466 Gatersleben), Germany according to the standard protocols. Per $F_{2:3}$ lines, 30 seeds were provided along with the parents of the cross. Total genomic DNA was extracted from pools of ten plants per $F_{2:3}$ lines. Following the extraction of the DNA, whole genome amplification (WGA) (BARKER *et al*., 2004) was performed for the SNP analyses in order to obtain DNA of equal quality for all samples. The objective is to amplify a limited DNA sample and to generate a new sample that is indistinguishable from the original but with a higher DNA concentration. The ideal WGA technique would amplify a sample up to a microgram level while respecting the original sequence representation. The SNP markers were analyzed using the SNPlex technology (TOBLER *et al.,* 2005) which is an SNP analysis method that permits the analysis of up to 48 SNP markers in a single reaction. In total, 142 SNP markers (three multiplexes of 48/47/47 markers) were analyzed. They were derived from a proprietary SNP marker set that has been generated at TraitGenetics. They were identified through amplicon resequencing method and quality of these markers has been validated through the analysis of many maize lines at TraitGenetics (GANAL *et al.,* 2009). SNPlex analysis was performed on an ABI 3730xl DNA sequencer, whereby internal and external standards were used for size determination.

SSR fragment analysis was performed on capillary DNA sequencers (ABI 3100) using dye-labeled primers. For size determination, internal and external standards were used. 65 of the 69 pre-screened SSR markers were successfully mapped. The four remaining markers were either not functional/not useful or not polymorphic between the parents of the mapping population.

Status of marker data, linkage map, percentages of homozygosity, and genome of the Parent 1 were calculated by PLABQTL computer program (UTZ and MELCHINGER, 1996) using the *first* statement. Probabilities for the Chi-square tests are calculated according to the Wilson-Hilferty approximation. For the mapping procedure, the data of both marker systems were combined and mapped using the MapChart program (VOORRIPS, 2002). The map was constructed using Haldane's mapping function and 121 molecular markers (56 SNP and 65 SSR). Due to brevity, original marker names were abbreviated, whereby "bn", "dp", "p", and "u" stand for "bnlg", "dup", "phi" and "umc" SSR markers, respectively. SNP markers were denoted with "Z".

## RESULTS AND DISCUSSION

56 of the 142 tested SNP markers (39%) were polymorphic between the parents of the mapping population and were mapped. The remaining markers were either not functional (5 = 3.5%) or not polymorphic (81 = 57%). No mapped SNP marker showed more than 10% missing data (Table 1). On average, the SNP markers showed only 1.5% of missing data. One marker was of dominant inheritance, and five had significantly distorted segregation (8.9%).

*Table 1. Status of SNP marker data, linkages and percentages of homozygosity and genome of Parent 1.*

| | | | | Number of observed segregation | | | Allele frequency of *m* | |
| | | Chrom. | Number of missing | Parent1 | | Parent2 | | |
| | Marker | number | genotypes | *mm* | *Mm* | *MM* | Frequency | $X^2$ |
|---|---|---|---|---|---|---|---|---|
| 1 | Z0451 | 1 | 0 | 63 | 142 | 89 | 0.46 | 2.3 |
| 2 | Z1300 | 1 | 3 | 57 | 163 | 71 | 0.48 | 0.67 |
| 3 | Z0876 | 1 | 3 | 62 | 168 | 61 | 0.50 | 0 |
| 4 | Z0845 | 1 | 2 | 69 | 161 | 62 | 0.51 | 0.17 |
| 5 | Z1096 | 1 | 4 | 71 | 147 | 72 | 0.50 | 0 |
| 6 | Z0230 | 2 | 7 | 103 | 125 | 59 | 0.58 | 6.75 |
| 7 | Z0356 | 2 | 6 | 53 | 152 | 83 | 0.45 | 3.12 |
| 8 | Z0820 | 2 | 6 | 56 | 145 | 87 | 0.45 | 3.34 |
| 9 | Z1359 | 2 | 5 | 55 | 147 | 87 | 0.44 | 3.54 |
| 10 | Z0195 | 2 | 3 | 63 | 146 | 82 | 0.47 | 1.24 |
| 11 | Z1368 | 2 | 5 | 80 | 129 | 80 | 0.50 | 0 |
| 12 | Z1372 | 2 | 0 | 18 | 198 | 78 | 0.40 | 12.24* |
| 13 | Z0823 | 2 | 4 | 63 | 146 | 81 | 0.47 | 1.12 |
| 14 | Z1364 | 3 | 2 | 39 | 162 | 91 | 0.41 | 9.26 |
| 15 | Z1290 | 3 | 4 | 53 | 131 | 106 | 0.41 | 9.69 |
| 16 | Z1376 | 3 | 0 | 28 | 173 | 93 | 0.39 | 14.37* |
| 17 | Z0237 | 3 | 6 | 60 | 149 | 79 | 0.47 | 1.25 |
| 18 | Z1268 | 3 | 12 | 75 | 133 | 74 | 0.50 | 0 |
| 19 | Z0366 | 3 | 5 | 60 | 147 | 82 | 0.46 | 1.67 |
| 20 | Z1386 | 3 | 1 | 67 | 116 | 110 | 0.43 | 6.31 |
| 21 | Z1261 | 4 | 1 | 66 | 146 | 81 | 0.47 | 0.77 |
| 22 | Z0967 | 4 | 5 | 68 | 138 | 83 | 0.47 | 0.78 |
| 23 | Z0839 | 4 | 4 | 80 | 121 | 89 | 0.48 | 0.28 |
| 24 | Z1362 | 4 | 5 | 60 | 159 | 70 | 0.48 | 0.35 |
| 25 | Z0819 | 4 | 0 | 56 | 153 | 85 | 0.45 | 2.86 |
| 26 | Z0391 | 4 | 7 | 77 | 116 | 94 | 0.47 | 1.01 |
| 27 | Z0215 | 5 | 6 | 73 | 147 | 68 | 0.51 | 0.09 |
| 28 | Z0322 | 5 | 7 | 75 | 140 | 72 | 0.51 | 0.03 |

| 29 | Z0893 | 5 | 4 | 95 | 129 | 66 | 0.55 | 2.9 |
|----|-------|---|---|-----|-----|-----|------|------|
| 30 | Z1312 | 5 | 8 | 72 | 147 | 67 | 0.51 | 0.09 |
| 31 | Z1384 | 5 | 2 | 66 | 159 | 67 | 0.50 | 0 |
| 32 | Z0141 | 5 | 12 | 56 | 156 | 70 | 0.48 | 0.7 |
| 33 | Z0304 | 5 | 11 | 56 | 0 | 227 | Dominance | |
| 34 | Z0287 | 5 | 7 | 64 | 153 | 70 | 0.49 | 0.13 |
| 35 | Z1371 | 5 | 7 | 71 | 154 | 62 | 0.52 | 0.28 |
| 36 | Z1260 | 5 | 4 | 73 | 153 | 64 | 0.52 | 0.28 |
| 37 | Z0826 | 5 | 2 | 136 | 96 | 60 | 0.63 | 19.78* |
| 38 | Z0831 | 5 | 2 | 73 | 156 | 63 | 0.52 | 0.34 |
| 39 | Z0904 | 5 | 3 | 64 | 156 | 71 | 0.49 | 0.17 |
| 40 | Z0189 | 5 | 2 | 62 | 163 | 67 | 0.49 | 0.09 |
| 41 | Z0901 | 5 | 3 | 61 | 162 | 68 | 0.49 | 0.09 |
| 42 | Z0900 | 6 | 3 | 68 | 127 | 96 | 0.45 | 2.69 |
| 43 | Z0960 | 6 | 2 | 65 | 147 | 80 | 0.47 | 0.77 |
| 44 | Z1367 | 6 | 6 | 71 | 167 | 50 | 0.54 | 1.53 |
| 45 | Z0314 | 6 | 8 | 68 | 100 | 118 | 0.41 | 8.74 |
| 46 | Z0173 | 7 | 12 | 49 | 106 | 127 | 0.36 | 21.57* |
| 47 | Z1299 | 7 | 2 | 45 | 161 | 86 | 0.43 | 5.76 |
| 48 | Z1352 | 7 | 5 | 46 | 140 | 103 | 0.40 | 11.24 |
| 49 | Z0936 | 8 | 2 | 72 | 150 | 70 | 0.50 | 0.01 |
| 50 | Z0353 | 8 | 8 | 83 | 130 | 73 | 0.52 | 0.35 |
| 51 | Z0825 | 8 | 0 | 76 | 144 | 74 | 0.50 | 0.01 |
| 52 | Z0943 | 9 | 2 | 90 | 157 | 45 | 0.58 | 6.93 |
| 53 | Z0385 | 9 | 9 | 96 | 151 | 38 | 0.60 | 11.8* |
| 54 | Z0363 | 10 | 6 | 69 | 164 | 55 | 0.52 | 0.68 |
| 55 | Z1315 | 10 | 3 | 78 | 159 | 54 | 0.54 | 1.98 |
| 56 | Z0968 | 10 | 1 | 78 | 144 | 71 | 0.51 | 0.17 |

• Distorted segregation significant if probability<0.001

Most of the SSR markers showed no major problems during fragment analysis and mapping. Some other SSR markers showed unfixed alleles mainly coming from mapping Parent 2 or were not that stable and showed on average much more missing data than the other markers (Table 2). On average, the markers showed 3.4% of missing data. Three SSR markers were of dominant inheritance, and other three had significantly distorted segregation (4.65%).

*Table 2. Status of SSR marker data, linkages and percentages of homozygosity and genome of Parent 1.*

|     | Marker | Chrom. number | Number of missing genotypes | Number of observed segregation | | | Allele frequency of $m$ | |
|-----|--------|---------------|------------------------------|------|------|------|-----------|----------|
|     |        |               |                              | Parent1 | | Parent2 | | |
|     |        |               |                              | *mm* | *Mm* | *MM* | Frequency | $X^2$ |
| 1   | bn1014 | 1 | 10 | 54 | 141 | 89 | 0.44 | 4.31 |
| 2   | bn1429 | 1 | 6 | 58 | 149 | 81 | 0.46 | 1.84 |
| 3   | p00064 | 1 | 2 | 80 | 0 | 212 | Dominance | |
| 4   | bn1866 | 1 | 96 | 18 | 144 | 36 | 0.45 | 1.64 |
| 5   | p109275 | 1 | 4 | 51 | 171 | 68 | 0.47 | 1 |
| 6   | bn2086 | 1 | 3 | 56 | 183 | 52 | 0.51 | 0.05 |
| 7   | bn0615 | 1 | 13 | 65 | 144 | 72 | 0.49 | 0.17 |
| 8   | p96100 | 2 | 4 | 54 | 169 | 67 | 0.48 | 0.58 |
| 9   | bn1302 | 2 | 19 | 53 | 153 | 69 | 0.47 | 0.93 |
| 10  | p00083 | 2 | 2 | 41 | 182 | 69 | 0.45 | 2.68 |
| 11  | bn1831 | 2 | 19 | 48 | 143 | 84 | 0.43 | 4.71 |
| 12  | bn1329 | 2 | 2 | 58 | 148 | 86 | 0.45 | 2.68 |
| 13  | bn1662 | 2 | 15 | 66 | 136 | 77 | 0.48 | 0.43 |
| 14  | bn1940 | 2 | 6 | 67 | 149 | 72 | 0.49 | 0.09 |
| 15  | bn1325 | 3 | 4 | 51 | 154 | 85 | 0.44 | 3.99 |
| 16  | bn1523 | 3 | 10 | 38 | 172 | 74 | 0.44 | 4.56 |
| 17  | bn1904 | 3 | 5 | 44 | 212 | 33 | 0.52 | 0.42 |
| 18  | p00099 | 3 | 2 | 48 | 158 | 86 | 0.43 | 4.95 |
| 19  | bn2047 | 3 | 5 | 47 | 154 | 88 | 0.43 | 5.82 |
| 20  | bn1456 | 3 | 7 | 44 | 159 | 84 | 0.43 | 5.57 |
| 21  | p00053 | 3 | 7 | 41 | 138 | 108 | 0.38 | 15.64* |
| 22  | bn1605 | 3 | 14 | 57 | 151 | 72 | 0.47 | 0.8 |
| 23  | bn1257 | 3 | 2 | 67 | 141 | 84 | 0.47 | 0.99 |
| 24  | bn1182 | 3 | 4 | 117 | 113 | 60 | 0.60 | 11.2* |
| 25  | p00096 | 4 | 2 | 77 | 139 | 76 | 0.50 | 0 |
| 26  | bn1265 | 4 | 6 | 70 | 137 | 81 | 0.48 | 0.42 |
| 27  | u2027 | 4 | 7 | 53 | 171 | 63 | 0.48 | 0.35 |
| 28  | bn1784 | 4 | 16 | 69 | 141 | 68 | 0.50 | 0 |
| 29  | bn1189 | 4 | 6 | 66 | 167 | 55 | 0.52 | 0.42 |
| 30  | dp28 | 4 | 16 | 68 | 146 | 64 | 0.51 | 0.06 |
| 31  | p00024 | 5 | 4 | 54 | 176 | 60 | 0.49 | 0.12 |
| 32  | bn1046 | 5 | 1 | 80 | 153 | 60 | 0.53 | 1.37 |
| 33  | bn1208 | 5 | 8 | 51 | 177 | 58 | 0.49 | 0.17 |
| 34  | p00087 | 5 | 5 | 237 | 0 | 52 | Dominance | |
| 35  | bn1740 | 6 | 3 | 51 | 0 | 240 | Dominance | |
| 36  | p00126 | 6 | 5 | 74 | 156 | 59 | 0.53 | 0.78 |
| 37  | bn0426 | 6 | 11 | 79 | 136 | 68 | 0.52 | 0.43 |

| 38 | u1887 | 6 | 9 | 60 | 158 | 67 | 0.49 | 0.17 |
|----|-------|---|---|----|-----|----|------|------|
| 39 | p00031 | 6 | 7 | 62 | 178 | 47 | 0.53 | 0.78 |
| 40 | p00078 | 6 | 2 | 62 | 158 | 72 | 0.48 | 0.34 |
| 41 | u1545 | 7 | 20 | 74 | 132 | 68 | 0.51 | 0.13 |
| 42 | bn1094 | 7 | 32 | 35 | 147 | 80 | 0.41 | 7.73 |
| 43 | bn1808 | 7 | 14 | 44 | 150 | 86 | 0.43 | 6.3 |
| 44 | bn1070 | 7 | 6 | 47 | 142 | 99 | 0.41 | 9.39 |
| 45 | p00116 | 7 | 2 | 64 | 154 | 74 | 0.48 | 0.34 |
| 46 | p00051 | 7 | 2 | 63 | 148 | 81 | 0.47 | 1.11 |
| 47 | bn1194 | 8 | 2 | 70 | 164 | 58 | 0.52 | 0.49 |
| 48 | p00119 | 8 | 5 | 84 | 152 | 53 | 0.55 | 3.33 |
| 49 | p100175 | 8 | 2 | 69 | 166 | 57 | 0.52 | 0.49 |
| 50 | bn1176 | 8 | 38 | 65 | 135 | 56 | 0.52 | 0.32 |
| 51 | bn1834 | 8 | 13 | 132 | 95 | 54 | 0.64 | 21.65* |
| 52 | bn1782 | 8 | 8 | 71 | 144 | 71 | 0.50 | 0 |
| 53 | bn1131 | 8 | 5 | 75 | 150 | 64 | 0.52 | 0.42 |
| 54 | bn2122 | 9 | 16 | 77 | 142 | 59 | 0.53 | 1.17 |
| 55 | p00017 | 9 | 10 | 82 | 153 | 49 | 0.56 | 3.83 |
| 56 | bn0244 | 9 | 1 | 85 | 160 | 48 | 0.56 | 4.67 |
| 57 | p00065 | 9 | 2 | 87 | 164 | 41 | 0.58 | 7.25 |
| 58 | u1675 | 9 | 3 | 73 | 151 | 67 | 0.51 | 0.12 |
| 59 | bn0619 | 9 | 35 | 55 | 135 | 69 | 0.47 | 0.76 |
| 60 | bn0128 | 9 | 4 | 73 | 145 | 72 | 0.50 | 0 |
| 61 | bn1129 | 9 | 10 | 61 | 144 | 79 | 0.47 | 1.14 |
| 62 | u1152 | 10 | 20 | 71 | 139 | 64 | 0.51 | 0.18 |
| 63 | bn1526 | 10 | 18 | 69 | 160 | 47 | 0.54 | 1.75 |
| 64 | bn1839 | 10 | 4 | 74 | 155 | 61 | 0.52 | 0.58 |
| 65 | bn1360 | 10 | 12 | 69 | 148 | 65 | 0.51 | 0.06 |

As expected, the level of missing data for SNPs was considerably lower than that for SSRs, corroborating herewith the findings of JONES *et al*., (2007). They found that SNP marker data had more than a fourfold lower level of missing data compared to SSR markers. Generally, it has been observed that SNPs are more reliable than SSRs (GUPTA *et al*., 2001). LUI *et al.* (2005) compare microsatellites and SNPs in the context of population structure inference.and found that although SNPs are less informative than microsatellites on average, among the most informative markers, SNPs usually constitute the majority. However, SNPs revealed more markers with segregation distortion, than SSRs (Tables 1 and 2), but not in a special region of the map or in a special direction (not only concerning one of the two alleles or the heterozygous genotypes). Additionally, although the ratio of the respective two alleles in heterozygous samples was variable (from very weak allele 1 and strong allele 2 to very strong allele 1 and weak allele 2), the mapping procedure was not significantly affected.

For the mapping procedure, the data of both marker systems were combined and mapped (Figure 1). Without any major constraints, ten stable linkage groups were received with a good distribution of both SNP and SSR markers over all groups. The markers mapped predominantly on the expected position as detected on the IBM mapping population. This is consistent with results of Jones *et al.* (2009). Two of the SSR markers (p00064 and bn1740) were grouped as expected but not mapped on the correct position, probably because of their dominant inheritance as well as one SNP marker which was not grouped as expected. The current position of the marker seems to be correct. A map was produced with length of 484.6 cM, and average distance between markers was 4.4 cM. The percentage of genome within 20 cM to the nearest marker equals 100%. The markers mapped predominantly on the expected position similar to the MaizeGDB maps.
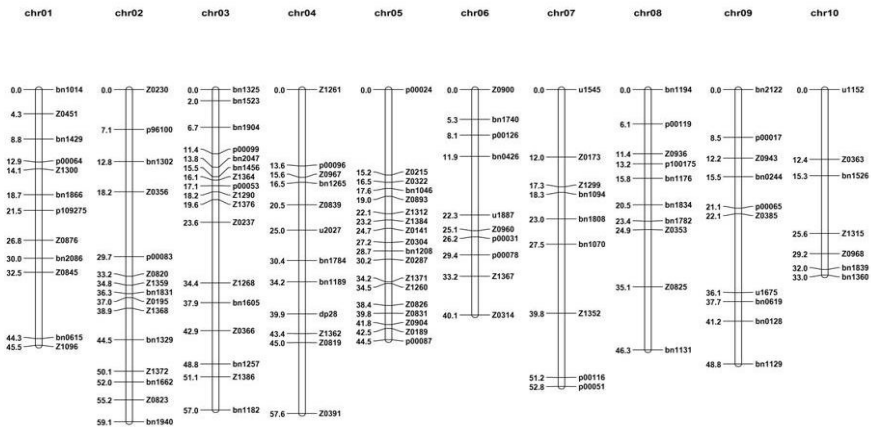


Figure 1. Linkage map based on polymorphic 121 marker loci (65 SSR and 56 SNP markers).
Right of bars are names of SSR and SNP markers, slightly abbreviated.
Left of bars are their relative positions in centi Morgan (cM). Graphs are made with MapChart (VOORRIPS, 2002).

A comparison of the mapping data with the publicly available mapping data on microsatellite markers and the proprietary mapping data generated by TraitGenetics indicates that the provided map is of good quality and that the map position of almost all markers agrees with their published map position. Thus, the marker information can be used for further QTL analysis.

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# ANALIZA I MAPIRANJE SNP I SSR MARKERA U POPULACIJI KUKURUZA

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Iako su trenutno visoko polimorfni SSR markeri izbora, novi markeri SNP se od nedavno koriste veoma intezivno u programima oplemenjivanja kukuruza u svetu. Cilj ovog istraživanja je ispitivanje primene SSR i SNP markera za mapiranje populacije kukuruza koje su prilagođene  uslovima Jugoistočne Evrope. Ukupno 294 $F_{2:3}$ linija dobijeno iz biparentalne mapirajuće populacije je analizirano sa 121 polimorfnim SNP i SSR markerom. SNP markeri su analizirani primenom SNPlex tehnologije. Pedeset šest od 142 ispitanih SNP markera (39%) je bilo polimorfno između roditelja mapirajuće populacije i uspešno je mapirano. Preostali markeri su bili ili nefunkcionalni (5=3,5%) ili nisu bili polimorfni (81=57%). Nemapirani SNP markeri su imali više od 10% podataka koji su nedostajali. U proseku, nivo podataka koji su nedostajali za SNP (1,5%) je bio značajno niži nego za SSR (3,4%). Za mapiranje kombinovani su SNP i SSR podaci. Poređenje mapirajućih podataka sa javno dostupnim mapirajućim podacima za SSR i zaštičenim mapirajućim podacima ukazuje da je mapa dobrog kvaliteta i da se pozicije na mapi skoro svih markera slažu sa njihovim prethodno objavljenim pozicijama. Otuda, informacije dobijene primenom oba marker sistema se mogu korisititi za buduće QTL analize.