Genetic Variation and Relationship Between Turkish Flint Maize Landraces by RAPD Markers

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Abstract: A comparative characterization of 17 flint maize landraces (Zea mays L.) was carried out using RAPD markers. Fourteen primers giving reliable and consistent polymorphic bands amplified 125 fragments (89%) with an average of 8.90 fragment per primer. Genetic variation in 17 maize landraces was characterized based on dissimilarity matrix by UPGMA dendogram which has no association into distinct grouping with respect to locations and many small clusters formed. The similarity was in range from 0.05 to 0.88. It is interesting that there was close relationship among yellow and yellow-orange endosperm accessions with the dissimilarity between 0.08 and 0.20. The group containing mostly white endosperm accessions displayed a range of genetic distance. It is considered that isolated accessions showed the highest genetic distance has a uncertain origin derived from a sample collected by landholder farmer. Turkish flint accessions had a high variability crossing from the different maize genetic resources. Only, isolated small landholder farmers kept the landraces without pollination from the different source of germplasm. These results will be able to help to maintain a germplasm collection with the genetic diversity among maize landraces for breeding program of maize in Turkey.

Key words: Turkish flint landrace, maize, RAPD, color

INTRODUCTION

The genetic information of a landrace can allow the possible explanation of genes for traits such as disease resistance, tolerance to environmental stresses by conservation of landraces which have high genetic variability with the fitness to the environments where they have originated[1]. Genetic knowledge of germplasm diversity among local populations has a significant impact on the improvement of plants not only a valuable source of useful traits but also a bank of highly adapted genotypes[2,3]. The genetic diversity has been assessed more efficiently relating polymorphism from the morphological, biochemical and DNA labels. Genetic variability in landraces has been studied by morphological traits [4,5,6,7,8] and isozyme [9,10,11,12,13,14], DNA polymorphism assays are powerful tools for characterizing and studying germplasm resources[15]. The first molecular markers used in maize breeding programme are RFLP[16, 17]. Markers based on polymerase chain reaction have been used in analysis of genetic distance such as RAPD [18]. RAPD markers are commonly used enabling genetic diversity analysis for plant populations in breeding program and germplasm collections [19]. RAPD markers were better where easy applications and cost were considered comparing RFLP [20, 21]. In maize, RAPD markers have been used in describing genetic diversity between maize accessions[7,22].

Maize is the most extensively cultivated crop in Turkey after wheat [23]. The introduction of maize (Zea mays L.) to Turkish cultivation system goes back around 1600 A.D. thought Ottoman traders following Spanish explorers to the Mediterranean coasts[12,24]. Up to date, newer landraces have originated from introduced populations with adaptation to local conditions. On the other hand, planned introduction of maize accessions to Turkey were made by Agricultural Institutions in Turkey until 1950s [24]. Farmers used open-pollinated varieties of maize with traditional farming practices until last quarter of this century when hybrids were introduced to Turkey.

Black Sea Region of Turkey is one of the areas that maize grows. The structure of regions with rainy climate and narrow growing area allows growing the maize at the coastal sides. Maize is growth in this area for long time, traditionally. The seeds are produced from the former plant’s seeds with limited seed exchange with other farmers. Most of the people use
maize for human and animal consumption and they do not use hybrids except plain areas with the low altitude. So, the region conserves the maize resources with traditional practices. The genetic diversity between flint Turkish maize landraces growing at the north of Turkey is not known although it shows different color of kernel types. The purpose of the present study is to assess genetic diversity among populations of these landraces in 17 cultivated accessions of landraces by molecular markers.

**MATERIAL AND METHODS**

**Plant material:** Seeds of seventeen maize accessions were obtained from north of Turkey which showed the characteristics of flint maize group. The accessions were selected to represent the whole north of Turkey in different altitudes and maintained in reproductive isolation by traditional agricultural farming for years of farmer-directed selection. Each accessions were represented by 20 individuals and kernels randomly sampled in four different ears on the basis of morphology. Polymorphism degree and genetic relationships among 17 maize landraces were evaluated with RAPD markers. The collection of flint maize accessions included different kernel color which can be used in the germplasm evaluation with flowering time according to Nass and Paterniani [25]. Flowering time changes due to the environmental conditions and it occurs between 40 and 100 days, generally [26]. The accessions were also evaluated in the flowering time (Table 1).

Table 1: The genotype code, locality, kernel color of Turkish flint maize accessions.

| No. | Genotype code | Locality   | Kernel color     | Altitude (m) |
|-----|---------------|------------|------------------|--------------|
| 1   | 12            | Çatalarmut | Yellow           | 120          |
| 2   | 16            | Keresteci1 | Yellow           | 10           |
| 3   | 19            | Eğercili1  | White            | 10           |
| 4   | 20            | Eğercili2  | Yellow           | 10           |
| 5   | 21            | Rize-Pazar | Yellow           | 350          |
| 6   | 25            | Trabzon-Tonya | Yellow   | 1000         |
| 7   | 27            | Tokat-Nıksar1 | Yellow   | 350          |
| 8   | 28            | Tokat-Nıksar2 | White   | 530          |
| 9   | 31            | Çorum      | Yellow           | 550          |
| 10  | 43            | Artvin     | White            | 900          |
| 11  | 46            | Ordu-Kumru | Yellow-orange    | 500          |
| 12  | 49            | Keresteci2 | Yellow-orange    | 10           |
| 13  | 51            | Elıfi      | White            | 20           |
| 14  | 57            | Termre     | White            | 20           |
| 15  | 67            | Eğercili 6(3) | Yellow   | 500          |
| 16  | 68            | Ordu-İkizce | Yellow           | 600          |
| 17  | 86            | Asarcık    | White            | 600          |

**DNA extraction and amplification:** Maize leaves were collected from the healthy seedlings and frozen in liquid nitrogen. Genomic DNA was isolated from the leaf samples according to the protocol of CTAB described by Doyle&Doyle [27]. DNA samples were measured in a fluorometer (Eppendorf-Spectrofotometer) adjusting to 5 ng/µl. PCR reactions for RAPD were done in a volume of 25 µl containing 13 µl master mix (Promega), 25 ng DNA template, 20 ng of 10-mer primer (Operon Technology) and deionize water. The PCR mix was settled to Hybaid thermal cycles with the following program: initial denaturation step for 2 min following 40 cycles at 94 °C for 1 min, 38 °C for 2 min and 72 °C for 2 min and a final cycle at 72 °C for 10 min. The amplified products were separated by agarose electrophoresis in 1.5 % (SIGMA-low melting agarose) in 0.5xTAE buffer (Tris Base 0.04 M and EDTA 0.01 M pH 8.3), containing 0.10 µg/µl of ethidium bromide. The gels were runned under 100 V for 5 h and transferred to gel documentation system (Syngene) for analysis. Molecular sizes of the products were estimated by Lambda DNA (wide range marker-SIGMA).

**Data analysis:** PCR products of eleven polymorphic primers to represent a single locus and data were scored for presence (1) and absence (0) of the respective bands in each population. Genetic distance (GD) values were calculated between all pairs of accessions according to Jaccard’s Formula [28]. The dissimilarity matrix was used to assess the relationships among landrace populations with a dendogram constructed by cluster analysis using UPMGA using NTSYS computer program, Version 2.11 [29].

**RESULTS AND DISCUSSION**

**RAPD marker analysis:** Totally, 160 primers were screened and 14 of them were found to be valuable for RAPD analysis. One hundred and fourtisix primers (91%) yielded either monomorphic or unrepducible fragments. The remaining 14 primers giving reliable and consistent polymorphic bands were used to amplify genomic DNA of the 17 maize accessions (Table 2). To increase analysis precision, weak bands occurring from low homology between the primer and the pairing site were disregard [21,30]. A total 125 fragments, in range of 180 (OPC-11) to 1500 (OPC-9) bp were analysed with an average of 8.90 fragment per primer.
### Table 2: The primer sequence, number of total band, shared bands and size of fragments of used decamers

| Primer | Sequence | Size of Fragments |
|--------|----------|-------------------|
| OPC-1  | TTCGAGCCAG | 390-960           |
| OPC-02 | GTGAGGCGTC | 280-1050          |
| OPC-07 | GTCCCGAGGA | 550-1500          |
| OPC-08 | TGGACCGGTG | 350-1100          |
| OPC-09 | CTCACCGGTTC | 330-1200         |
| OPC-15 | GACGGATCAG | 180-980           |
| OPC-19 | GTGTCGAGCC | 300-1050          |
| OPD-03 | GTGCGCGTCA | 300-1500          |
| OPD-05 | TGAGCGGCAC | 300-1300          |
| OPD-08 | GTGTGCCCCCA | 330-830         |
| OPD-18 | GAGAGGCAAC | 360-1260          |
| OPD-20 | ACCGGCTCAC | 260-1200          |
| OPE-07 | AGATGCAGGC | 340-1400          |
| OPE-15 | ACGCACAACC | 200-1100          |

The percentage of polymorphism was calculated as 89%. It is good value of efficiency for genetic analysis that in the study done on Ethiopian highland maize accessions by Beyene et al., (2005) showed a all SSR loci and 89.5 % of AFLP bands were polymorphic. This value was found lower in southern Brazilian maize accessions[30], in the genetic analysis of endogamic maize lines[31], in the study of maize hybrids[32] and in the genetic analysis among varieties on native American maize[22].

### Genetic variation and relationships among maize landraces:

It is considered that Turkish maize accessions indicate large amount of genetic diversity in the most of the accessions analyzed by isozyme analysis of different maize races[12], discriminant analysis of different races of morphologic parameters[8] and Turkish maize accessions belonging to the north of Turkey (unpublished data).

The extent of genetic variation in 17 maize landraces was characterized based on dissimilarity matrix by UPGMA dendogram which has no association into distinct grouping with respect to locations, many small clusters formed (Figure 1). The accessions of Tokat-Niksar2, Termel, Eğercili1 and Rize-Pazar did not join to clustering with the largest genetic distance value. İlarslan et al.,[12] reported that the most of flint maize accessions took place in the large cluster groups analyzed by isozymes without grouping with respect to locations in Turkey. Genetic distance between studied Turkish flint maize accessions revealed that accessions Tokat-Niksar2, Termel, Eğercili2 and Rize-Pazar had the highest genetic dissimilarity due to other accessions. The dissimilarity ranged between 0.116 (Ordu-İkizce x Trabzon-Tonya) to 0.947 (Termel x Elifli).
yellow-orange maize accessions studied in Keresteci1 x Keresteci2 and Çorum x Ordu-Kumru. Whereas Trabzon-Tonya x Ordu-İkizce accessions had low genetic distance for yellow endosperm color and Elifli x Eğercili had for white endosperm.

To avoid from the replication of genetic material in the evaluation of accessions, molecular markers are important tools. The highest genetic similarity (0.88) was observed between the Keresteci1 and Keresteci2 landraces. These accessions possibly have been cultivated in either distinct regions or as a result of seed exchange between unrelated farmers with the same name which the landrace was collected. Similar results reported by [36] in the study done by isozymes analysis in 15 maize populations derived from three indigenous maize accessions. Carvalho et al., [30] also observed similar results from the study on the 81 maize accessions in Brazilian accession by RAPD markers. The authors reported that there was no connection between the accession name and genetic relationships.

Each cluster formed several small groups and revealed the genetic divergence within the yellow color and within the white color landraces. This implies that the Turkish flint maize landraces were crossed from the different maize genetic resources in the past in different times. This can be the affect of hybrids or introductions compared to Brazilian landraces that these landraces from United States after 1960s. The results were compared to Brazilian landraces that these landraces also was derived from crossing introductions from United States in the past and maize accessions cultivated for an extended period of time [30,37].

CONCLUSION

In the region, the white germplasms are produced for human consumption and the yellow germplasms are growth for human and animal consumption. Some farmers prefer the white color of maize for production, only in the region. On the other hand, it was seen in some ears that there was a mix of white and yellow color of seeds and it means that there is also a seed mixture by pollen mobility. Only, isolated small landholder farmers keep the landraces without pollination from the different source of germplasm. The farmers prefer landraces because of less expensive than the commercial hybrids. Therefore, improvement of landraces is important for traditional agriculture maintenance. This will help the conservation and management of this valuable Turkish flint maize germplasm for breeding program of maize.

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