

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

A. Okumus

Agriculture Faculty, Department of Animal Science\Biometry-Genetics Unit, Ondokuz Mayıs
University, 55139, Samsun, Turkey

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 dendrogram 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^[4]. 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 growths. 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-Niksar1	Yellow	530
8	28	Tokat-Niksar2	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	Elifli	White	20
14	57	Terme	White	20
15	67	Eğercili 6(3)	Yellow	10
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 deionized 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 run 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 dendrogram 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 fourty-six primers (91%) yielded either monomorphic or unreproducible 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 disregarded^[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	GTCCCGACGA	550-1500
OPC-08	TGGACCGGTG	350-1100
OPC-09	CTCACCGTCC	330-1200
OPC-15	GACGGATCAG	180-980
OPC-19	GTTGCCAGCC	300-1050
OPD-03	GTCCCGTCA	300-1500
OPD-05	TGAGCGGACA	300-1300
OPD-08	GTGTGCCCA	330-830
OPD-18	GAGAGCCAAC	360-1260
OPD-20	ACCCGGTCAC	260-1200
OPE-07	AGATGCAGCC	340-1400
OPE-15	ACGCACAACC	200-1100
Total/range		180-1500

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 dendrogram which has no association into distinct grouping with respect to locations, many small clusters formed (Figure 1). The accessions of Tokat-Niksar2, Terme, Eğercili2 and Rize-Pazar did not join to clustering with the largest genetic distance value. Ilarslan 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, Terme, 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 (Terme x Elifli).

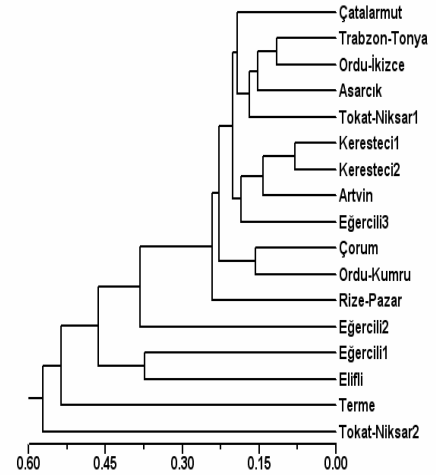


Fig. 1: Dendrogram of 17 Turkish flint maize landraces constructed using UPGMA method based on a dissimilarity matrix by NTYSYS 2.1 software

It is interesting that there was close relationships 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. However, Tokat-Niksar2, Terme, Eğercili1 and Elifli accessions remained isolated in the dendrogram with white endosperm. It is considered that isolated accessions (Tokat-Niksar2, Terme, Eğercili1 and Elifli) showed the highest genetic distance has a uncertain origin derived from a sample collected by landholder farmer. The similar results was showed in a study done by Carvalho et al.,^[30] in the Brazilian accessions on the endosperm color grouping by RAPD and in the same maize collections by inter simple sequence repeats (ISSR)^[31].

Moeller and Schall^[22] discussed the similarity index changing from 0.44 to 0.80 in Native American maize collections of Great Plains by RAPD markers. It was suggested that the high level of variability is an indicator of migration of maize accessions to other localities. In Brazilian accessions had a similarity varied between 0.78 to 0.91^[30]. The similarity in Turkish flint accessions was in range from 0.05 to 0.88 with high variability. Possibly, it represents a large fraction of the Turkish flint maize collection and Samsun territory is an origin of these accessions due to multivariate discriminant analysis (unpublished data).

The association between morphological and dendrogram prepared by RAPD among open pollinated varieties have been reported by^[30,33,34,35]. The low genetic distance was seen between the yellow and

yellow-orange maize accessions studied in Keresteci1 x Keresteci2 and Çorum x Ordu-Kumru. Whereas Trabzon-Tonya x Ordu-Ikizce accessions had low genetic distance for yellow endosperm color and Elifli x Eğercili1 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 from United States. In Black Sea Agricultural Research Institute-Samsun in Turkey, it is known that there were some practices with the accessions coming to Turkey 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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