

RELATIONSHIP BETWEEN MAIZE LANCASTER INBRED LINES  
ACCORDING TO SNP-ANALYSIS

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The modern division of maize genotypes into germplasms and subgermplasms went away from exclusively pedigree registration, hybridization analysis or phenotypic observations and nowadays is based on the utilization of DNA-technologies. DNA-markers of single nucleotide polymorphism (SNP-markers) are widely used for characterization of maize genetic polymorphism. They give information about relationship and genetic distances between genotypes, which is necessary in breeding schemes based on certain heterotic models (Richard et al., 2016, Wu et al., 2016).

The aim of our work is to evaluate the level of relationship on SNP-markers between maize Lancaster inbred lines from Ukrainian breeding programs themselves and with typical representatives of Lancaster group – lines Mo17 and Oh43.

**Materials and methods.** The following 28 maize (*Zea mays* L.) new elite inbred lines were used as research materials: DK633, DK267, DK212, DK6080, DK633266, DK298, DK3070, DK236, DK633325, DK296MV, DK1129, DK2668, DK3023zCzM, DK3626, DK6342, DK2965zCzM, DK4263, DKD9053, DKV3451, DK8143, DK517MV, DK534zCzM, DK117zCMV, DK231zC, DK6337zCzM, DK2953zCzM, DK366M and DK3044. These inbreds were produced in Institute of Grain Crops of National Academy of Agrarian Sciences (Dnipro, Ukraine) in climatic zone of Northern Steppe of Ukraine and were attributed as Lancaster by pedigree. As typical representatives of main subgroups of Lancaster germplasm public inbred lines Mo17 and Oh43 were chosen.

SNP-genotyping of maize inbred lines was performed through analyzing single nucleotide polymorphism on SNP-markers with GoldenGate test and Illumina VeraCode screening system (Fan et al., 2006) in BioDiagnostics, Inc. (USA). For genotyping the BDI-III panel with 384 SNP-markers developed by BioDiagnostics, Inc. (USA) on the base of Illumina VeraCode Bead Plate was used. SNP-markers of the BDI-III panel were selected *in silico* according to the results of analysis of 95 commercial maize inbred lines derived from USA breeding programs, they are biallelic, equally distributed on all 10 maize chromosomes and have designability rank score >0.6 (Venkatramana et al., 2012).

**Results.** The average homozygosity of breeding samples on SNP-markers analyzed was 99.61%. Heterozygous SNP-sites were not taken into account in further calculations. The part of markers with the frequency of missing data >20% amounted to 2.8 %. The frequency of monomorphic markers in the given set of inbreds was 6.3%, while the frequency of dimorphic ones was 93.7%. Minor allele frequency >0.2 was observed for 52.4% of dimorphic markers.

The indices of gene diversity of Lancaster lines (table 1) varied in the range of 0.1701-0.1873 (at the widest possible range of 0-0.5) confirmed the phylogenetic affinity of investigated inbreds.

Table 1

The indices of gene diversity for Lancaster inbred lines according to SNP-analysis

Inbred line	Gene diversity index	Inbred line	Gene diversity index
DK236	0.1873	DK1129	0.1769
DK6342	0.1862	DK633	0.1764
DK3626	0.1833	DK534zCzM	0.1763
Oh43	0.1821	Mo17	0.1759
DK8143	0.1816	DKD9053	0.1754
DK633266	0.1811	DK212	0.1751
DK2965zCzM	0.1810	DK6080	0.1740
DK3070	0.1808	DK2668	0.1738
DK117zCMV	0.1791	DKV3451	0.1736
DK3023zCzM	0.1787	DK3044	0.1734
DK296MV	0.1786	DK 231zC	0.1722
DK298	0.1784	DK267	0.1718
DK633325	0.1782	DK4263	0.1713
DK366M	0.1779	DK6337zCzM	0.1703
DK2953zCzM	0.1771	DK517MV	0.1701
X ± mt <sub>0.05</sub>		0.1774 ± 0.0016	
Lim		0.1701 ÷ 0.1873	

Index of polymorphic information content (PIC) of SNP-markers of the given panel ranged from 0 to 0.3750 with the mean value at 0.2420.

The least genetic distance (0.0249) was observed between inbred lines DK3023zCzM and DKD9053 while the largest one took place between DK8143 and Oh43. According to the results of SNP-analysis, the dendrogram of phylogenetic relationships between Lancaster inbreds by means of complete linkage method was built (Fig. 1). To complete linkage method, the object is included in an existing cluster if object's similarity to any of the elements of a cluster is not less than the specified threshold value. In this case, compact clusters of high similarity are formed (Sivolap et al., 2011).

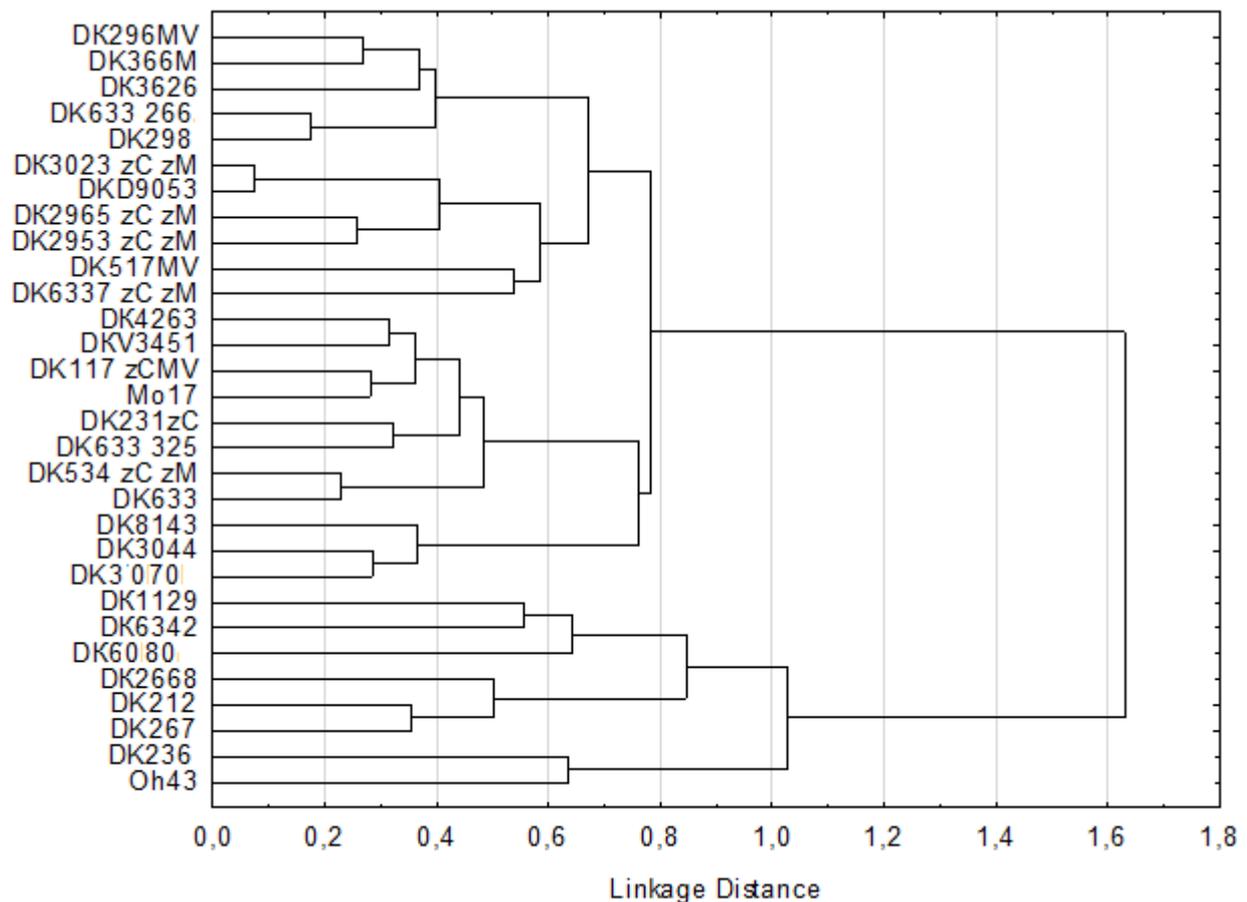


Figure 1. Dendrogram of phylogenetic relationships between Lancaster lines according to SNP-analysis (complete linkage method)

As shown in Fig. 1, two clusters were distinguished according to dendrogram. The first cluster combines 21 Lancaster inbred lines from Ukrainian breeding programs and public inbred line Mo17. The second cluster combines 7 Lancaster inbreds from Ukrainian breeding programs and public inbred line Oh43.

Phenotypically similar inbreds DK267, DK212 and DK6080 which had been selected from the initial population involving Oh43, have formed common cluster with Oh43, which indicates the compliance of pedigree, phenotypic and molecular observations. Inbred lines DK633266 and DK298, which had Oh43 and Mo17 in their pedigrees, have formed a common cluster with Mo17. Presumably, it confirms that they had received more hereditary information from Mo17 than from Oh43.

The relationship between two lines was determined as the percentage of SNP-markers which allelic status are the same in both lines. Table 2 shows the relationship between Lancaster inbreds from Ukrainian breeding programs and typical representatives of the group – Mo17 and Oh43 according to allelic status of SNP-markers of BDI-III panel.

As shown in table 2, the relationship of Lancaster inbred lines derived in Ukrainian breeding programs to the typical representatives of this germplasm varies from 52.2 to 89.7% for Mo17 and from 48.2 to 66.8% for Oh43. It means that more than half of the analyzed single nucleotide points in genomes of Ukrainian inbreds and typical representatives of this germplasm do match. The differences have arisen in a lot of cycles of hybridization which involved breeding material not related to Lancaster germplasm, recombinations, mutations etc. in synthetic populations which were initial for inbred production

Table 2

Relationship between Lancaster lines from Ukrainian breeding programs and Mo17 and Oh43 according to SNP-analysis

Inbred line	Relationship to Mo17, %	Relationship to Oh43, %
DK633	89.3 ± 3.8	53.3 ± 6.1
DK267	52.2 ± 6.1	58.8 ± 6.0
DK212	56.4 ± 6.0	66.8 ± 5.7
DK6080	64.4 ± 5.8	60.9 ± 5.9
DK633266	74.6 ± 5.5	55.6 ± 6.3
DK298	69.5 ± 5.6	55.1 ± 6.1
DK3070	87.1 ± 4.2	53.6 ± 6.2
DK236	56.0 ± 6.0	57.9 ± 6.0
DK633325	82.2 ± 4.6	54.4 ± 6.0
DK296MV	69.5 ± 5.5	53.8 ± 6.0
DK1129	64.4 ± 5.8	57.1 ± 6.0
DK2668	59.4 ± 5.9	55.8 ± 6.0
DK3023zCzM	79.1 ± 4.9	54.7 ± 6.0
DK3626	70.9 ± 5.5	53.6 ± 6.0
DK6342	65.8 ± 5.7	58.3 ± 6.0
DK2965zCzM	80.1 ± 4.9	51.9 ± 6.1
DK4263	81.0 ± 4.7	54.2 ± 6.0
DKD9053	80.9 ± 4.7	56.2 ± 6.0
DKV3451	84.9 ± 4.3	53.1 ± 6.0
DK8143	82.4 ± 4.6	48.2 ± 6.0
DK517MV	73.3 ± 5.6	58.2 ± 6.3
DK534zCzM	89.7 ± 3.7	53.5 ± 6.1
DK117zCMV	86.9 ± 4.1	53.8 ± 6.1
DK231zC	77.2 ± 5.1	49.3 ± 6.1
DK6337zCzM	71.1 ± 5.5	54.1 ± 6.1
DK2953zCzM	80.7 ± 4.8	50.9 ± 6.1
DK366M	71.6 ± 5.5	56.0 ± 6.1
DK3044	83.5 ± 4.5	56.1 ± 6.0
Oh43	54.9 ± 6.0	100.0
Mo17	100.0	54.9 ± 6.0

**Conclusions.** As a result of the research, the information content of SNP-markers of BDI-III panel was estimated, the relationship on allelic status of SNP-markers of Lancaster inbred lines from Ukrainian breeding programs between themselves and with typical representatives of the given germplasm was identified.

For the analyzed panel of SNP-markers and 30 Lancaster inbred lines genetic diversity index varied in 0.1701-0.1873. PIC was 0-0.3750 with the average value of 0.2420. Among SNP-markers involved in the investigation 90.9% were informative.

The construction of the dendrogram according to complete linkage method has allowed distinguishing two clusters that contain inbred lines close to Mo17 and inbred lines close to Oh43.

The relationship of Lancaster inbred lines from Ukrainian breeding programs to typical representatives of this germplasm ranged from 52.2 to 89.7% for Mo17 and from 48.2 to 66.8% for Oh43.

As modern lines of Ukrainian selection had been produced from initial synthetic populations of different complexity, often unrelated to Mo17 and Oh43, the estimation on SNP-markers of their similarity to initial forms and between themselves will permit to chose an appropriate strategy of alternative genotypes selection for application in synthesis of hybrids with high heterosis.

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