Inter- and Intra-Population Variation of Local Maize (*Zea mays* L.) Populations from Slovakia and Czech Republic

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Abstract: Evaluation of genetic variation was performed within 62 local maize populations originating from Slovakia and Czech Republic. In total 48 alleles at 22 analyzed isoenzyme loci with an average of 2.2 alleles per locus were revealed. The percentage of polymorphic loci ranged from 14% to 59% and the frequencies of detected alleles varied from null to four per locus. No polymorphism was detected at the loci *Dia2*, *Got3*, *Mdh4*, *Mmm*, and *Pgm1*. The highest number of alleles (four) was detected at loci *Acp1*, *Cat3*, *Pgm2*. No new alleles were identified, nevertheless the frequency of seven alleles was only about 1%. The expected heterozygosity ranged from null to 0.492 with an average of 0.197. The revealed isoenzyme polymorphism confirmed that all analyzed populations were heterogeneous and as many as 17 of them were completely heterogeneous. None of the analyzed populations was identical in the frequency of alleles at all 22 analyzed loci.

Keywords: maize; local populations; isoenzyme; genetic diversity

Maize (*Zea mays* ssp. *mays*) was introduced into Europe at the end of the 15th century by the expedition of Christopher Columbus. Dissemination from Spain, associated transmission over the old continent from different parts of America, and subsequent maize exploitation during the next five centuries contributed to the establishment of native maize genetic diversity in Europe. Even though the genetic variation of American populations will probably remain higher forever in comparison with European maize, alleles specific to European populations also emerged during adaptation to the local climate and environment (Rebourg *et al*. 2003). Contrasting climate, soils, agronomical practices, and breeder's effort created local populations progressively cultivated in favourable European regions. Local populations were grown by the end of the forties of the 20th century. After World War Two traditional local maize populations (landraces, obsolete cultivars) were progressively replaced by agronomically improved hybrids. At the same time there were efforts to preserve traditional populations (landraces) and collections of maize genetic resources were established in different countries. The maintenance of genetic resource collections is necessarily associated with relevant evaluation and characterization of genetic diversity. The maize germplasm belongs to the most studied. Extensive evaluations of morphological traits were complemented later by isoenzyme markers as the first type of applied molecular markers. Many laboratories have been investigating the polymorphic isoenzyme patterns in plants for more than twenty years (Brown 1979). They

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have found massive application in maize studies to survey adapted and exotic populations (Kahler *et al*. 1986; Rebourg *et al*. 2001), historically important lines (Smith *et al*. 1985), races (Goodman & Stuber 1983; Pflüger & Schlatter 1996), and other populations (Lefort-Buson *et al*. 1991). Recent development in molecular biology methods influenced also the concept of maize populations and maize genetic resource evaluation where DNA-based markers are frequently used, mainly RFLPs (Melchinger *et al*. 1991; Messmer *et al*. 1992; Gauthier *et al*. 2002), AFLPs (Marsan *et al*. 1998), and SSRs (Heckenberger *et al*. 2002).

The power of isoenzyme and DNA markers to evaluate genetic diversity within and among populations is certainly different. DNA loci definitely contain greater variation and discrimination ability. The total number of alleles and overall diversity detected by isoenzymes is lower but the magnitude of population differentiation, relative to the total diversity, should be similar for isoenzymes and RFLPs (Dubreuil & Charcosset 1998). This supports that isoenzyme loci are driven by similar evolutionary forces and are neutral. For these reasons the starch gel electrophoresis of maize isoenzymes is still widely used for several purposes. Moreover, it is the basic, relatively simple, and inexpensive tool for the analysis of genetic variation within and among maize populations including local populations – landraces (Kahler *et al*. 1986). Moreover, segregating isoenzyme loci in maize appeared to provide effective means for the identification of genomic regions influencing an important phenotypic characteristic. Edwards *et al*. (1987) explained 8–40% of the phenotypic variation in quantitative traits by cumulative effects of allozyme loci. Linkages between some isoenzyme loci and important quantitative agronomical traits were published by Zlokolica and Milošević (2001).

Such a tool was also used in the study of local maize populations originating from Slovakia and Czech Republic, considering that scientists, breeders, curators, and other enthusiasts have collected local populations in the last fifty or sixty years for further exploitation in breeding and conservation. Collected seed samples have usually been multiplied and evaluated by morphological traits according to national and international descriptors.

Therefore the aims of this study were: (i) to perform the quantitative and qualitative isoenzyme evaluation of diversity of original Slovak and Czech local maize populations as the first study of this type for populations originating from this territory, (ii) to identify new or rare isoenzyme alleles in these populations, (iii) to analyse intra- and inter-population variation within local populations, and (iv) to determine relatedness among local populations.

MATERIAL AND METHODS

A total of 62 local populations of maize (*Zea mays* L.) from the collection of maize genetic resources were analyzed. They were collected in different regions of Slovakia and Czech Republic and they were provided for analyses by the curator of the maize genetic resource collection (Sempol Holding Inc., Trnava). Our study was based on the analysis of twenty individuals per population originating from bulked seed samples. Bulks were generated by multiplication where the rules for the effective size of heterogenic local populations were taken into account as described by Ryšavá (1997).

Analyses were performed by horizontal starch gel electrophoresis according to Cardy *et al*. (1980), Stuber *et al*. (1988), Greneche and Giraud (1989), Bourgoin-Greneche and Lallemand (1993), and Bourgoin-Greneche *et al*. (1998). Twenty individual seeds were used for isoenzyme extraction and analysis. Seeds were randomly sampled from seed bulks representing maintained accessions.

Variation within each population was profiled at 22 loci of 11 isoenzymes: acid phosphatase (ACP, E.C. 3.1.3.2), alcoholdehydrogenase (ADH, E.C. 1.1.1.1), catalase (CAT, E.C. 1.11.1.6), diaphorase (DIA, E.C. 1.6.99.2), β-glucosidase (GLU, E.C. 3.2.1.21), glutamateoxaloacetatetransaminase (GOT, E.C. 2.6.1.1), isocitratedehydrogenase (IDH, E.C. 1.1.1.42), malatedehydrogenase (MDH, E.C. 1.1.1.37), 6-phosphogluconatedehydrogenase (PGD, E.C. 1.1.1.44), phosphoglucoisomerase (PGI, E.C. 5.3.1.9), and phosphoglucomutase (PGM, E.C. 2.7.5.1). Alleles at observed loci were classified according to their migration characteristics in starch gel. The null recessive alleles were labelled with *n*. *Mmm* is a modified locus the alleles of which affect the migration of certain MDH bands (Goodman *et al*. 1980; Newton & Schwartz 1980).

The expected heterozygosity as unbiased estimate was calculated according to Nei (1972). The genetic relatedness within populations was

evaluated by hierarchic cluster analysis using the statistical software package SPSS for Windows 8.0.1. (SPSS Inc.).

RESULTS AND DISCUSSION

In total 48 alleles at 22 analyzed isoenzyme loci with an average of 2.2 allele per locus were revealed across 62 local maize populations. This characteristic, i.e. the number of alleles per locus, should be considered as the basic quantitative trait of genetic diversity specific to any population. The studied local maize populations do not seem exceptional at the first sight. The average number of alleles per locus seems less variable than the same parameter in maize populations originating from other regions, e.g. south-western China (3.7 alleles per locus) (Lu *et al*. 2002), USA (2.8) (Kahler *et al*. 1986), Bolivia (5.2) (Goodmann & Stuber 1983), or Mexico (7.1) (Doebley *et al*. 1985). However, it should be emphasized that the results of our and above-mentioned studies were obtained by the analysis of different numbers of populations and individuals within them, and different and more or less compatible loci, which affects the comparison of genetic variation. Populations possessing a high range of alleles and a high average number of alleles per locus are generally considered as germplasms valuable for the broadening of genetic variation in modern maize. Germplasms coming from the centre of origin and adjacent areas are traditionally regarded as reservoirs of high inherent genetic variation. The study of Matsuoka *et al*. (2002) indicated that the high genetic diversity of maize arose as single domestication in highlands of southern Mexico, which supports high isoenzyme variation of these populations (Doebley *et al*. 1985; Goodmann & Stuber 1983). Other factors also influence the incidence of a higher number of alleles in populations originating from outside of the centre of origin, e.g. growing of populations instead of hybrids, small-scale farming systems, and self-seed generation. On the contrary, reasons for lower genetic variation can be recent founding of the population from a small number of individuals, reduction of population size, restricted gene flow among populations (Heywood & Fleming 1986) as well as later introduction of maize into a specific region, large area management of growing and mass seed production for a long time, and more active maintenance of homogeneity in the fields. Local population improvement by recurrent selection also increases the frequency of favourable alleles and reduces overall variation. Such an effect of recurrent and reciprocally recurrent selection in maize was described at the isoenzyme and RFLP loci (Stuber *et al*. 1982; Labate *et al*. 1999; Pinto *et al*. 2003). Discrepant variation between maize populations of different origin may also be a consequence of small sample size collected (Reedy *et al*. 1995) and founder effects (Doebley *et al*. 1983). Based on the quantitative evaluation of isoenzyme diversity of the Slovak and Czech local maize populations they appertain into the range of populations originating from other areas. This is confirmed for instance by the studies of Brazilian and other tropical maize populations where average numbers of alleles per loci were lower than within our populations – 1.6 and 2.0–2.4, respectively (Gimenes & Lopes 2000; Pinto *et al*. 2003). This parameter is also generally affected (decreased) by a low number of analyzed populations (Dubreuil & Charcosset 1998).

Another approach to evaluate the isoenzyme variation of populations is a qualitative one, i.e. individual allele occurrence. Different numbers and types of alleles were detected in our study at the analyzed loci *Acp1* (alleles 2, 3, 4, 6), *Adh1* (4, 6), *Cat3* (7, 9, 12, n), *Dia1* (8, 12), *Dia2* (4), *Glu1* (1, 2, 6–7), *Got1* (4, 6), *Got2* (2, 4), *Got3* (4), *Idh1* (4, 6), *Idh2* (4, 6), *Mdh1* (1, 6, 10.5), *Mdh2* (3, 6), *Mdh3* (16, 18), *Mdh4* (12), *Mdh5* (12, 15), *Mmm* (M), *Pgd1* (2, 3.8), *Pgd2* (2.8, 5), *Pgi1* (2, 4, 5), *Pgm1* (9), and *Pgm2* (1, 3, 4, 8), i.e. 77% of the analyzed loci were polymorphic. The percentage of polymorphic loci ranged from 14% in local populations Vrbové and Chynorany to 59% in local population No. 92. The frequencies of detected alleles varied from null to four per locus (Figure 1). No polymorphism was detected at the loci *Dia2*, *Got3*, *Mdh4*, *Mmm*, and *Pgm1*. Reversely, the highest number of alleles (four) was detected at three loci (*Acp1*, *Cat3*, *Pgm2*). The frequency of some alleles was only about 1%. Even though only a limited number of local populations was analyzed, i.e. seven, 15% of rare alleles were revealed. The occurrence of such rare, low-frequented alleles, e.g. *Acp1*:*3*, *Acp1*:*6*, *Cat3*:*n*, *Mdh1*:*1*, and others could be interesting mainly from the forensic and genetic point of view. The frequencies of alleles in the Slovak and Czech local populations were compared with relevant results reported in Bolivian, (Goodman & Stuber 1983), European, Asian (Lavergne 1988; Lefort-Buson *et al*. 1991), Spanish (Revilla *et*

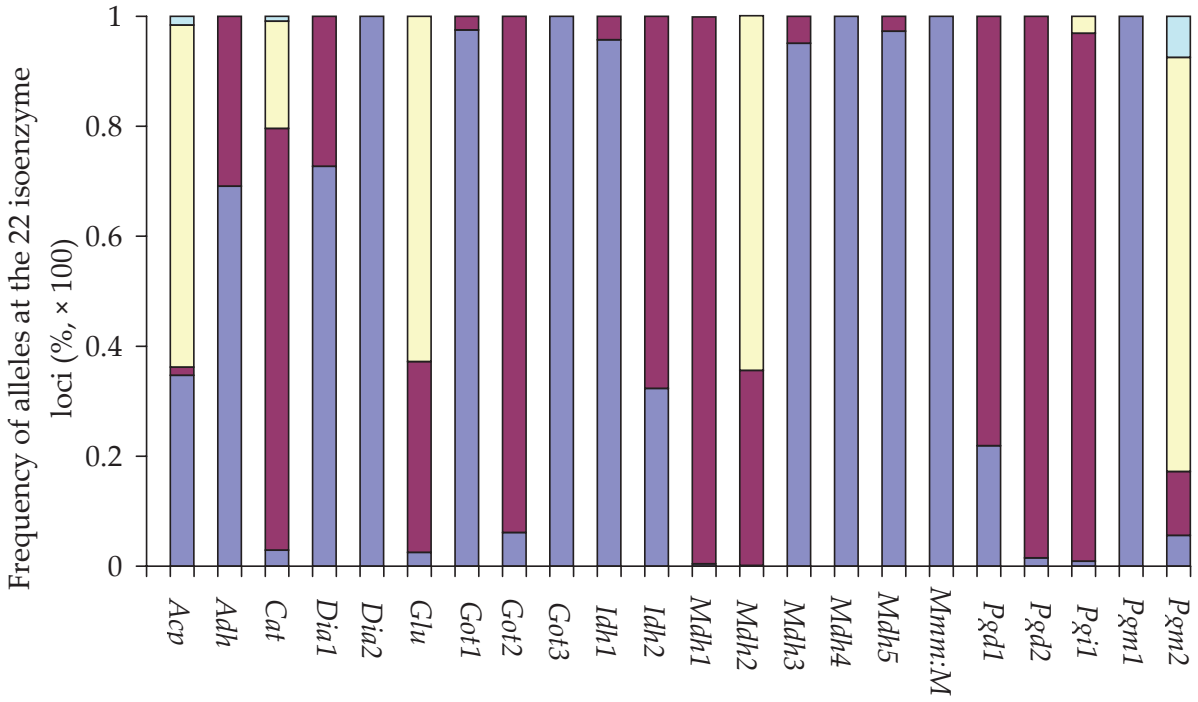

Figure 1. Frequency of alleles in 62 local maize populations

al. 1998), U.S. (Kahler *et al.* 1986), and south-western Chinese (Lu *et al.* 2002) maize populations. According to those results neither new allele nor special distinctness was revealed in our study. Nevertheless, differences were observed in the frequency of alleles. The low frequency of allele *Acp1:3* (1.5%) in our populations is similar to Spanish (Lefort-Buson *et al.* 1991) and other European populations (Llaurado *et al.* 1993) and very different from the frequency of this allele in western African (Sanou *et al.* 1997), south-western U. S. (Doebley *et al.* 1988), and Brazilian (Gimenes & Lopes 2000) ones. The almost identical status in comparison with those populations was in the frequency of allele *Mdh1:1*, less frequented was allele *Mdh5:15* and more frequented was allele *Pgd1:2*.

The highest internal isoenzyme homogeneity was detected within the local population Čerhov, which contains only 6 different isoenzyme lines. On the

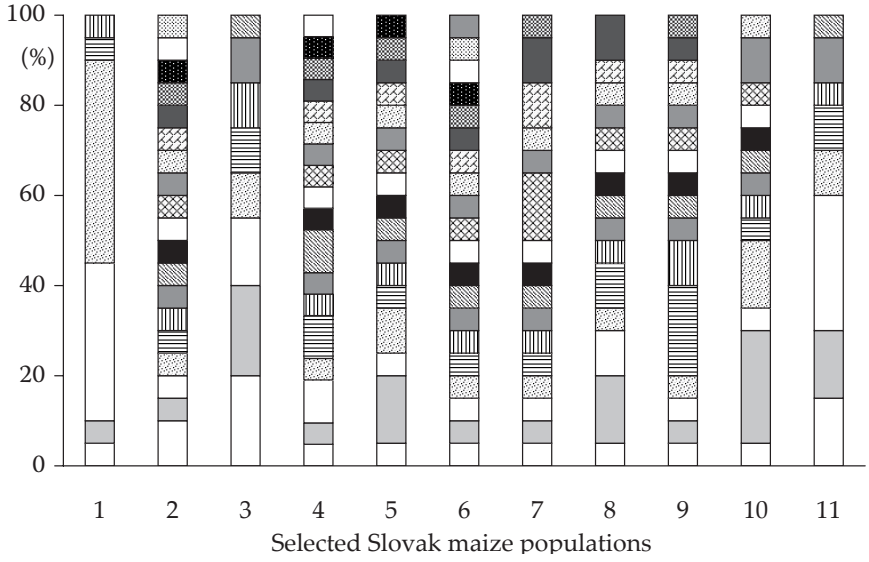

Figure 2. The divergence in intra-population heterogeneity of selected local populations

contrary, as many as 17 maize populations (27.4% of all analyzed ones) were completely heterogeneous, i.e. each of twenty individual grains revealed different isoenzyme patterns (samples in Figure 2). Prevalence of populations with higher heterogeneity and tendency to increase intra-population variation is an understandable trait of maize. A very significant factor in the process of increasing or decreasing the intra-population variation is the relation between native tendency of maize to increase its heterogeneity as open-pollinated species and antagonistic effort of breeder. It is a very important feature mainly for local populations grown in developing countries where they are not cultivated yet and selection for homogeneity has lagged behind. High heterogeneity within the analyzed Slovak and Czech local populations could be an effect of grower practices and isolation distances used in the years 1950–1970.

The expected heterozygosity (*He*) revealing both allele numbers and allele frequencies ranged from null to 0.492 (locus *Acp1*) with an average of 0.197 (Table 1), which is less than average *He* in south-western Chinese (0.29) (Lu *et al*. 2002), U.S. (0.46) (Kahler *et al*. 1986), Bolivian (0.23) (Goodmann & Stuber 1983) or Mexican (0.21) (Doebley *et al*. 1985) but similar to Brazilian (0.195) (Gimenes & Lopes 2000) populations. Considering that 15% of alleles were rare in the Slovak and Czech local populations a lower level of heterozygosity is explainable by this fact.

The revealed isoenzyme polymorphism in our local populations confirmed that all of them are heterogeneous. The analyses also showed that none of the analyzed populations was identical in the frequency of alleles at all 22 analyzed loci.

Table 1. Genetic variation at 22 loci in 62 Slovak and Czech local populations

Locus	H_e*	H_o	Locus	H_e*	H_o
Acp1	0.492	0.341	*Mdh1*	0.010	0.001
Adh1	0.427	0.299	*Mdh2*	0.458	0.334
Cat3	0.373	0.121	*Mdh3*	0.093	0.044
Dia1	0.397	0.233	*Mdh4*	0	0
Dia2	0	0	*Mdh5*	0.053	0
Glu1	0.485	0.102	*Mmm*	0	0
Got1	0.049	0.046	*Pgd1*	0.342	0.139
Got2	0.115	0	*Pgd2*	0.030	0
Got3	0	0	*Pgi1*	0.077	0.017
Idh1	0.082	0.027	*Pgm1*	0	0
Idh2	0.437	0.252	*Pgm2*	0.411	0.097
Mean H_e	0.197				
Mean H_o	0.093				

*unbiased estimate according to Nei (1972)

H_o – observed heterozygosity

The isoenzyme occurrence was different across all local populations and showed the presence of a variable number of involved isoenzyme genotypes and phenotypes (Figure 3).

Graphical presentation of diversity among populations (Figure 4) confirms the more or less compact branching of individuals of randomly chosen five local populations. Individuals from different populations were clustered into common groups. A dendrogram shows intra- and inter-population isoenzyme diversity and heterotic groups which

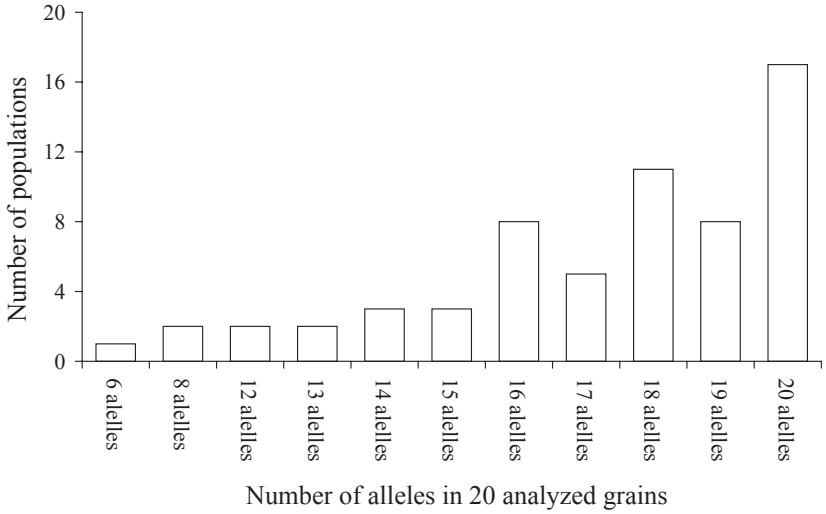

Figure 3. Distribution of isoenzyme heterogeneity within 62 analyzed maize local populations

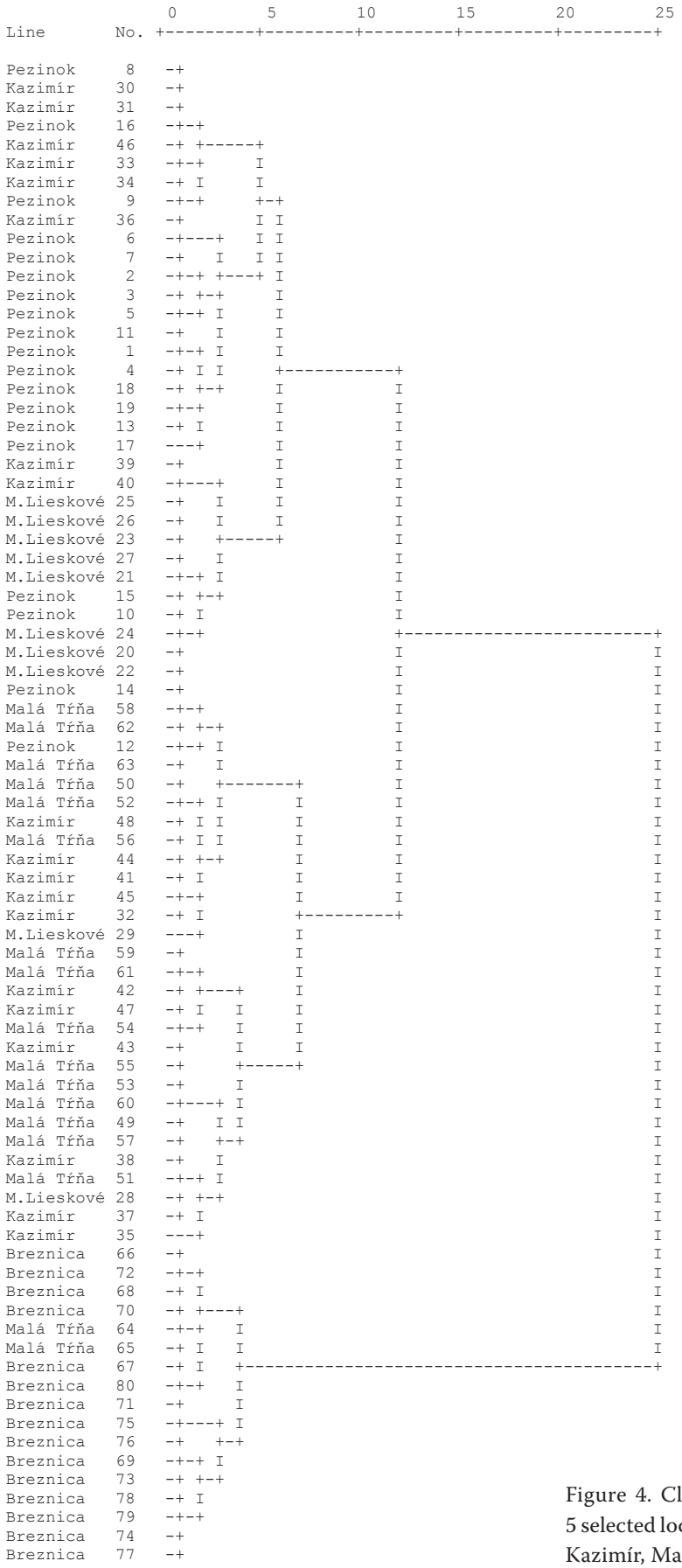

Figure 4. Clustering of isoenzyme phenotypes among 5 selected local populations (Pezinok, Moravské Lieskové, Kazimír, Malá Tŕňa, and Breznica)

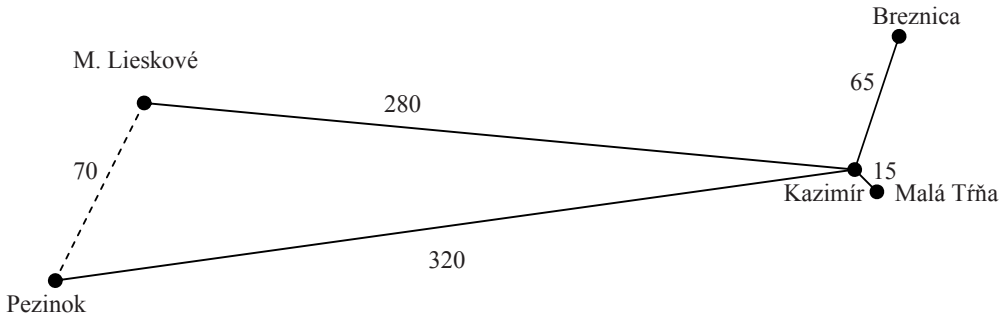

Figure 5. Drawing of inter-location distances between the places of local population origins (distances in kilometres)

should be consequently used in hybrid breeding. The pedigree of populations was unknown. Nevertheless, the grouping of populations reflects geographic (microgeographic) relations between them. Individuals in the framework of populations Pezinok, M. Lieskové, and Breznica are grouped more tightly and these populations are more evidently separated from the others. Individuals from the populations Malá Tŕňa and Kazimír were more or less mixed together in the dendrogram composition, which could correspond to a small distance between places where these populations were collected (Figure 5) due to gene flow between both near located places caused by the transfer of genetic variability by farmers.

The Slovak and Czech local populations of maize represent original ecotypes of this crop which are not grown in agricultural practice at the present time. They can serve as valuable biological materials for experimental research and also for breeding. Presented results show high genotypic (phenotypic) variation within analyzed local maize populations. All populations have a different composition at isoenzyme loci, nevertheless no new unknown allele was found. We believe that the proteomic classification of local populations by an isoenzyme analysis could increase interest in their utilization in research, breeding programmes, European maize landraces survey and database.

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