Genetic Diversity of Croatian Common Bean Landraces

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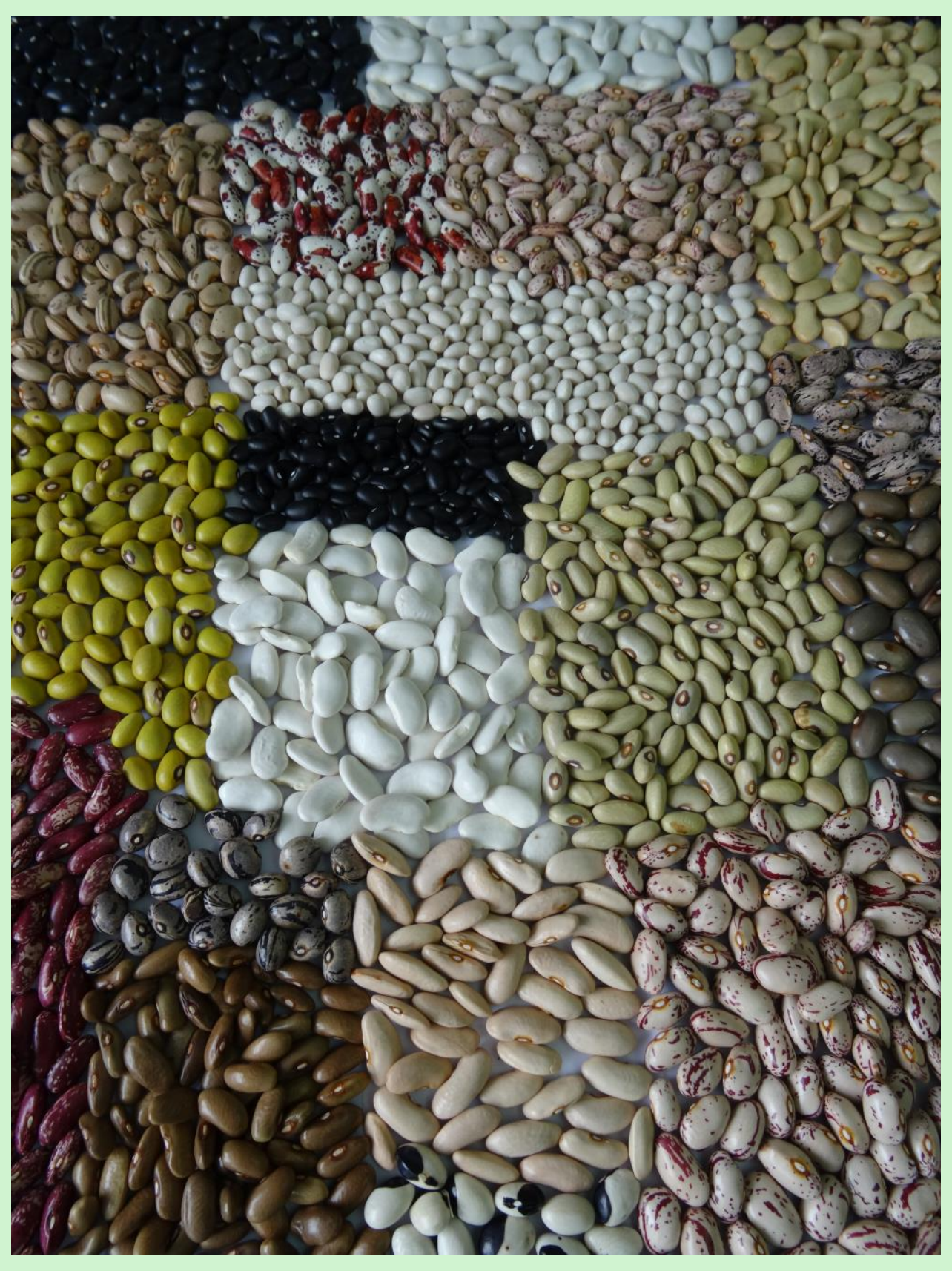

Fig. 1. Croatian common bean landraces

Abstract

In Croatia, common bean is a traditional grain legume, grown by small farmers in low input agriculture systems. The majority of the production is still based on local landraces (Fig.1) adapted to the specific growing conditions and agro-environments and showing a great morphological diversity. Local landraces are in danger of genetic erosion caused by complex socio-economic changes in rural communities. The low profitability of farms and their small size, the advanced age of farmers and the replacement of traditional landraces with modern bean cultivars and/or other more profitable crops have been identified as the major factors affecting genetic erosion.

Three-hundred accessions belonging to seven most widely used morphotypes (landraces) were genotyped by microsatellite markers in order to assess the genetic diversity and population structure of Croatian common bean germplasm. PCR-based phaseolin type analysis was used to trace the origin of accessions to Mesoamerican and Andean domestication centres. One-third of accessions showed phaseolin type I ("S"), predominant in germplasm of Mesoamerican origin while the rest of accessions belonged to Andean domestication centre characterized by the phaseolin type II ("H" or "C"; 22,95%) or III ("T"; 48,09%). A model-based structure analysis based on microsatellite markers revealed the presence of three clusters, for the most part, in congruence with the results of phaseolin type analysis.

Introduction

In Croatia, common bean is a traditionally grown in low input production systems. The production is based on landraces displaying high levels of morphological diversity.

The diversity of DNA sequences that encode structural and functional features of phaseolin renders is useful as an evolutionary marker supporting the hypothesis of two major gene pools, the Mesoamerican and Andean (Gepts et al., 1986) while microsatellite markers have been widely used in cultivar identification and the analysis of genetic structure (Benchimol et al 2007; Blair et al 2009).

The aim of our research was the assessment of genetic diversity of landraces using phaseolin genotyping as well as microsatellite markers to trace the origin and structure of Croatian common bean germplasm.

Materials and methods

The PCR amplification of phaseolin sequences was carried out using primers developed by Kami et al. (1995). Twenty-six primers (Yu et al., 2000; Blair et al., 2009) were used for microsatellite analysis. Both distance-based and model-based approaches were applied for the interpretation of microsatellite marker data. The proportion-of-shared-alleles distance (Bowcock et al., 1994) between pairs of accessions was calculated using MICROSAT (Minch et al., 1997). Cluster analysis was performed using the Fitch-Margoliash least-squares algorithm in PHYLIP (Felsenstein, 2004). A model-based clustering method was applied to infer genetic structure and define the number of clusters using the software STRUCTURE (Pritchard et al., 2000).

Results and discussion

Microsatellite analysis of accessions (collected all over Croatia) of common bean, being predominantly a self-pollinated species, revealed a total of 183 different multi-locus genotypes in the panel of 300 accessions: 53 (28.96%) of phaseolin type I (Mesoamerican; "S"), 42 (22.95%) of II (Andean; "H" or "C") and 88 (48.09%) of III (Andean; "T"). The duplicated accessions were excluded from further analyses.

The distance-based clustering unambiguously defined two distinct clusters corresponding to Mesoamerican and Andean origin of accessions as identified by phaseolin analysis (Fig. 2). Moreover, a subcluster containing the great majority of phaseolin type III accessions could be identified within the Andean cluster.

The model-based clustering method as implemented in STRUCTURE indicated K = 2 as the most likely number of clusters, followed by K = 3. As expected, at K = 2, the most of the accessions of Mesoamerican origin (phaseolin type I) were assigned to cluster A, while the accession of Andean origin (phaseolin type II and III) were assigned to cluster B. At K = 3, the newly formed cluster (cluster C) clearly grouped the phaseolin type III accessions.

A total of 16 accessions could be considered as of 'mixed origin', having the membership probabilities Q < 75% for all clusters at K = 3. Furthermore, a total of 13 accessions did not show the correspondence between phaseolin type and the membership according to model-based clustering analysis based on microsatellite loci (i.e. phaseolin type I = cluster A; II = B, III = C). As four accessions were classified as both of 'mixed origin' and 'non-corresponding', a total of 25 accessions (13.66%) could be considered as putative hybrids between gene pools.

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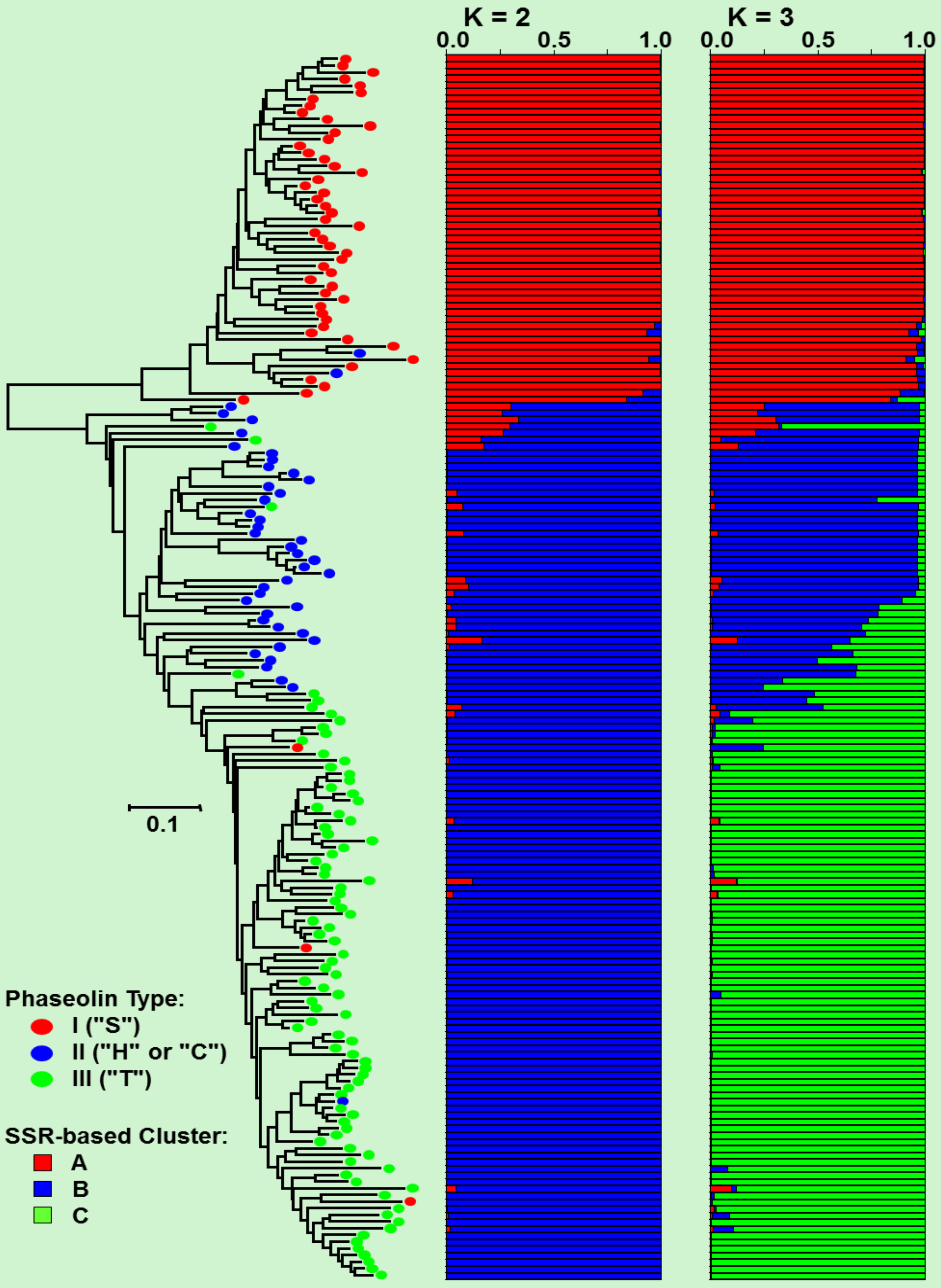

Fig. 2. Fitch-Margoliash tree based on proportion-of-shared alleles distance between 183 common bean accessions. Average proportions of membership for K = 2 and 3 clusters are given as estimated by STRUCTURE. Classification based on phaseoline type is indicated on branches of the tree.

Conclusions

For the most part, the classification of common bean accessions according to phaseolin type analysis is in congruence with the results of both distance-based and model-based analyses of microsatellite marker data. Nevertheless, putative hybrids constitute a significant proportion of Croatian common bean germplasm representing a valuable resource for expanding the genetic diversity that could be used in future breeding programmes.

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