

Klaudija CAROVIĆ-STANKO^{1,2}
Zlatko LIBER^{2,3}
Filip VARGA^{1,2}
Martina GRDIŠA^{1,2}
Mihailo RISTIĆ^{4†}
Zlatko ŠATOVIĆ^{1,2}

HOW DO MORPHOTYPES AND CHEMOTYPES RELATE TO GENOTYPES? INTRASPECIES DIVERSITY OF *OCIMUM BASILICUM* L.

¹University of Zagreb Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska cesta 25, HR-10000 Zagreb, Croatia
²Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, HR-10000 Zagreb, Croatia
³University of Zagreb Faculty of Science, Division of Biology, Department of Botany, Marulićev trg 9a, HR-10000 Zagreb, Croatia
⁴Institute for Medicinal Plant Research "Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia
*kcarovic@agr.hr

Abstract

Basil (*Ocimum basilicum* L.) is a widely known member of Lamiaceae family with a long tradition of cultivation. Cultivation for different market requirements led to great intraspecific variation at both morphological and biochemical level. The aim of this research was to assess diversity among basil accessions maintained at the Collection of Medicinal and Aromatic Plants as a part of the National Bank of Plant Genes. Eighty basil accessions belonging to five basil morphotypes (True basil, Small-leaf, Lettuce-leaf, Purple basil A and Purple basil B) and five chemotypes (High linalool, Linalool/trans- α -bergamotene, Linalool/methyl chavicol, Linalool/trans-methyl cinnamate and High methyl chavicol) were analysed using AFLP markers. Sixteen AFLP primer combinations yielded a total of 2,114 polymorphic markers. Analysis of molecular variance (AMOVA) revealed that most of the genetic diversity was attributable to differences among accessions within morphotypes and within chemotypes. Bayesian Analysis of Population Structure (BAPS) resulted in assignment of the investigated accessions to four clusters. The accessions belonging to 'green' morphotypes (True basil, Small-leaf, Lettuce-leaf) were assigned to Clusters A or B and Purple basil A accessions to Clusters A or C. Purple basil B morphotype was genetically the most admixed, having accessions belonging to all four genetic clusters.

Introduction

Basil (*Ocimum basilicum* L.) has long been acclaimed for its diversity as a source of essential oils, its flavour and delicacy as spice, and its beauty and fragrance as an ornamental (Simon et al., 1990). It is extensively used by the perfume, pharmacy and food industries for its natural aroma and flavour (Darrah, 1980). Because of the constant human demand for new flavours, many *O. basilicum* cultivars have been bred during the long cultivation of the herb. As a consequence, a high genetic, chemical, and morphological variability currently exist in this species, and its taxonomy remains unresolved.

Morphological diversity of basil accessions held at the Collection of Medicinal and Aromatic Plants (University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology) as a part of the National Bank of Plant Genes has been assessed using the descriptor list based on morphological traits developed by the International Union for the Protection of New Varieties of Plants (UPOV, 2003). The accessions were grouped into five morphotypes (True basil, Small-leaf, Lettuce-leaf, Purple basil A and Purple basil B).

Based on essential oil composition 80 *O. basilicum* accession were classified into five chemotypes: (A) High-linalool, (B) Linalool/trans- α -bergamotene, (C) Linalool/methyl chavicol, (D) Linalool/trans-methyl cinnamate and (E) High-methyl chavicol chemotype (Varga et al., 2017).

Amplified fragment length polymorphism markers (AFLPs; Vos et al., 1995) have been successfully used for the assessment of genetic relationships and diversity in basil (Carović-Stanko et al., 2010; 2011) by generating genetic data from a large number of loci without requiring prior knowledge of DNA sequence.

The aim of this study was to: (A) determine genetic relationships among basil accessions using AFLP markers, and (B) compare the patterns of genetic with morphological and chemical variability.

Materials and methods

Eighty basil accessions belonging to five morphotypes were analysed using sixteen AFLP primer combinations. AFLP amplified fragments were scored for the presence (1) or absence (0) of homologous bands.

Molecular diversity within morphotypes was assessed by calculating the percentage of polymorphic markers (%P), the Shannon's information index (Lewontin, 1972) and the number of private markers (Npr). In order to quantify rare markers within each morphotype we calculated the rarity index (RI; Novoselović et al., 2016), which is expected to be higher for a morphotype which overall rare markers were more frequent among accessions.

Pairwise distances were calculated using Dice's coefficient (Dice, 1945). Within each morphotype the average Dice's distance among accessions was calculated. Principal co-ordinate analysis (PCoA) based on Dice's distance matrix was performed using PAST version 2.01 (Hammer et al., 2001).

The analysis of molecular variance (AMOVA; Excoffier et al., 1992) was used to partition the total AFLP diversity (a) between groups of morphotypes ('green' vs. 'purple'), among morphotypes within groups and within morphotypes as well as (b) among and within chemotypes. The variance components were tested statistically by non-parametric randomisation tests using 10,000 permutations using Arlequin ver. 2.000 (Schneider et al., 2000).

The presence of distinct evolutionary clusters among accessions has been evaluated using a Bayesian approach (Corander et al., 2003) as implemented in BAPS 5.2 (Corander et al., 2004). For each accession, the proportion of its genome derived from the different clusters was estimated. Accessions having more than 75% of their genome estimated to belong to a germplasm group were considered to belong to the germplasm group, while accessions with membership probabilities < 75% for all groups were considered to be of 'mixed origin' (Matsuoka et al., 2002).

Table 1. Molecular diversity of basil morphotypes

Morphotypes	n	%P	Sh	Npr	RI	Davg
True basil	34	95,60	0,618	2	0,942	0,365
Small-leaf basil	10	76,96	0,576	0	0,980	0,387
Lettuce-leaf basil	7	72,52	0,583	0	1,128	0,391
Purple basil A	10	80,84	0,616	0	1,034	0,402
Purple basil B	19	89,40	,0631	4	1,049	0,377

n - No. of accessions; %P - Proportion of variable markers; Sh - Shannon's information index; Npr - No. of private alleles; RI - Rarity index; Davg - Average Dice's distance among accessions

Results and discussion

Sixteen AFLP primer combinations yielded a total of 2,114 polymorphic markers. Dice's distances ranged from 0.365 to 0.402. Molecular diversity indices of basil morphotypes are given in Table 1. The highest number of private markers was observed for the Purple basil B morphotype as well as the genetic diversity measured by Shannon's information index. The highest RI was observed for the Lettuce-leaf basil morphotype suggesting that these morphotype was the most isolated.

Analysis of molecular variance (AMOVA) revealed that most of the genetic diversity was attributable to differences among accessions within morphotypes (90,06%), while the remaining variability was almost equally distributed among morphotypes within groups of morphotypes (6.16%) classified according to anthocyanin coloration on stems ('green' vs. 'purple' morphotypes) and among the groups (5.61%). Also, AMOVA showed that most of the genetic diversity was attributable to differences among accessions within chemotypes (95,70%).

Bayesian analysis of genetic structure resulted in congruent assignment of the investigated accessions to four clusters. The accessions belonging to 'green' morphotypes (True basil, Small-leaf, Lettuce-leaf) were assigned to Clusters A or B and Purple basil A accessions to Clusters A or C. Purple basil B morphotype was genetically the most admixed, having accessions belonging to all four genetic clusters (Figure 1.).

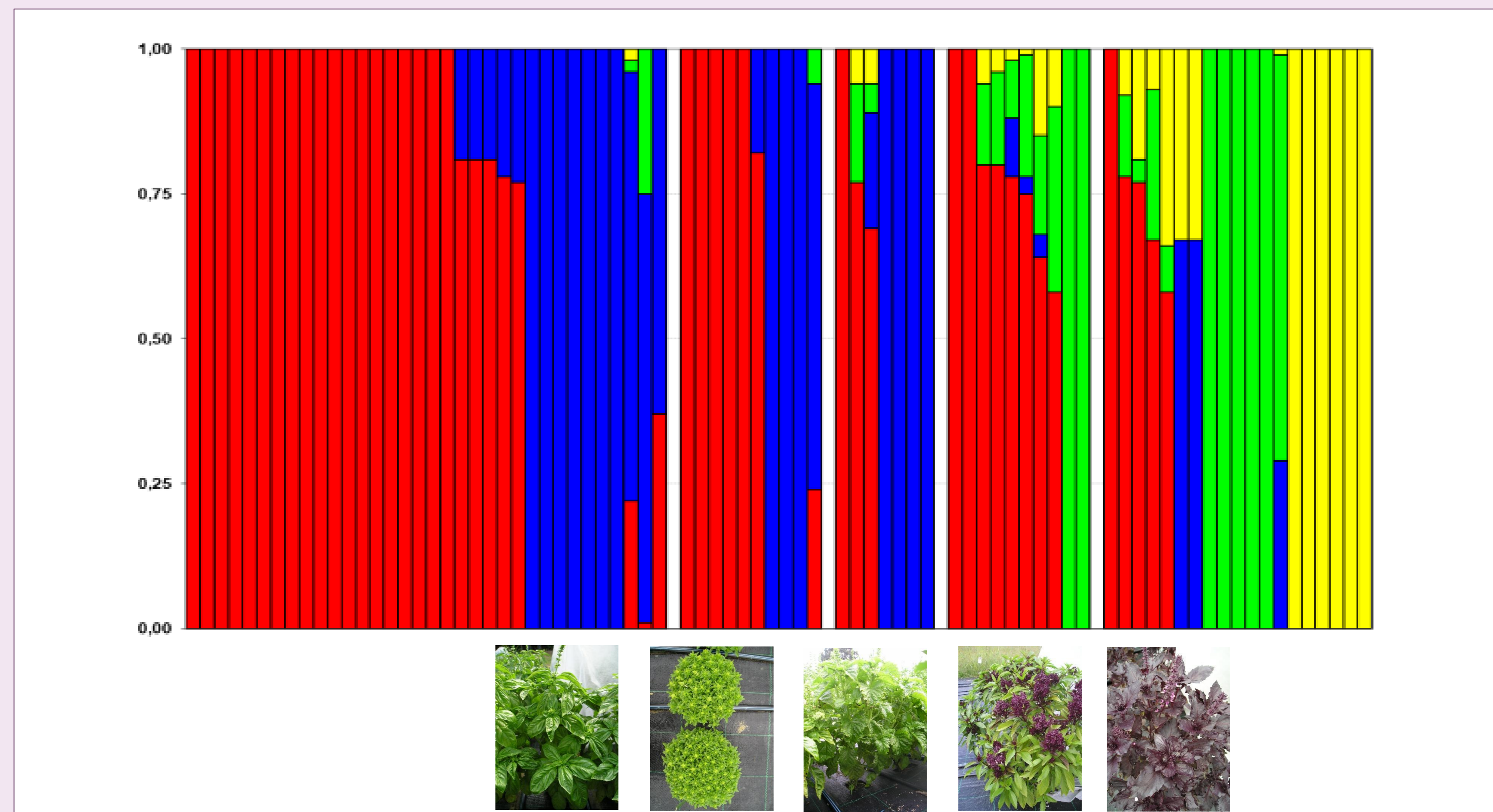


Figure 1. Population structure of basil accessions based on BAPS analyses at K=4

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