

*Comparative analysis of the genetically determined isoenzyme polymorphism in populations of selected *Crocus* species distributed in Bulgaria*

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Abstract. The genus *Crocus* is represented by 15 species in the flora of Bulgaria. The present research is based on isoenzyme analysis, and the genetic heterogeneity in populations of selected species was studied: *C. adamioides*, *C. chrysanthus*, *C. flavus*, *C. olivieri*, *C. pallasii*, *C. pulchellus*, and *C. randjeloviciorum*. The probable genetic control of four polymorphic enzyme groups - non-specific esterases (EST), malate dehydrogenases (MDH), malic enzymes (ME), and superoxide dismutases (SOD) was established, which appear as suitable markers for characterizing intra- and inter-population genetic variability. Fourteen variable loci were analyzed, and specific biochemical markers were established for distinguishing *Crocus* species. A comparative analysis of the studied populations' gene pool and genotypic structure was conducted. The results show that malate dehydrogenases, malic enzymes, superoxide dismutases, and non-specific esterases are polymorphic biochemical genetic markers suitable for inter-population comparisons and for studying intra-population heterogeneity in *Crocus* species.

Key words: Iridaceae, *Crocus*, polymorphic enzymes, EST, MDH, ME, SOD.

Introduction

The evolutionary process underlies genetic diversity in nature, representing molecular genetic heritable differences within and between populations and species. Natural selection facilitates the adaptation of populations to specific conditions, leading to genetic differentiation between geographically separated populations. When studying the molecular bases of the phylogeny of organisms, the studies of different protein systems occupy an important place. Allozyme markers are often used in systematic or population investigations (Hamrick & Godt, 1990) and are reliable enough and informative in the studies of gene variability within and between plant populations (Zeidler, 2000). Various isozyme systems have been used in the studies of plants. Peroxidase profiles have been applied, e.g., as taxonomic markers

and measures of interpopulation variability in the *Sphagnum subsecundum* complex (Krzakowa & Melosik, 1999), *Thymus capitatus* (Ali & Mustafa, 2021), and the variability between the Bulgarian members of the genus *Phelipanche* (Stoyanov & Stoyanova, 2007). Eleven enzymes have been used to describe isozyme variation between closely related species of the genus *Elytrigia* (Angelov, 2003).

Methods for electrophoretic separation of proteins are suitable for studying the genetic structure of populations, as well as for establishing genetic proximity and distance at the intraspecific and interspecific levels. In most plant species, NAD-dependent malate dehydrogenase (MDH, EC 1.1.1.37) is a highly polymorphic enzyme, with multiple molecular forms. The MDH polymorphism was found to be genetically determined by several loci with a large number of alle-

les (Yudina, 2012). NADP-dependent malate dehydrogenase (ME, EC 1.1.1.40) is a key enzyme in plant metabolism that participates in many biochemical processes. The malic enzyme has multiple isoforms that are mainly found in the cytoplasmic stroma, mitochondria, and chloroplasts and are important for plant growth and development (Badia et al., 2017). Superoxide dismutases (SOD, EC 1.6.4.3) play a major role in the resistance to toxic oxygen radicals, which are generated as byproducts of many metabolic oxidation processes. Most plant species contain multiple isoforms of superoxide dismutases that differ in their active metal ions. Many studies have shown that high SOD activity and a greater number of isoforms determine a greater potential to scavenge toxic radicals (Berwal & Ram, 2019). Non-specific esterases (EC 3.1.1.11) are some of the most active enzymes that serve many metabolic processes. Esterases are widely used biochemical-genetic markers in population studies. The reason is the high degree of intraspecific variability and the possibility of simultaneously studying several genes controlling the esterase spectrum. Null alleles at the esterase loci determining the absence of esterase expression in the homozygous state have been reported (Kawase & Sakamoto, 1984; Manganaris & Alston, 1992; Nakagahra, 1977).

Recent studies indicate that *Crocus* species exhibit considerable morphological and genetic diversity across the Balkans, reflecting complex evolutionary processes and environmental adaptations (Raycheva & Stoyanov, 2025; Raca et al., 2025). In the Bulgarian flora, the genus *Crocus* shows pronounced morphological and taxonomic variation (Stoyanov & Raycheva, 2024; Raycheva & Stoyanov, 2025).

The present study aims to analyze comparatively the genetically determined isoenzyme polymorphism in populations of 7 selected species of the genus *Crocus*, distributed in Bulgaria, characterizing the genotypic structure and the gene pool based on selected biochemical genetic markers – non-specific esterases (EST), malate dehydrogenases (MDH), malic enzymes (ME), and superoxide dismutases (SOD).

Materials and methods

Plant samples

According to the planned research objectives, more than 250 individuals of the species *Crocus*

pallasii Goldbl., *C. chrysanthus* Herb., and *C. adamioides* Kernd. & Pasche, *C. pulchellus* Herb., *C. flavus* West. and *C. randjeloviciorum* Kernd., Pasche, Harpke & Raca were used. The plants were collected from 23 localities in Bulgaria. The voucher specimens were deposited in the Herbarium of the Agricultural University - Plovdiv (SOA). Their description is given below (Fig. 1):

Crocus adamioides: Toundja Hilly Country:

35TMG56: Golemiya Kamak Locality, 130 m, N42.09814 E26.50484, 2020-02-03 (coll. T.R. & K.S.) SOA 062719; **35TMH41:** Panaretovtsi, 240 m, N42.55333 E26.29883, 2020-02-16 (coll. I. Kostadinov) SOA 062837

Crocus chrysanthus subsp. *chrysanthus*:

Rhodopi Mts. (central): 35TKG95: a rock near Perestitsa Locality, 808 m, N42.030938 E24.534379, 2020-02-22 (T.R. & K.S.) SOA 062739; Thracian Lowland: **35TKG96:** Shirokia Peak above Novo Selo, 262 m, N42.10026 E24.47349, 2019-02-17 (coll. T.R. & K.S.) SOA 062600; **35TLG82:** Knizhovnik, 250 m, N41.8118889 E25.6003889, 2020-02-16 (coll. V. Trifonov) SOA 062731.

Crocus flavus: Black Sea Coast (southern):

35TNG39: Chekiliya Hill, 2020-02-15 (coll. T.R. & K.S.) SOA 062801; Forebalkan (eastern): **35TLH75:** Tsinga, 300 m, N42.9646111, E25.4290278, 2021-02-08 (coll. T.R. & K.S.) SOA 063087; Rhodopi Mts. (eastern): **35TLF79:** Sokolino, 400 m, N41.52492 E25.44383, 2020-03-05 (coll. V.Trifonov) SOA 062821; Thracian Lowland: **35TKG99:** Pyasachnik Dam reservoir, 293 m, N42.39103 E24.56797, 2019-02-19 (coll. T.R. & K.S.) SOA 062619; **35TLG83:** Galabets, 350 m, N41.8275 E25.635, 2020-02-29 (coll. V.Trifonov) SOA 062815; Toundja Hilly Country: **35TMG36:** Hlyabovo, 262 m, N42.098301 E26.233179, 2021-02-23 (V. Trifonov) SOA 063085; **35TMG55:** Oustrem, 128 m, N42.0324641 E26.4344543, 2020-02-23 (V. Trifonov) SOA 063086.

Crocus olivieri: Black Sea Coast (southern):

35TNG85: Sekana Koriya locality near Sinemorets, 32 m, N42.06109 E27.96935, 2020-02-14 (coll. T.R. & K.S.) SOA 062809.

Crocus pallasii: Rhodopi Mts. (western):

35TKG86: Elenski Peak near Ognyanovo, 492 m, N42.12522 E24.414196, 2018-11-14 (coll. K.S.) SOA 062444; Tracian lowland: **35TKG96:** Shirokiya Peak above Novo Selo, 292 m, N42.09729 E24.46419, 2019-11-10 (coll. T.R. & K.S.) s.n. 2019.101; **35TLG16:** Youth Hill, Plovdiv, 199 m,

N42.136104 E24.729023, 2018-11-01 (coll. K.S.) SOA 062432.

***Crocus pulchellus*: Rhodopi Mts (western): 35TKG86:** Bessapara Hills, Elenski Peak, 492 m, N42.12486 E24.41953, 2018-11-14 (coll. K.S.) SOA 062444; Rhodopi Mts. (eastern): 35TLF79: Near Karamfil village, 521 m, N41.4767 E25.5483, 2019-11-15 (coll. T.R. & K.S.) SOA 062674; Toundja Hilly Country: 35TLG86: Near the border of Propadna-

loto Blato protected locality, N42.125029 E25.64003, 2019-10-09 (coll. T.R. & K.S.) s.n. 2019.095;

***Crocus randjeloviciorum*: Stara Planina (eastern): 35TMH42:** Karandila, 1005 m, N42.7182111 E26.3605139, 2020-03-14 (coll. I. Kostadinov) SOA 062762; Toundja Hilly Country: 35TMH22: Tepyata Locality, 256 m, N42.63839 E26.13462, 2020-01-17 (coll. I.Kostadinov, T.R. & K.S.) SOA 062718.

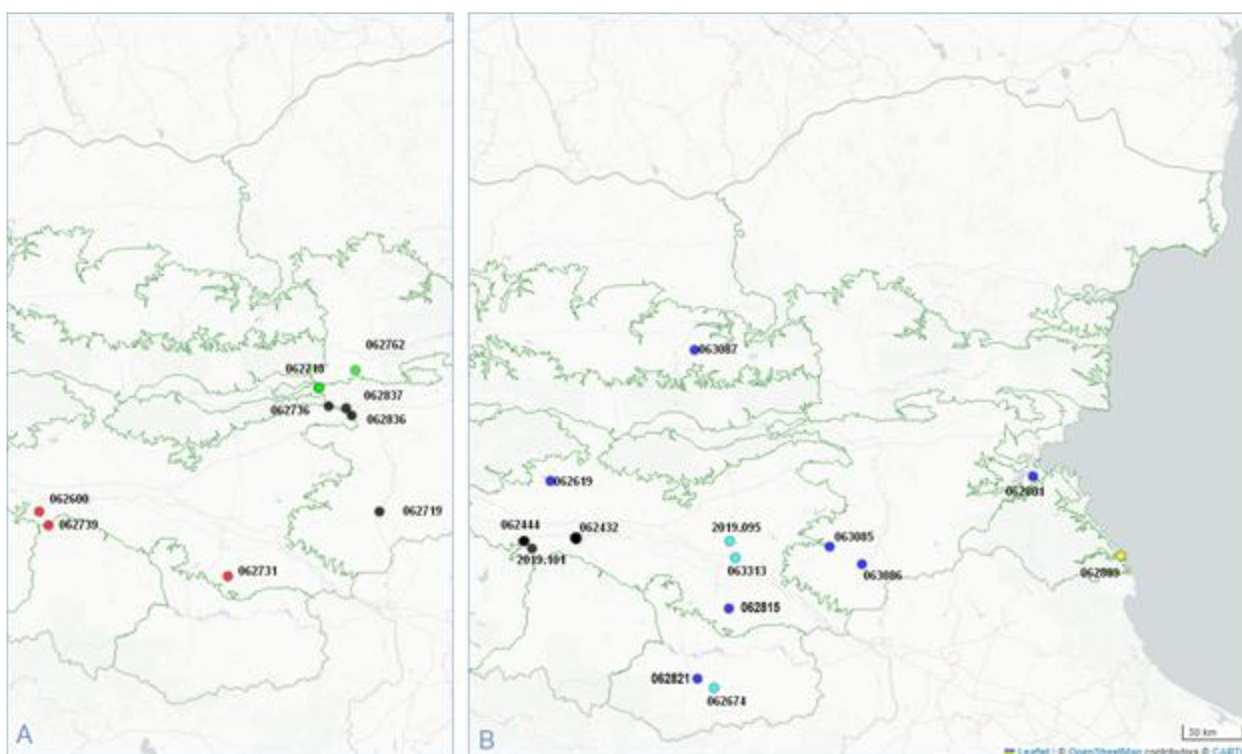


Fig. 1. Maps of the evaluated specimens: A - *Crocus adamioides*, *C. randjeloviciorum* and *C. chrysanthus*; B - *C. flavus*, *C. olivieri*, *C. pallasii* and *C. pulchellus*.

Methods

The maps of the investigated populations were drawn over a layer of the floristic regions (Bozhilova & Boyadzhieva, 2023) using Leaflet 1.9.4 (<https://leafletjs.com/>) over a map tile from <https://carto.com/>.

Samples were analyzed using polyacrylamide gel electrophoresis according to Maurer's method (1971), with modifications according to Ivanova (1996). A two-layer gel was used to concentrate and separate samples. Based on the experiments conducted, the 11% concentration of the separating polyacrylamide gel was adopted as a model.

For statistical analysis, the software product BIOSYS-1 (Swofford & Selanger, 1981) was used,

through which the following parameters were calculated: allelic frequencies; the average number of alleles per locus; the degree of polymorphism; observed and expected heterozygosity and Nei genetic distance (Nei, 1972); the fixation index characterizing the degree of intra-population and inter-population variability.

The matrix and relational graphs and the cluster analysis (Ward's method) trees were built using PAST 4.17c (Hammer et al., 2001).

Results and Discussion

Enzyme electrophoresis allows the molecular phenotype of the studied individual to be seen in a spectrum and given an objective assessment, unlike most morphological phenotypes, which

are the result of complex interactions, both within the organism itself and between it and the environment, and which have a polygenic character.

All alleles of each isoenzyme group, with their frequencies, are shown for each specimen in Fig. 2.

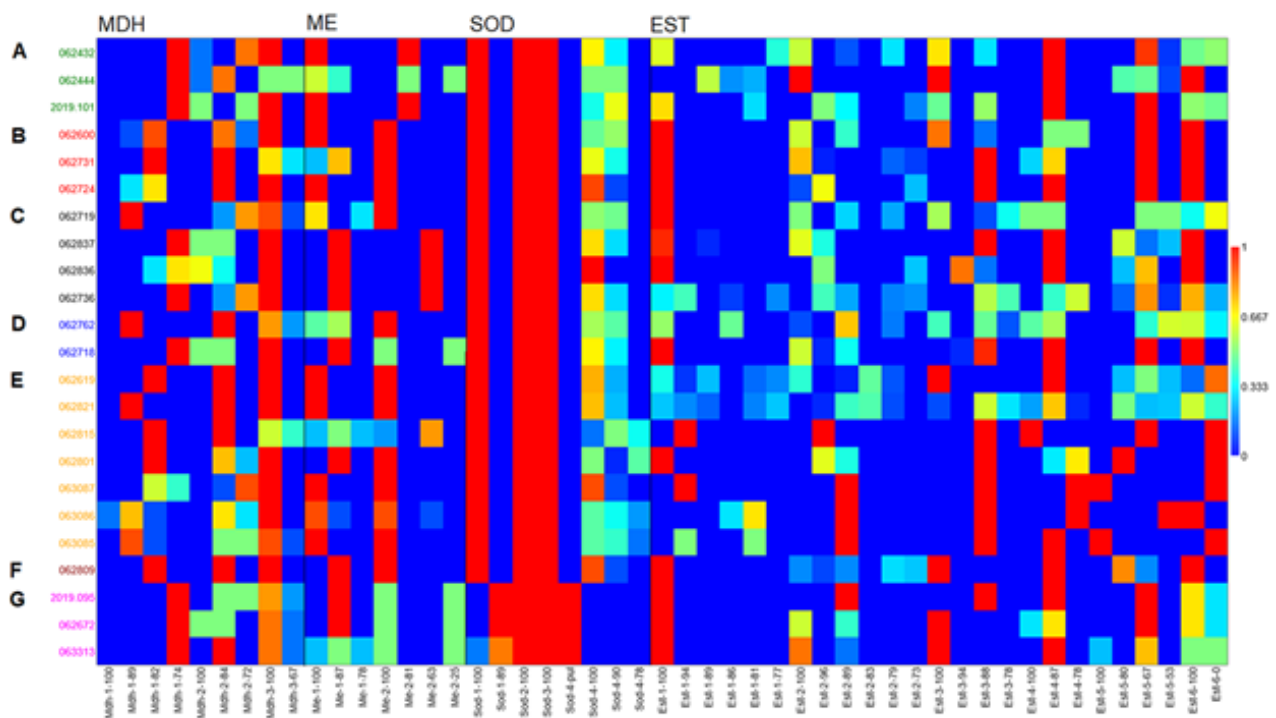


Fig. 2. Alleles and allele frequencies in the evaluated isoenzyme groups: MDH, ME, SOD, EST. A- *C. pallasii*; B - *C. chrysanthus*; C - *C. adamioides*; D - *C. randjeloviciorum*; E - *C. flavus*; F - *C. olivieri*; G - *C. pulchellus*.

NAD-dependent malate dehydrogenases (MDH, EC 1.1.1.37)

The MDH specters of *Crocus* individuals were visualized in a total of ten fractions with different electrophoretic mobility. Differences regarding the number and intensity of appearance of the MDH

fractions were found (Fig. 3). The nature of their expression gives a reason to assume that the genetic control of these isoenzymes is carried out by three polymorphic loci - Mdh-1, Mdh-2, and Mdh-3 (Fig. 4).

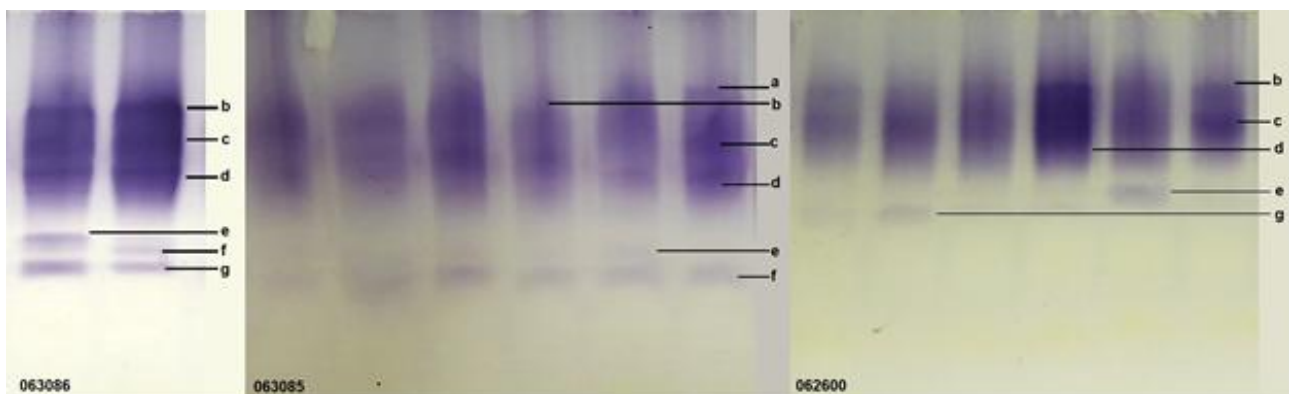


Fig. 3. MDH spectres in *Crocus flavus* (SOA 063086 and 063085) and *C. chrysanthus* (SOA 062600): a- Mdh-3⁶⁷, b- Mdh-3¹⁰⁰, c- Mdh-2⁷², d- Mdh-2⁸⁴, e- Mdh-1⁸², f- Mdh-1⁸⁹, g- Mdh-1¹⁰⁰.

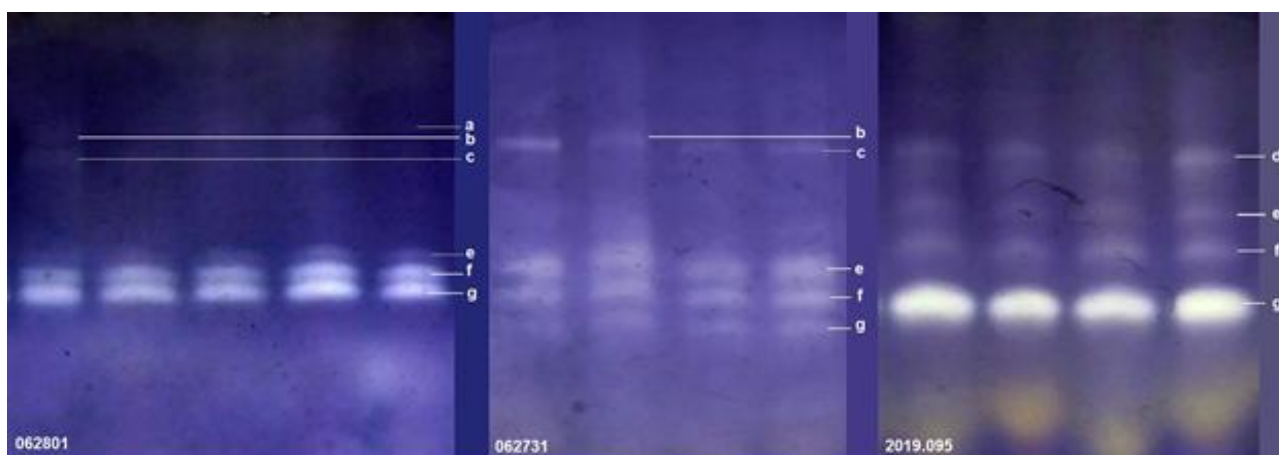


Fig. 7. SOD spectres in *Crocus flavus* (SOA 062801), *C. chrysanthus* (SOA 062731) and *C. pulchellus* (sn 2019.095): a- Sod-4⁷⁸, b- Sod-4⁹⁰, c- Sod-4¹⁰⁰, d- Sod-4^{pul}, e- Sod-3, f- Sod-2, g- Sod-1.

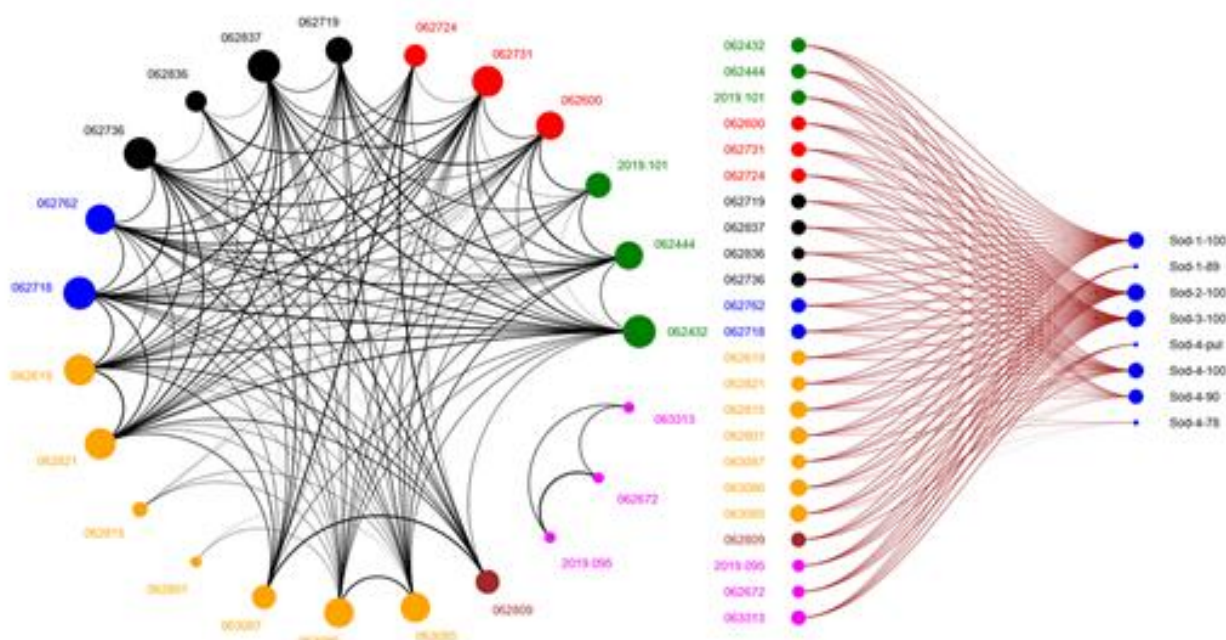


Fig. 8. Relations between evaluated samples according to the SOD polymorphism. See the Materials and Methods section for the specimen numbers. The size of the circles is proportional to the frequency of each allele.

A high frequency of the Sod-4¹⁰⁰ allele has been reported in populations of *C. adamioides* (SOA 062736, 062836, 062837), *C. randjeloviciorum* (SOA 062718), *C. olivieri* (SOA 062809), *C. chrysanthus* (SOA 062731, 062739), and *C. pallasii* (SOA 062432). Due to the dimeric structure of the enzyme, in some electrophoregrams, the appearance of intermediate fractions was observed, which appeared to be interlocus heterodimeric products. A taxonomy-dependent specificity of the genetic control of SOD in *C. pulchellus* was found (Fig. 8). A new

fraction with higher electrophoretic mobility appeared in the spectrum of individuals of this species, indicating the expression of another locus – Sod-4^{pul} (Fig. 7). Differences regarding the intensity of appearance of the fractions in the SOD zones for individual species of crocuses were also found. The variation in the expression of the Sod-3 locus was particularly distinct. The observed variability in enzyme expression may be associated with environmental conditions. It is well established that environmental factors influence

the physiological and biochemical processes of plants, thereby contributing to differentiation at both the population and species levels (Jangpangi et al., 2025).

Non-specific esterases (EC 3.1.1.11)

In the present study, comparing the specters of individuals from different populations of the *Crocus* genus, esterase expression is distributed in six zones. A specificity in the population, regarding the number and intensity of esterase fractions, was observed (Fig. 9). The results observed showed that the synthesis of non-specific esterases in *Crocus* is controlled by six loci: Est-1, Est-2, Est-3, Est-4, Est-5, and Est-6, and all of them were

polymorphic (Fig. 10). Six-allelic polymorphism at the Est-1 (Est-1¹⁰⁰, Est-1⁹⁴, Est-1⁸⁹, Est-1⁸⁶, Est-1⁸¹, and Est-1⁷⁷) and Est-2 (Est-2¹⁰⁰, Est-2⁹⁶, Est-2⁸⁹, Est-2⁸³, Est-2⁷⁹, and Est-2⁷³) loci, four-allelic polymorphism at the Est-3 (Est-3¹⁰⁰, Est-3⁹⁴, Est-3⁸⁸, and Est-3⁷⁸) and Est-5 (Est-5¹⁰⁰, Est-5⁸⁰, Est-5⁶⁷, and Est-5⁵³) loci, a triallelic polymorphism at the Est-4 locus (Est-4¹⁰⁰, Est-4⁸⁷, and Est-4⁷⁸), and a biallelic polymorphism at the Est-6 locus (Est-6¹⁰⁰ and Est-6⁰) was found. The presence of null alleles, displayed by the absence of esterase expression determined by the homozygous state, was reported. In some of the studied *C. flavus* (SOA 063087, 062815) populations, the Est-1 locus was monomorphic, with a fixed Est-1⁹⁴ allele.

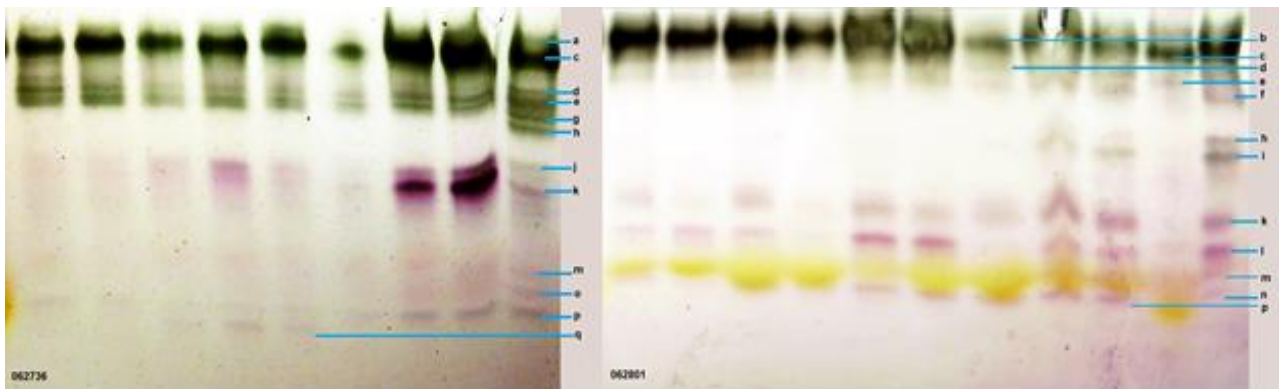


Fig. 9. EST spectres in *Crocus adamioides* (SOA 062736) and *C. flavus* (SOA 062801): a- Est-6¹⁰⁰, b- Est-5⁶⁷, c- Est-5⁸⁰, d- Est-4⁷⁸, e- Est-4⁸⁷, f- Est-4¹⁰⁰, g- Est-3⁷⁸, h- Est-3⁸⁸, i- Est-3¹⁰⁰, j- Est-2⁷³, k- Est-2⁷⁹, l- Est-2⁹⁶, m- Est-1⁷⁷, n- Est-1⁸¹, o- Est-1⁸⁶, p- Est-1⁹⁴, q- Est-1¹⁰⁰.

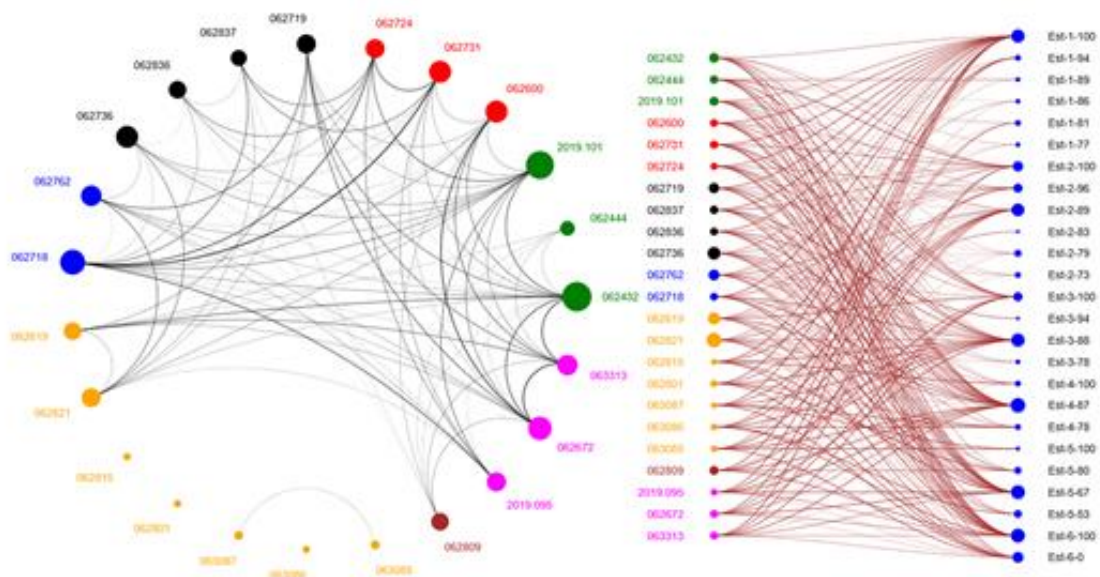


Fig. 10. Relations between evaluated samples according to the EST polymorphism. See the Materials and Methods section for the specimen numbers. The size of the circles is proportional to the frequency of each allele.

The Est-1⁸¹ allele demonstrated a high frequency in the populations of *C. flavus* (SOA 063085, 063086). The highest frequency of the Est-2¹⁰⁰ allele was found in the gene pool of the *C. adamioides* (SOA E), *C. chrysanthus* (SOA 062731, 062724), and *C. pallasii* (SOA 062432, 062444) populations. In the genotypic structure of the studied populations, homo- and heterozygous combinations on the alleles of the Est-2 locus (Fig. 9) were observed. The Est-3⁹⁴ allele showed high frequency in *C. adamioides* (SOA 062836) population. In the genotypic structure of the *C. adamioides* (SOA 062719) populations, only Est-4¹⁰⁰/4¹⁰⁰ and Est-4⁷⁸/4⁷⁸ type homozygotes were found. In the genotype structure of *C. chrysanthus* (SOA 062600), the homozygotes were Est-4⁸⁷/4⁸⁷. In all other populations with visible polymorphism at the Est-4 locus, heterozygous combinations of its allelic variants were found. The Est-5⁶⁷ allele demonstrated a high frequency in the gene pool of most analyzed populations and was fixed in some of them. The Est-5⁸⁰

allele occurred at the highest frequency in populations of *C. adamioides* (SOA 062837), *C. olivieri* (SOA 062809), and *C. flavus* (SOA 062801, 062821). In the gene pool of some of the studied populations, the Est-6 locus was monomorphic, with a fixed Est-6¹⁰⁰ allele. In the genotypic structure of these populations, only Est-6¹⁰⁰/6¹⁰⁰ type homozygotes were observed.

Level of selectiveness

The selectivity level of each enzyme system was displayed using cluster analysis (Fig. 11).

The reported variability in the expression of malate dehydrogenases (MDH) in the genus *Crocus* allows us to define this polymorphic isoenzyme system as a suitable marker for reporting the degree of genetic heterogeneity and analyzing the composition of the gene pool and the genotypic structure of the studied populations. The MDH markers showed a high selectiveness on the population but not at the species level.

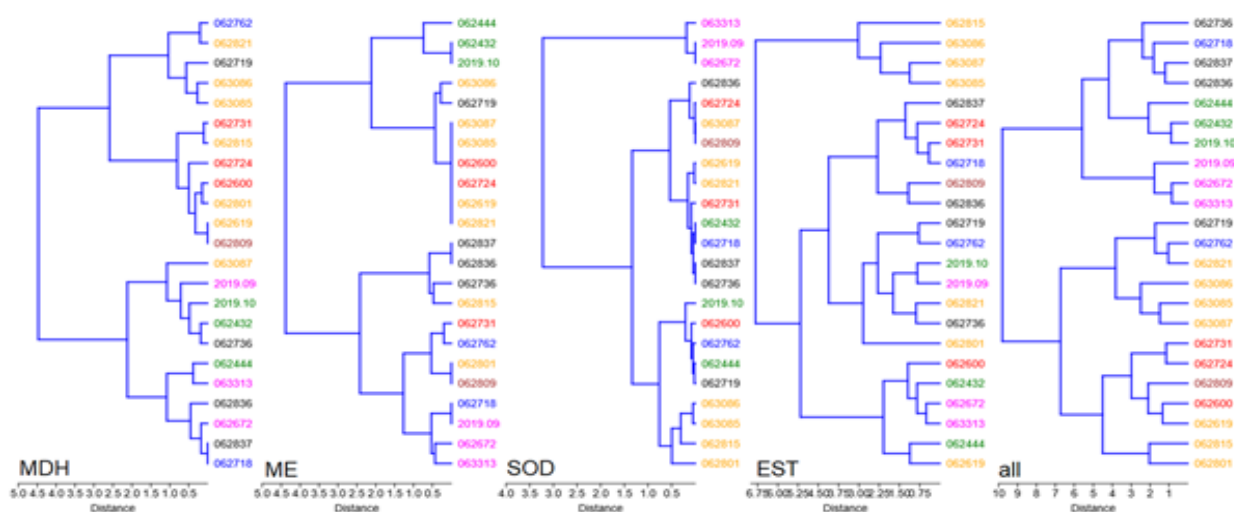


Fig. 11. Clustering of the evaluated specimens by MDH, ME, SOD, EST, and all loci.

The specificity in the recorded variability in the expression of the malic enzyme (ME) in the representatives of the genus *Crocus* gives a reason to accept this isoenzyme system as a suitable biochemical-genetic marker for studying intra-population variability and for comparative analysis at the inter-population level. The results showed low taxonomic selectiveness for some species, such as *C. pallasii* and *C. pulchellus*, and high selectiveness at the populational level.

The findings for the polymorphism of the superoxide dismutases (SOD) established them as

suitable biochemical-genetic markers for the characterization and discrimination of *C. pulchellus*. Differences, regarding the fraction intensity in the SOD zones for the investigated species, were also found. The observed differences in the fraction intensity in the SOD zones may be determined by the differences in the environmental conditions.

The results concerning esterase loci (EST) in the genus *Crocus* reveal them as suitable markers for genetic polymorphism among and between populations, based on ecological conditions and geographical characteristics of the populations.

This marker is not selective at the species level. As can be seen in Fig. 11, the combination of isoenzyme markers could represent the taxonomical and populational differences.

Polymorphism and heterozygosity

A moderate to low percentage of polymorphism (26.7% - 60.0%) was observed in all the populations of the genus *Crocus* (Table 1).

A lower percentage of polymorphic loci was found in populations of *C. chrysanthus* - SOA 062739 (1.3) and *C. flavus* - SOA 063087 (1.2), 063085 (1.3), and 062801 (1.3). These results correlated with the fixation of some alleles (e.g., at the Me-1, Me-2, Mdh-3, Sod-1, Sod-2, Est-1, Est-6 loci).

Table 1 presents information on the obtained and expected heterozygosity values. Heterozygosity, considered as an indicator of genetic diversity, is a convenient parameter for studying genetic variability (Ji et al., 2011). The highest level of heterozygosity was observed in populations of *C. adamioides* - SOA 062836 (0.233), 062837 (0.217), *C. pallasii* - SOA 062444 (0.222), and *C. pulchellus* - SOA 063313 (0.215). The obtained heterozygosity was higher than expected in some of the populations and varied from 0.039 to 0.233. The high values of reported heterozygosity in some populations can be explained by the diversity in habitat conditions and the high level of displacement, which favors distant interbreeding.

Table 1. Average sample size and number of alleles per locus, degree of polymorphism (%), observed (H_o), and expected (H_e) heterozygosity in populations of *Crocus* species.

Species and Population (cited by voucher specimen)	Average sample size per locus	Average number of alleles per locus	Degree of polymorphism	Average heterozygosity	
				Observed (H_o)	Expected (H_e)
<i>C. adamioides</i>					
SOA 062719	19.3 ±2.1	1.7±0.2	53.3	0.039±0.020	0.255±0.070
SOA 062736	24.0 ±2.6	2.1±0.3	53.3	0.153 ±0.071	0.280 ±0.078
SOA 062837	16.9 ±1.3	1.5±0.2	40.0	0.217 ±0.101	0.184 ±0.067
SOA 062836	10.0 ±0.0	1.4±0.1	40.0	0.233 ±0.098	0.176 ±0.059
<i>C. chrysanthus</i>					
SOA 062600	13.3±0.6	1.4±0.2	33.3	0.087±0.068	0.143±0.056
SOA 062731	14.1 ±1.2	1.5 ±0.2	33.3	0.111±0.051	0.150±0.057
SOA 062739	10.3 ±0.1	1.3±0.2	26.7	0.087±0.047	0.106±0.050
<i>C. flavus</i>					
SOA 062619	12.9 ±0.6	1.9±0.4	46.7	0.112±0.052	0.239±0.077
SOA 062801	9.2 ±0.3	1.3±0.1	33.3	0.167 ±0.081	0.147 ±0.057
SOA 062815	10.0 ±0.0	1.5±0.2	33.3	0.180±0.098	0.178±0.069
SOA 062821	16.7 ±1.3	2.2±0.4	46.7	0.057±0.030	0.267±0.081
SOA 063085	10.0 ±0.0	1.3 ±0.2	26.7	0.107 ±0.070	0.126 ±0.061
SOA 063086	10.0 ±0.0	1.5 ±0.2	40.0	0.133 ±0.064	0.169 ±0.061
SOA 063087	10.0 ±0.0	1.2 ±0.1	20.0	0.040 ±0.021	0.059 ±0.036
<i>C. olivieri</i>					
SOA 062809	12.8 ±0.9	1.5±0.3	33.3	0.060 ±0.038	0.111 ±0.056
<i>C. pallasii</i>					
SOA 062432	18.7±2.0	1.7±0.2	53.3	0.069±0.041	0.229±0.064
SOA 062444	14.4±0.8	1.7±0.2	53.3	0.222±0.090	0.278±0.073
sn 2019.101	17.3 ±1.4	1.6±0.2	46.7	0.140 ±0.082	0.244±0.071
<i>C. pulchellus</i>					
sn 2019.095	10.0 ±0.0	1.3 ±0.1	26.7	0.080 ±0.067	0.122 ±0.055
SOA 062674	12.9 ±1.3	1.5±0.1	53.3	0.188 ±0.093	0.241 ±0.062
SOA 063313	16.4 ±1.2	1.7±0.2	66.7	0.215 ±0.090	0.268 ±0.058
<i>C. randjeloviciorum</i>					
SOA 062718	12.4 ±1.1	1.5±0.2	40.0	0.200 ±0.099	0.176 ±0.063
SOA 062762	16.3 ±1.2	1.9±0.2	60.0	0.093±0.036	0.299±0.067

Fixation index

The fixation index (F_{ST}) was used to determine genetic differentiation in populations based on allelic frequencies. The parameters, marked as F_{IS} and F_{IT} , represent inbreeding coefficients and are used to determine the genetic variability, respectively, within the population and for all the populations. The summarised results show that the F_{IS} values for the individual loci (Table 2) in the genus *Crocus* vary widely: from 0.773 to 1.000.

A high mean F_{IT} value (0.7145) was found, indicating a heterozygous deficiency in most populations. The resulting mean F_{ST} value was 0.5496 (between 0.1987 and 0.9386 for the different loci), indicating that 45.04% of the total genetic variability occurred between populations and 54.96% within populations. This value indicates a high level of genetic differentiation between populations at the polymorphic loci in the genus *Crocus*.

Table 2. Summarized F-statistics for the studied polymorphic loci in the genus *Crocus*.

Loci	F_{IS}	F_{ST}	F_{IT}
Sod-1	0.2083	0.9386	0.9514
Sod-3	-0.1296	0.5982	0.5461
Sod-4	0.6245	0.1987	0.6991
Est-1	0.5729	0.4810	0.7784
Est-2	0.3770	0.3999	0.6261
Est-3	0.6210	0.4967	0.8092
Est-4	0.2558	0.3760	0.5356
Est-5	0.5049	0.5409	0.7727
Est-6	1.0000	0.4816	1.0000
Mdh-1	0.3732	0.8853	0.9281
Mdh-2	-0.1482	0.5194	0.4482
Mdh-3	-0.0256	0.2525	0.2333
Me-1	-0.0791	0.7675	0.7491
Me-2	-0.7730	0.7841	0.6172
Mean value	0.3661	0.5496	0.7145

Conclusions

The analysis carried out in the present study provides new information on genetic polymorphism and the level of genetic heterogeneity in populations of *Crocus* species. The results show that malate dehydrogenases, malic enzymes, superoxide dismutases, and non-specific esterases are polymorphic biochemical-genetic markers suitable for inter-population comparisons and investigation of intra-population heterogeneity in *Crocus* species. The F_{ST} data demonstrate high levels of genetic differentiation among populations of the *Crocus* species. These findings support the view that environmental factors and genetic structure jointly influence variability in plant populations (Gavrilova et al., 2025) and are consistent with evidence of significant genetic differentiation among *Crocus* populations revealed by ISSR marker analysis (Apostolova et al., 2022), highlighting the complex variability and adaptive potential of the genus.

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