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Comparative analysis of the genetically determined isoenzyme polymorphism in populations of plants of the genus Crocus distributed in Bulgaria.

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Introduction

The genetic heterogeneity in populations of plants of the genus Crocus was studied through the conducted research based on isoenzyme analysis. The probable genetic control of four polymorphic enzyme groups - non-specific esterases (EST), malate dehydrogenases (MDH), malate enzymes (ME) and superoxide dismutases (SOD) was established, appearing as appropriate markers for characterizing intra- and inter-population genetic variability.
A comparative analysis of the gene pool and genotypic structure of the studied populations was carried out.

Material and Methods

According to the objectives of the planned study, 250 individuals were used from the species *Crocus pallasii* Goldbl., *C. chrysantus* Herb., *C. adamioides* Kernd. & Pasche, *C. pulchellus* Herb., *C. flavus* West. and *C.* cf. *biflorus* auct. bulg. The plants were collected from 23 localities in Bulgaria. Voucher specimens were deposided in the Herbarium of Agricultural university – Plovdiv (SOA)

The samples were examined by electrophoresis in polyacrylamide gel by the method of Maurer (1971), with modifications according to Ivanova (1996). The allelic frequency, polymorphism, levels and genetic distance by Nei were calculated using the BIOSYS-1 software package (Swofford & Selander, 1981).

Discussion

Using the BIOSYS-1 software package (Swofford & Selander, 1981), we calculated allele frequencies, polymorphism and heterozygosity levels, and Nei genetic distance. In all investigated populations of the Crocus genus, we reported a moderate to low percentage of polymorphism (26.7% – 60.0%) (Table 1.). We found a lower percentage of polymorphic loci in populations of *C. chrysanthus* and *C. flavus*. The obtained heterozygosity was higher than expected in some of the studied populations and varied from 0.039 to 0.233. The highest level of observed heterozygosity was observed in populations of *C.* cf. *biflorus* and *C. pulchellus*.

Results

Electrophoretic analysis showed that the genetic control of MDH isoenzymes is carried out by three polymorphic loci (Fig. 1). We reported quadri-allelic polymorphism at the Mdh-1 locus and tri-allelic polymorphism at the Mdh-2 and Mdh-3 loci. The obtained results give us reason to assume that ME synthesis in crocodilians is controlled by two loci (Fig. 2). We reported triallelic polymorphism at the Me-1 locus and four-allelic polymorphism at the Me-2 locus. Data from the study indicate that the genetic control of SOD in Crocus individuals is mediated by four loci. We found biallelic polymorphism at the Sod-1 locus, triallelic polymorphism at the Sod-4 locus and monomorphism of the Sod-2 and Sod-3 loci. (Fig. 3). Based on the obtained results, we assume that the synthesis of non-specific esterases in crocodilians is controlled by six loci: Est-1, Est-2, Est-3, Est-4, Est-5 and Est-6 (Fig. 4).

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity	
				Observed Heterozygosity (Ho)	Expected Heterozygosity (He)
C. pallasii 2018002	18.7±2.0	1.7±0.2	53.3	0.069±0.041	0.229±0.064
C. pallasii 2018004b	14.4±0.8	1.7±0.2	53.3	0.222±0.090	0.278±0.073
C. pallasii 2019101	17.3 ±1.4	1.6±0.2	46.7	0.140 ±0.082	0.244±0.071
C. chrysanthus 2019021	13.3±0.6	1.4±0.2	33.3	0.087±0.068	0.143±0.056
C. chrysanthus 2020010	14.1 ±1.2	1.5 ±0.2	33.3	0.111±0.051	0.150±0.057
C. chrysanthus 2020222	10.3 ±0.1	1.3±0.2	26.7	0.087±0.047	0.106±0.050
C. adamioides 2020015	19.3 ±2.1	1.7±0.2	53.3	0.039±0.020	0.255±0.070
C. adamioides 2020170	16.3 ±1.2	1.9±0.2	60.0	0.093±0.036	0.299±0.067
C. flavus 2019025	12.9 ±0.6	1.9±0.4	46.7	0.112±0.052	0.239±0.077
C. flavus 2020132	16.7 ±1.3	2.2±0.4	46.7	0.057±0.030	0.267±0.081
C. flavus 2020118	10.0 ±0.0	1.5±0.2	33.3	0.180±0.098	0.178±0.069
C. flavus 2020033	9.2 ±0.3	1.3±0.1	33.3	0.167 ±0.081	0.147 ±0.057
C. flavus 2021005	10.0 ±0.0	1.2 ±0.1	20.0	0.040 ±0.021	0.059 ±0.036
C. flavus 2021017	10.0 ±0.0	1.5 ±0.2	40.0	0.133 ±0.064	0.169 ±0.061
C. flavus 2021016	10.0 ±0.0	1.3 ±0.2	26.7	0.107 ±0.070	0.126 ±0.061
C. cf. biflorus 2020045	16.9 ±1.3	1.5±0.2	40.0	0.217 ±0.101	0.184 ±0.067
C. cf. biflorus 2020044	10.0 ±0.0	1.4±0.1	40.0	0.233 ±0.098	0.176 ±0.059
C. cf. biflorus 2020005	12.4 ±1.1	1.5±0.2	40.0	0.200 ±0.099	0.176 ±0.063
C. cf. biflorus 2020026	24.0 ±2.6	2.1±0.3	53.3	0.153 ±0.071	0.280 ±0.078
C. olivieri 2020030	12.8 ±0.9	1.5±0.3	33.3	0.060 ±0.038	0.111 ±0.056
C. pulchellus 2019095	10.0 ±0.0	1.3 ±0.1	26.7	0.080 ±0.067	0.122 ±0.055
C. pulchellus 2019104	12.9 ±1.3	1.5±0.1	53.3	0.188 ±0.093	0.241 ±0.062
C. pulchellus 2019096	16.4 ±1.2	1.7±0.2	66.7	0.215 ±0.090	0.268 ±0.058

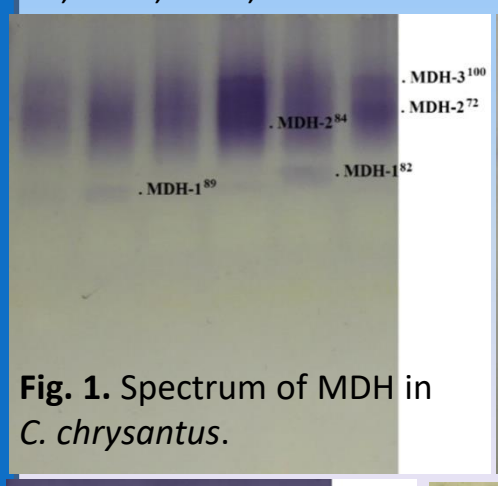

Fig. 1. Spectrum of MDH in *C. chrysantus*.

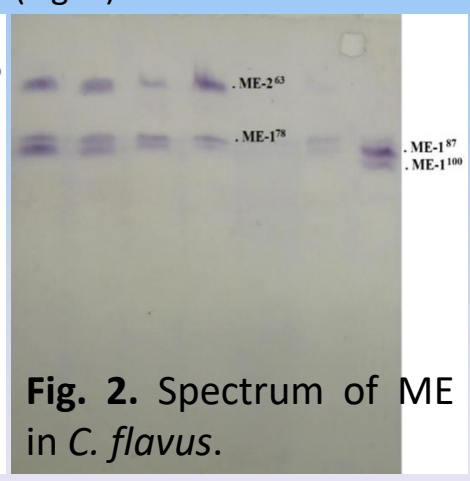

Fig. 2. Spectrum of ME in *C. flavus*.

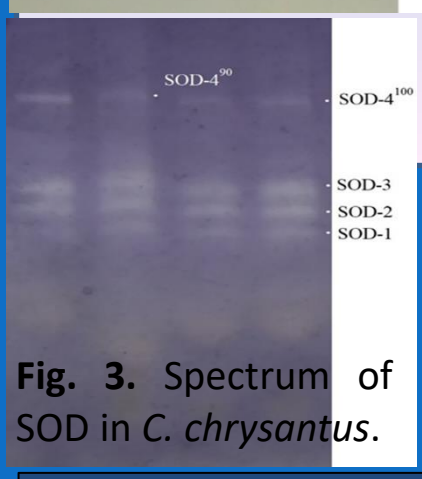

Fig. 3. Spectrum of SOD in *C. chrysantus*.

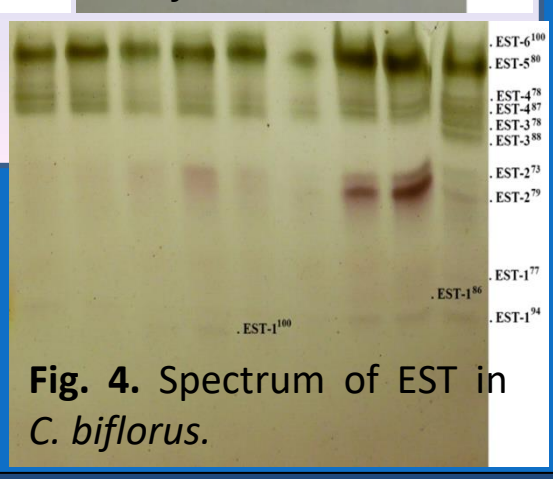

Fig. 4. Spectrum of EST in *C. biflorus.*

Conclusions

The analysis carried out in the course of the present study provides new information on genetic polymorphism and the level of genetic heterogeneity in populations of *Crocus* species. The obtained results show that malate dehydrogenases, malate enzymes, superoxide dismutases and non-specific esterases are polymorphic biochemical-genetic markers that are suitable for inter-population comparisons and investigation of intra-population heterogeneity in *Crocus* species.

Acknowledgments

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