

Preliminary study of genetic variation of Crocus taxa, ser. *biflori* (Iridaceae Juss.) from Bulgaria by ISSR markers

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One of the rather heterogeneous groups is *Crocus biflorus* s.l., belonging to *C*. sect. *Nudiscapus* B. Mathew, ser. *Biflori* B. Mathew. Recent phylogenetic analyses have proved several units of this series to be para- or polyphyletic, causing subsequent taxonomic problems (Petersen et al. 2008, Harpke et al. 2013). A DNA-based investigation showed that several subspecies of *C. biflorus* s.l. according to Mathew (1982), have been ranked as species instead of subspecies (Harpke et al. 2016). The aggregate of *C. biflorus* has been a subject of recent studies and the rank of its infraspecific taxa is also still unclear. The previous analysis based on ITS sequences confirms that the population belongs to *C. adamioides*. The obtained data confirmed that *C. chrysanthus* was a separate taxon. The results also verified the genetic divergence of the *C.* cf. *biflorus*. The ISSR analysis showed the need for additional DNA-based methods for taxonomic revisions of the genus *Crocus* L.



The present study was aimed to evaluate the genetic diversity and pattern of variation throughout the ranges of polymorphic aggregate group *C. biflorus* in Bulgaria. For this purpose, samples from five *Crocus* L. species collected from 15 natural populations were assessed using ISSR molecular markers (Fig. 1; Table 1). Cluster dendrogram were created using the Wars's method (PAST 4.03), then combined into consensus tree using Consense (PHYLIP 3.69) (Fig. 2).



Figure 1. ISSR profiles of three *C. biflorus* populations obtained with primers L1, L2, L3, L6, L7 and L8. Electrophoresis of ISSR products was done in a 2% agarose gel. M – molecular weight marker (SM1331, Thermo Fisher Scientific).

Table 1. List of primer sequencesused for ISSR analysis.		
Primer	Sequence 5' – 3'	04
L1	CACACACACACACAA(AG)G	06
L2	GAGAGAGAGAGAGAGA(CT)C	
L3	AGAGAGAGAGAGAGAG(CT)C	
L6	AGAGAGAGAGAGAGAGC	
L7	CTCTCTCTCTCTC(TG)C	<u>н</u> с
L8	AGAGAGAGAGAGAGAGAG(AGC)C	

	And a second
3 062725	cf. biflorus
-039 062712	cf. bflorus
1 062715	cf. biflorus
5 062817]cf. biflorus
062716	adamioides
062794	cf. biflorus
062524	?adamioides
062856	randjeloviciorum
4 062843	chrysanthus
2 06 2601	chrysanthus



References

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C. cf.biflorus - 042

Figure 2. Consensus tree built using CONSENSE (PHYLIP) - confirmed with ITS sequence.

Our results showed significant levels of genetic variation of the *C. biflorus* group corresponding with morphological diversity. Dendrogram showed that the samples of *C. adamioides* were in the group of *C. biflorus* but cannot be grouped with *C. biflorus* subsp. *adamii*.

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