Genetic diversity in high-mountain *Thymus* species in Bulgaria revealed by ISSR genetic markers

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SUMMARY

High mountain populations of the plant species possess particular interest from both evolutionary and conservation points of view. The mode of distribution, limited gene flow and severe environmental conditions act as evolutionary forces shaping the level and distribution of genetic diversity within and among populations. The paper reports results of a study on four populations of *Thymus praecox* aggr., including the taxa *T. vandasii* and *T. jankae*. Two populations are located in Rila Mts (Belmeken dam and Yastrebets), one – in Pirin (Vihren hut) and one – in the Rhodopes (Perelik hut). Eleven inter simple sequence repeat (ISSR) markers were applied to document the genetic diversity within and among populations. The level and distribution of the diversity correspond to the values reported in other studies on the species of the genus *Thymus* and other species with similar life-history characteristics. The populations from Rila and Pirin were genetically closer to each other, while the population from the Rhodopes was the most differentiated. The results are discussed in the light of the conservation and sustainable use of the species resources.

Thymus vandasii

Thymus jankae

Table 1. Populations studied and their geographic coordinates

Population	Mountain ridge	Geographic coordinates	Altitude (m)	No of samples
Belmeken	Rila	42° 09' 02" N 23° 46' 31" E	1950	15
Yastrebetz	Rila	42° 13' 27" N 23° 34' 47" E	2350	15
Vihren hut	Pirin	41° 45' 40" N 23° 25' 02" E	1950	12
Perelik	Rhodopes	41° 36' 27" N 24° 35' 36" E	1970	10

DNA extraction was done by Invisorb® Spin Plant Mini Kit (Invitek Molecular, Berlin, Germany) following the instructions of the producer. DNA quality and concentration were measured by a spectrophotometer Nanodrop Lite (Thermo Fisher Scientific).

Table 2. List of the primers, their sequences, annealing temperature and bands scored

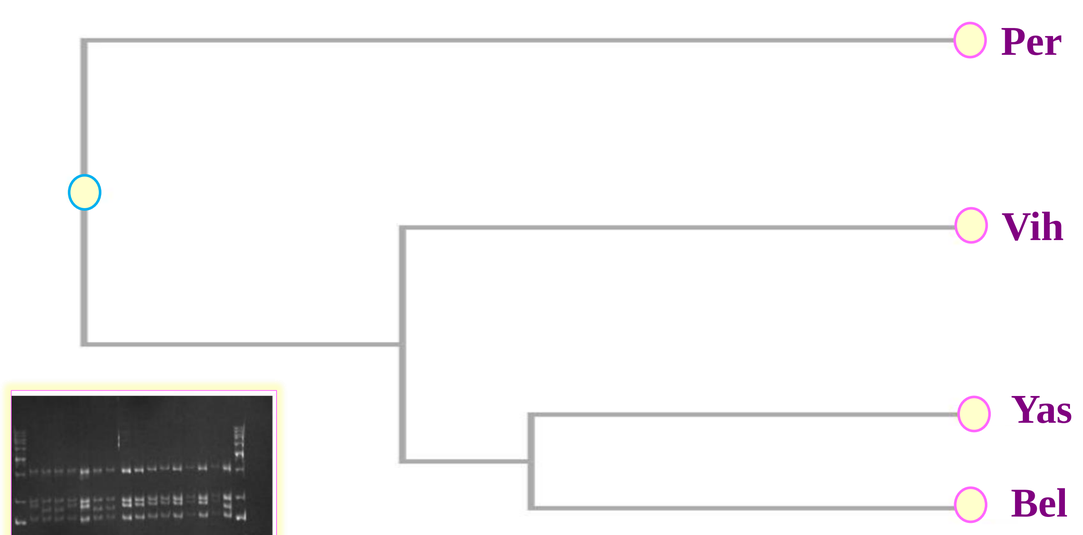
Primer code	Primer sequence	T_a	Total No of bands	No of polymorphic bands	Percent of poymorphic bands
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50	13	9	69
UBC-811	5'-GAGAGAGAGAGAGAGAC-3'	50	16	12	75
UBC-814	5'-CTCTCTCTCTCTCTCTA-3'	48	15	10	67
UBC-827	5'-ACACACACACACACACG-3'	48	15	11	73
UBC-834	5'-AGAGAGAGAGAGAGAGCTT-3'	46	15	12	80
UBC-845	5'-CTCTCTCTCTCTCTCTAG G-3'	48	17	12	70
UBC-846	5'-CACACACACACACACAAGT-3'	50	17	14	82
UBC-848	5'-CACACACACACACACAAG C-3'	50	15	11	73
UBC-852	5'-TCTCTCTCTCTCTCTCAG A-3'	48	14	10	71

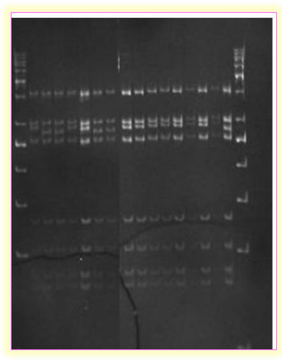
Polymerase chain reactions were performed in a volume of 20 µl, containing 15 µl PCR master mix (Canvax Biotech, Cordoba, Spain), 1 µM of the primer, 2 µl of the extracted (template) DNA solution (~ 50 ng) and 2 µl sterile deionized water. The primers (Biomers, Ulm, Germany) were selected based on literature survey (Yousefi et al., 2012; György et al. 2020). They are presented in Table 2, together with the annealing temperature of the reactions and number of bands.

PCR products were analyzed by means of 1 % agarose gel in 0.5 TBE buffer. The gels were stained with ethidium bromide and visualized on a Gel documentation system. Standard genetic diversity parameters were scored. Among-population genetic diversity was evaluated by Jaccard similarity coefficient and a dendrogram was constructed by using UPGMA method.

Table 3. Parameters of within-population diversity

Population	Percent of polymorphic loci	Shannon Index of diversity
Belmeken	33	0.189
Yastrebetz	44	0.210
Vihren hut	44	0.201
Perelik	33	0.168





Conclusions

The level of genetic diversity within and among populations of the studied species correspond to the values established in other studies on the species of genus Thymus.

Significant relationship between the geographic distance and genetic differences was detected – populations from different mountain ridges tend to be genetically different, too.

ISSR genetic markers can be a useful tool for studying the diversity in the genus Thymus and for characterizing its genetic resources.

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