FSFIS "The Labor Red Banner Order Nikita Botanical Gardens – National Scientific Center of the Russian Academy of Sciences"

Molecular characterization of population level genetic diversity of several endemic species of *Thymus* L. (Lamiaceae) by using ISSR markers

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Aim

The goals of this work were preliminary study of population level genetic polymorphism of a narrow endemic species of Crimean Flora, belonging to the Western Yailae group – *Thymus pseudohumillimus* Klokov et Des.-Shost., and a forecast on it's population sustainability.

Motivation

A *Thymus* L. is one of the largest Lamiaceae genera with great practical value. Studies of perspective thyme species introduction is problematic due to the extra endemism coupled with wide infra-generic hybridization possibilities and numerous spontaneous hybrids known, high morphological polymorphism and unclear taxonomic status of a most species.

Matherial

Thymus pseudohumillimus Klokov et Des.-Shost. is ultranarrow endemic species of the Western Yailae group in Crimean penninsula.
Fresh leaves of *Th. pseudohumillimus* (six individuals), agg. *Th. roegnerii* K. Koch (two individuals) and putative hybrid of theirs (two individuals) were harvested from natural populations at the Nikitian Yayla mountains.

Figure 2. Thyme at nature:
A - *Th. psudohumillimus*, B - agg. *Th. roegnerii*

Methods and Algorithms

DNA was extracted from fresh young leaves according to the CTAB protocol with addition of 2% polyvinylpyrrolidone. Quality and quantity of DNA extracted were analyzed on NanoPhotometer NP80 (Implen, Germany). For PCR, BioMaster HS-Taq PCR (2×) commercial kit (Biolabmix, RF) was used. DNA was amplified with 35 cycles using C1000™ Thermal Cycler (Bio-Rad, Singapore). For UBC-824 and UBC-826 annealing temperature was 55ºC, and for UBC-864 and UBC-880 – 52ºC.

Gels after electrophoresis were analyzed and on binary matrix base, UPGMA and Neighbor Joining dendrograms performed

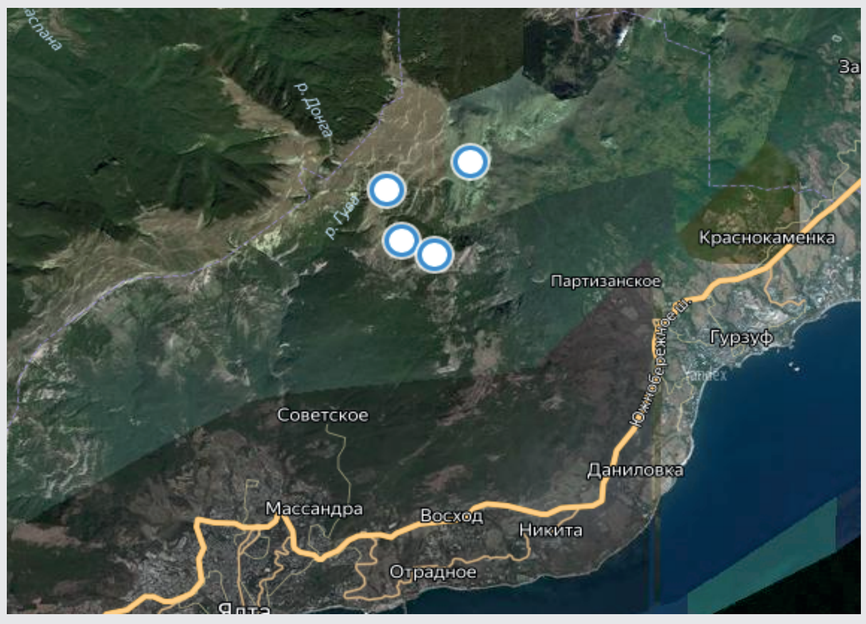

Figure 1. Natural localisations of thyme observed

Table 1. Purity of DNA extracted from ten Thymus accessions

Accession	C, ng/μL	$A_{260/230}$	$A_{280/260}$
Th. pseudohumillimus	91	2,102	2,205
Th. pseudohumillimus	417,75	2,130	2,291
Th. pseudohumillimus	287	2,038	2,362
Th. pseudohumillimus	661,45	2,070	2,422
Th. pseudohumillimus	518,9	1,827	1,841
Th. pseudohumillimus	671,8	2,070	2,248
Th. roegnerii	359,05	2,144	2,279
Th. roegnerii	171,95	2,132	2,206
Th. pseudohumillimus x Th. roegnerii	13,85	2,052	1,530
Th. pseudohumillimus x Th. roegnerii	278,6	2,132	2,273

Figure 3. Gel with electrophoresis products, amplified using University of British Columbia primers set No.9. The 4th is negative control and the 5th is ladder

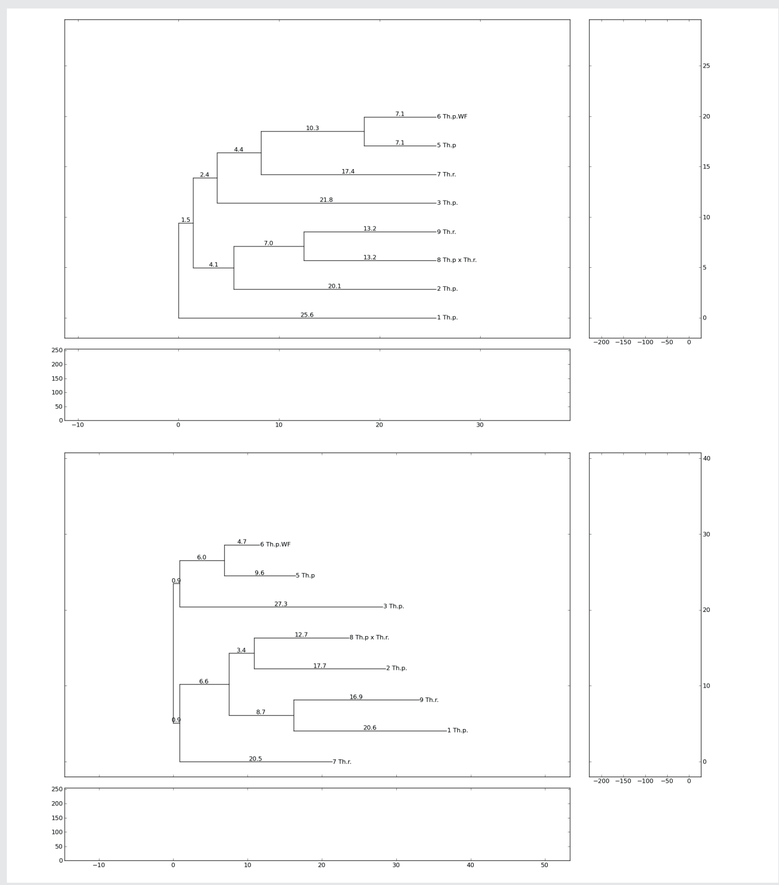

Figure 4. UPGMA (top) and NB (bottom) dendrogramms plotted using open-sorce PyElph software

Conclusion

ISSR PCR analysis has showed that the Thymus pseudohumillimus Klokov et Des.-Shost. is highly polymorphic and has molecular similarities to the Th. roegnerii K. Koch.

Acknowledgements

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