

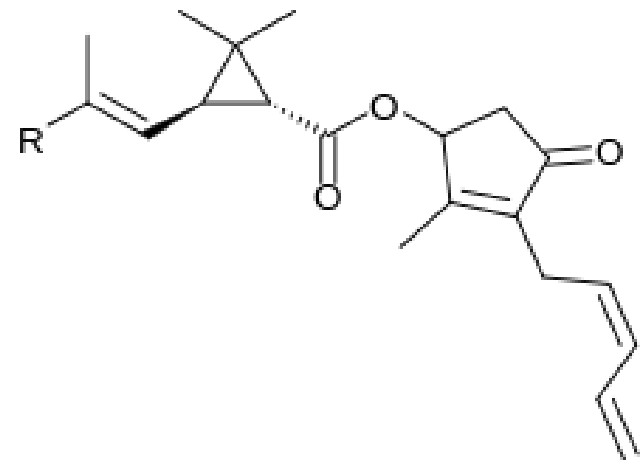
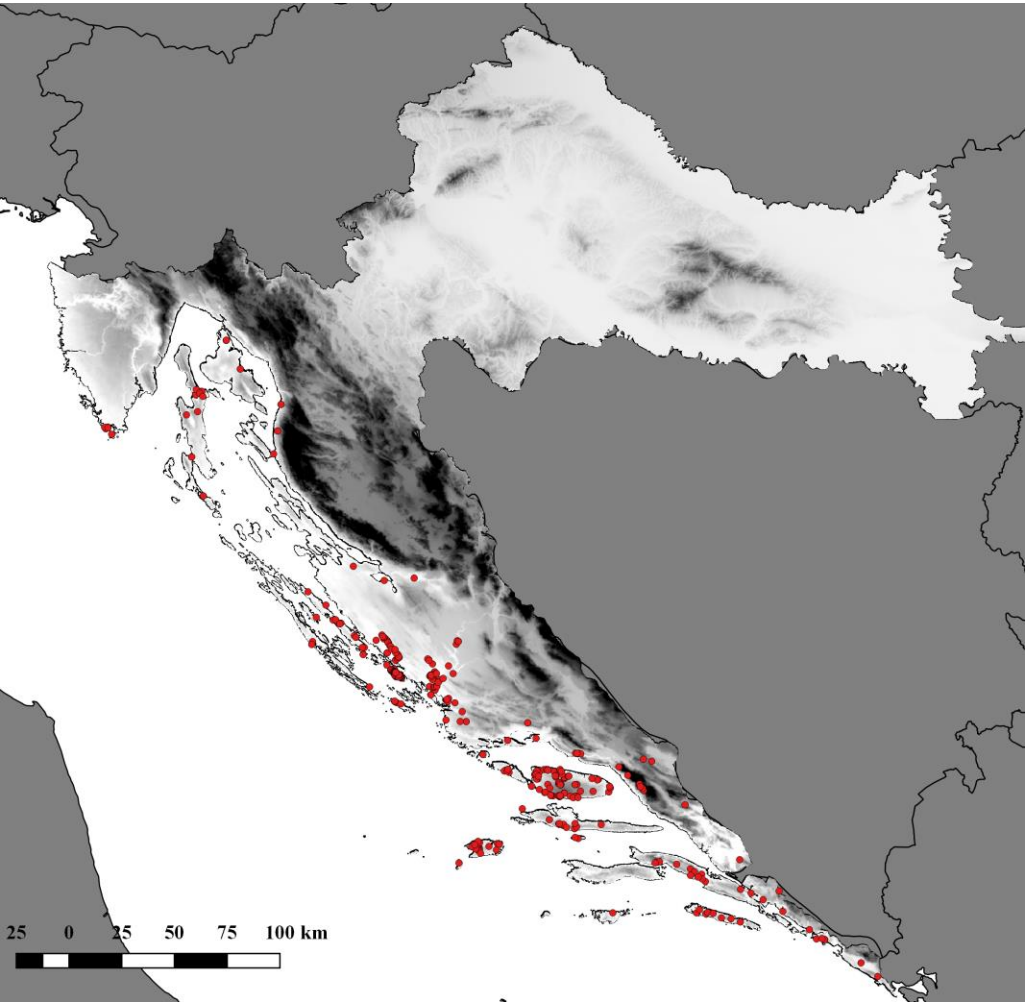
RAZVITAK MIKROSATELITNIH BILJEGA DALMATINSKOG BUHAČA POMOĆU SEKVENCIRANJA VISOKE PROPUSNOSTI



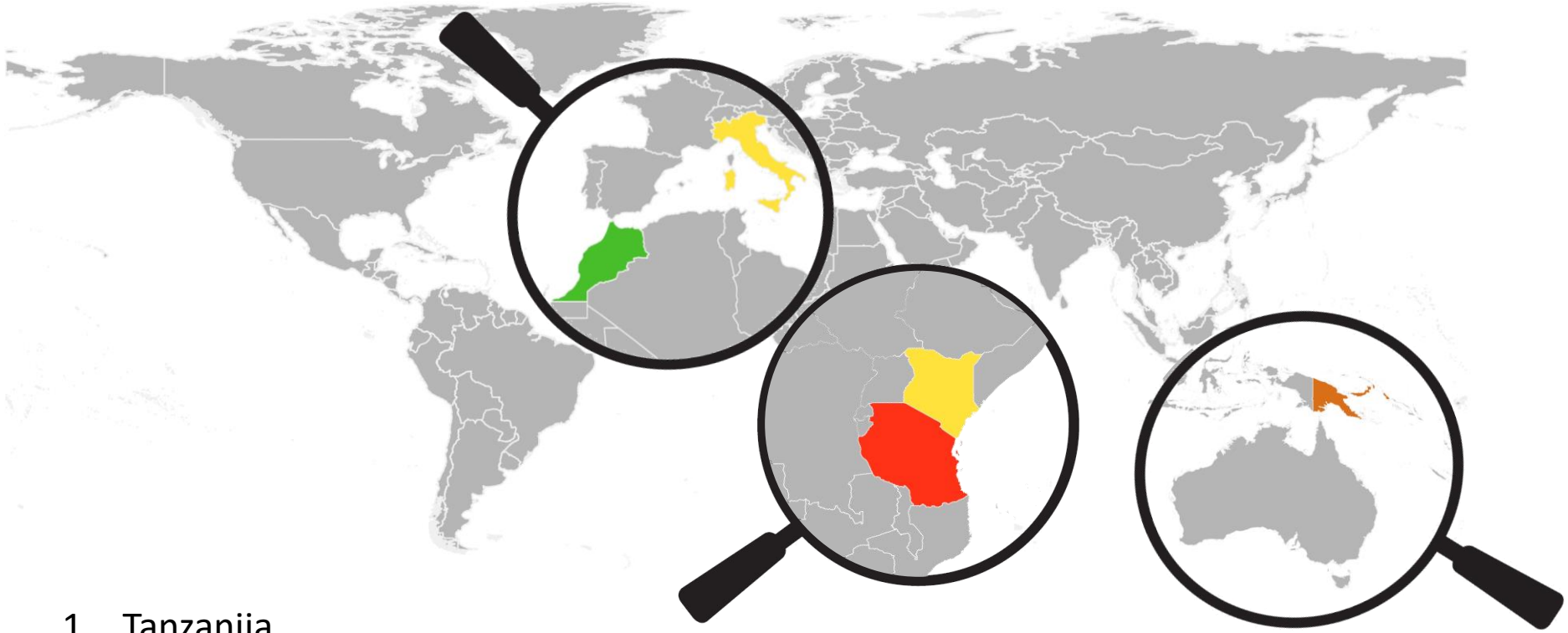
Filip Varga, mag. biol. exp.

Sveučilište u Zagrebu, Agronomski fakultet, Zagreb
Znanstveni centar izvrsnosti za bioraznolikost i molekularno oplemenjivanje bilja, Zagreb
E-mail: fvarga@agr.hr

Tanacetum cinerariifolium (Asteraceae)

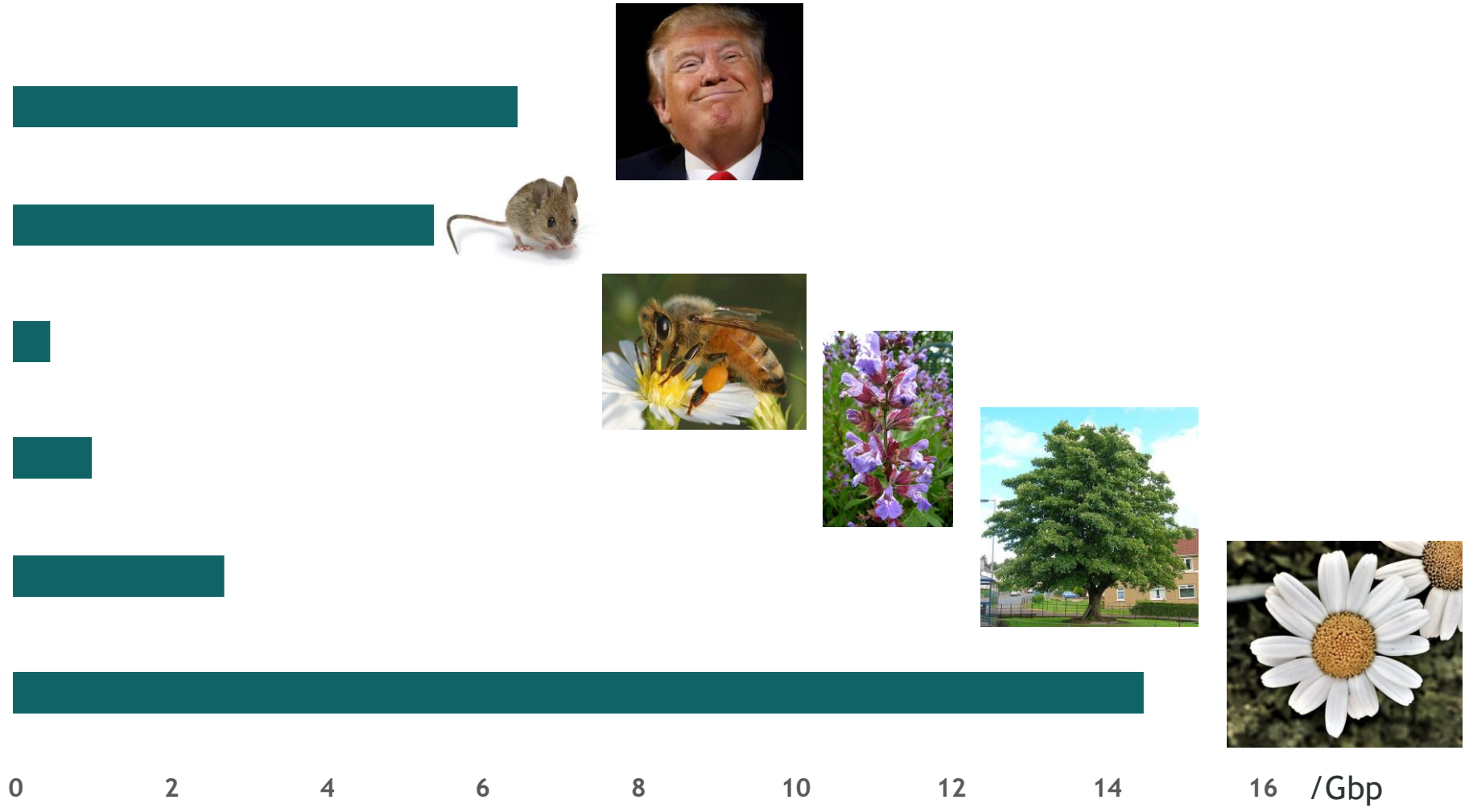


PROIZVODNJA U SVIJETU (2015. godina)



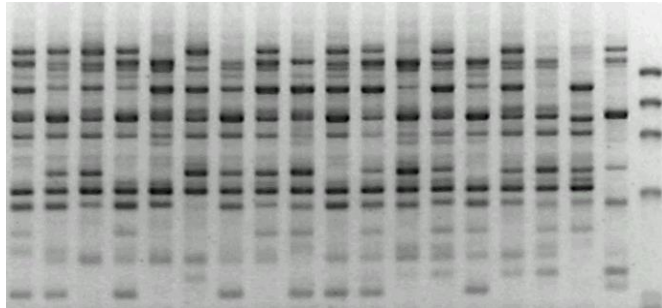
1. Tanzanija
2. Papua Nova Gvineja
3. Kenija
4. Italija
5. Maroko

VELIČINA GENOMA DALMATINSKOG BUHAČA (2n)

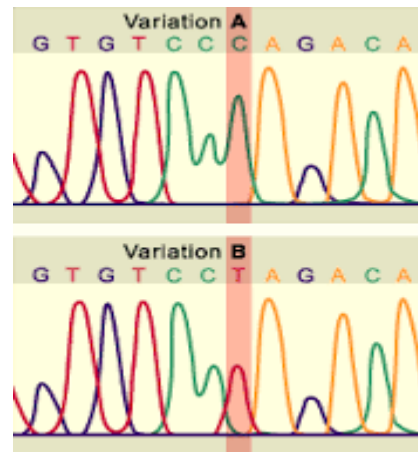


0 2 4 6 8 10 12 14 16 /Gbp

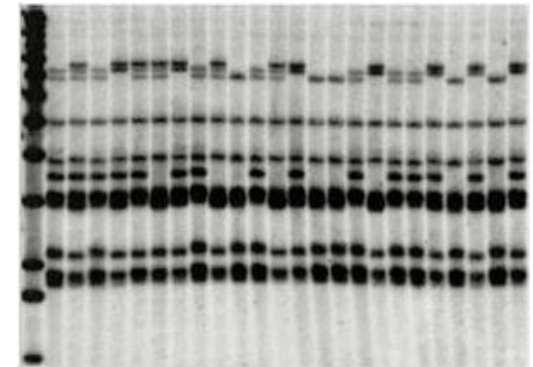
MOLEKULARNI BILJEZI



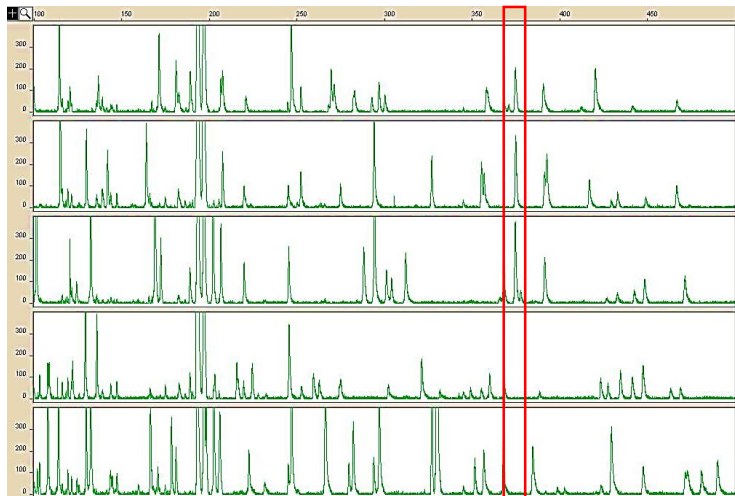
RAPD



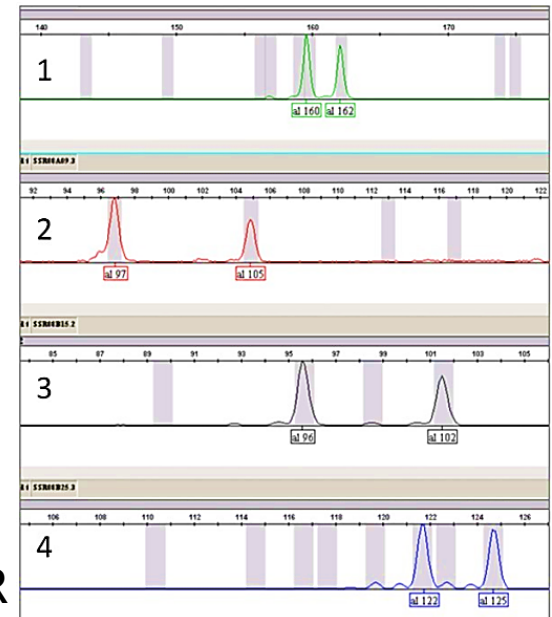
SNP



RFLP



AFLP



SSR

MIKROSATELITNI BILJEZI

- temelje se na umnažanju kratkih ulomaka DNA u kojima se svojstveni motiv (motif) sastavljen od 1 do 6 nukleotidnih baza ponavlja

(AC)₇

```
GACTCGAACCCACGACCTGCACTACACACACACACACCCCTCTTTCCATTATGC
TAGAACCATTTATCTGTTTATGTTTCTTTTCAATACTTATTTAAGTCGATTTTT
TTTATTTTAGTGTATAAGCTGTTTCAGCTTATATTCGGCTTATTTGATAAGCTA
AATTTTTGTTCTTATTTTTTCTTAAATACTAGATTAAATTTACACTTTCTGTAA
TTTTTTGGTAATTTTTTACTATTCATCTACTATTTATTTTAATTTAATTTTCGTTT
TTTAATTAAAAAATTCATAATAAATAAAATATTAATCAAAAAAATATAAACTTT
TTTTTAAAAATCTTATTTTTTAATTGTAGTTTCTGTGAAAATT
```

1. jednostavni (simple): (AT)_n
2. sastavljeni (compound): (AT)_n(CA)_m
3. prekinuti (interrupted): (AT)_n-CCA-(AT)_m

- česti mikrosatelitni motivi kod biljaka:
(GA), (GT), (AGA), (ACA), (ACC), (AGG)

SEKVENCIRANJE NOVE GENERACIJE



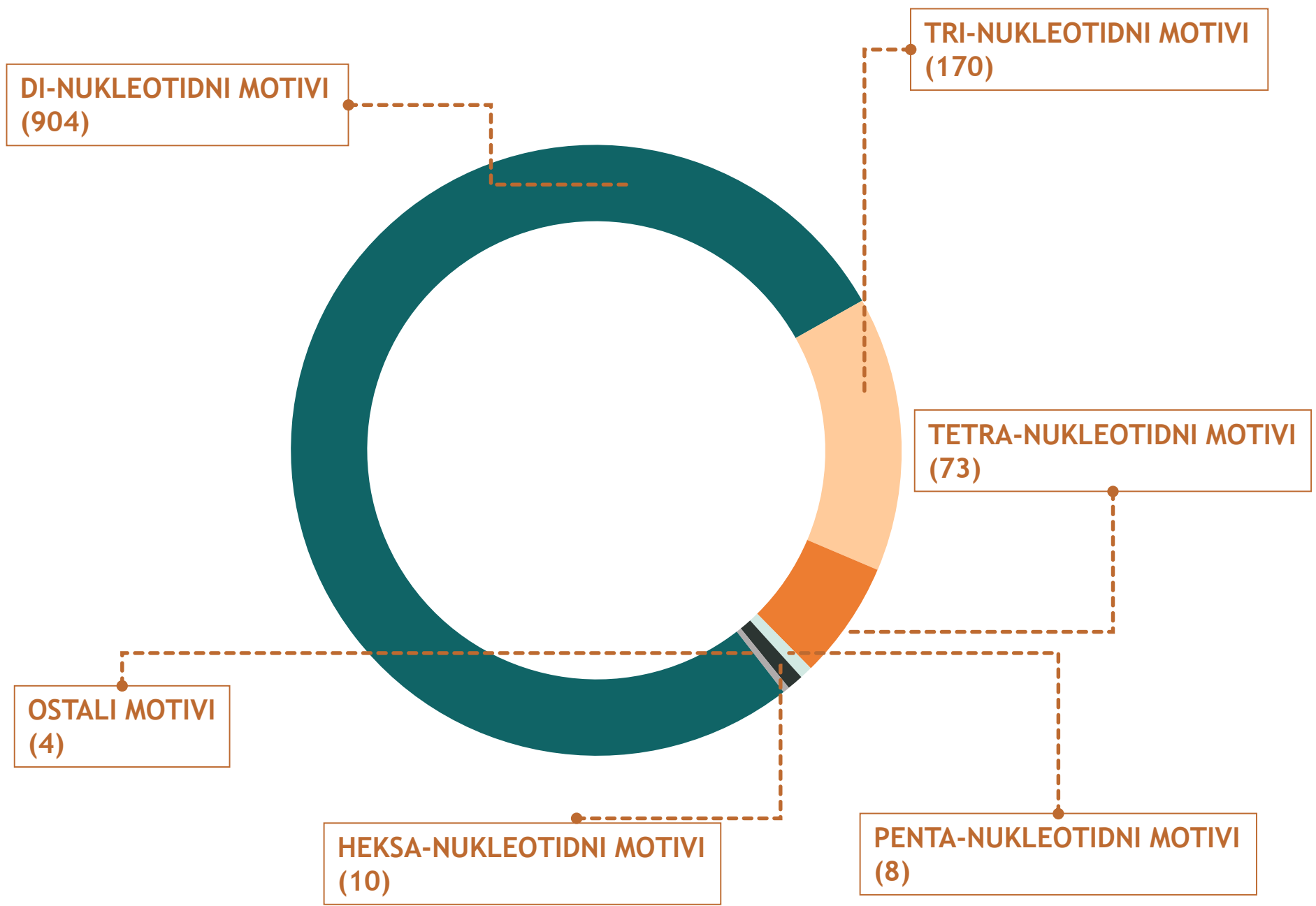
(1) Izolacija DNA (Qiagen Dneasy Plant Mini Kit)

(2) Sekvenciranje (Illumina NExtSeq sustav, UK)

- rezultat 6.619.805 očitavanja visoke kvalitete (1,98 Gbp genoma)
- daljnje pročišćavanje podataka i sastavljanje 35.758 kontiga (N50=360 bp, Avg=348 bp, max=37.391 bp)

(3) Identifikacija SSR biljega (MlcroSAteLLite identification tool)

- identificirano 1169 mikrosatelitnih sekvenci



(4) Dizajn početnica (PRIMER3)

No	SSR Marker	Repeat Motif	Product Size	Left Primer	Right Primer	OriginalSeqID
1	TcUz001	(TC)8	127	GTGTGTTTTGCTGCGTGTTT	CGATGGAAGAAGAAGATGGTGG	Tan_OPT1_contig_487
2	TcUz002	(GA)6	127	ACGCAGACAATGACACGGTA	GGGCTGCGTTTTGTCATATT	Tan_OPT1_contig_4268
3	TcUz003	(AC)6	143	ACCAGAGAAAGTGATCAAAGTCA	TTTTGGCATGCAAGTTAGGC	Tan_OPT1_contig_1893
4	TcUz004	(AG)6	143	GAAAACGTTTCGAGATTTGTTCA	TCAACAAGCACCTTCTTAGCTT	Tan_OPT1_contig_1970
5	TcUz005	(CA)7	150	TGCTAGCCAAACTATTACCCCT	TGTTTTACGTGCATCATTCCGC	Tan_OPT1_contig_75
6	TcUz006	(AT)8	151	CGGGGATTGCCTTCTCTTCT	AAAGCCTTCAGAGCCTCAGT	Tan_OPT1_contig_46
7	TcUz007	(AT)6	152	GCGTCTTCTCCTTGGCAAAG	GCCAGCACTGCACAAATAT	Tan_OPT1_contig_57_2
8	TcUz008	(CA)7	152	TGAAAAATGTTCCCAATGC	AAAGTCCCAAGGGCAGAGAC	Tan_OPT1_contig_9369
9	TcUz009	(AT)6	153	AAACCGTCTTGCATCAATAAACA	ACAAGTTC AAGATCAGGTTTAGC	Tan_OPT1_contig_1612
10	TcUz010	(CA)7	160	TGGTTCGTGTGTAGGGAGAG	TGGTCCCCTATTCCCTGTATCC	Tan_OPT1_contig_1269
11	TcUz011	(GT)8	180	CAGAAAGTGCAATAAAGCATGA	TGACCTCTACCCACCTCGAC	Tan_OPT1_contig_7243
12	TcUz012	(AT)7	188	TGACTTCATTTTGGGCGGAT	GCCAGCACTGCACAAATAT	Tan_OPT1_contig_57_3
13	TcUz013	(AT)7	194	ATGGCCCATGATGATTA AAA	TGGATTTGGTTTTTGGGAAG	Tan_OPT1_contig_34036
14	TcUz014	(GT)6	195	CAAGGGCGCGAGTGTTAAG	TTTTGGGCTTAATTTCTGCTT	Tan_OPT1_contig_1622
15	TcUz015	(AC)7	216	TTCAAAACATTCTCCACCACA	GGATTGATGAGATGGCATGAT	Tan_OPT1_contig_20748
16	TcUz016	(TA)11	217	GCACATGTATTTTCTGTCATCG	TCATAAGCGATATGAAGAGTGC	Tan_OPT1_contig_5862
17	TcUz017	(TA)44	220	AGTGTGGATCCATGACAAACA	TGGGTA AAATGCCGAACTGC	Tan_OPT1_contig_709
18	TcUz018	(TG)6	241	TGGTGGTTACAGCTAGTGTGTG	AAAAAGCGATCTTAGCCCTCT	Tan_OPT1_contig_30112
19	TcUz019	(GT)6	245	AAAGTCCCAAGGGCGAGAT	CATGTTGATTGCTTCTGTCTGA	Tan_OPT1_contig_8820
20	TcUz020	(TA)7	246	ACAAGAGCATGTTATTTCCGGC	AGTTATCTTTGTTTCATGCCCGA	Tan_OPT1_contig_991
21	TcUz021	(GTT)6	112	CCTGAGCATTTCCAGTCCCA	GCTAGTTGTTATGAGTGC GGG	Tan_OPT1_contig_711
22	TcUz022	(TGG)6	114	GGAGGAGGTTCTTGAGCAGA	TTCCCCTAAA ACTCCACCGG	Tan_OPT1_contig_547
23	TcUz023	(TTC)5	118	ACGAAGATACCTGAACCACCA	TCTGTCATGAGTGTGGAGAGA	Tan_OPT1_contig_649
24	TcUz024	(AAT)6	122	TGTTTCTTGTCTTGAGCCGG	AGGCTCGGGATTACTTCC	Tan_OPT1_contig_193
25	TcUz025	(GAG)7	127	TGAGATGGATGTAGAGGCCG	CTCAAATGGCTCCGTCTCCT	Tan_OPT1_contig_634
26	TcUz026	(TTA)5	131	TCCGAAACA ACTCAACTACGA	TGATGTTGCAGTTGTTTAAAGGA	Tan_OPT1_contig_177
27	TcUz027	(CTC)5	133	GTAGGTACCACAGGAGAGTCG	ATGGT GATGATGAGCCCTCC	Tan_OPT1_contig_210
28	TcUz028	(GTT)7	141	CTCCGCACTCAAAACAACCA	GTCCTTGCTACCTAAGTGC	Tan_OPT1_contig_528
29	TcUz029	(ACA)7	147	GAAACCAAGCTGCTAACCCC	AATGACCCTGAGCTCCACAC	Tan_OPT1_contig_441
30	TcUz030	(GGT)5	157	ACCTTACGGCTGCTTTGAGA	GTGGCAGCAGACCGAAAC	Tan_OPT1_contig_34
31	TcUz031	(CAA)6	180	TCAACATGGCCCGTGA ACTA	CCTGCCTCCTGTTTTGTTGT	Tan_OPT1_contig_1557
32	TcUz032	(ATG)5	187	CGAAGCCGTCCTATCGTCTA	ACTCACTTGGGTTTGGGACA	Tan_OPT1_contig_1909
33	TcUz033	(AAT)5	195	GGTGTAGCAGGAAGCTTTCC	CGACAATTCGGGTATCGTTT	Tan_OPT1_contig_2158
34	TcUz034	(GTT)5	202	CCTGAGCATTTCCAGTCCCA	TGCAACAAGATTGGCCACTT	Tan_OPT1_contig_712
35	TcUz035	(TTC)5	208	GCCTCAACTACTTTTTGCA TT	GGTGAGTTTGAGCTTCTTTGG	Tan_OPT1_contig_11937
36	TcUz036	(GGT)5	208	GGACCAAAAAGGGATATGGTG	CCCCTTTTACCCACACACAC	Tan_OPT1_contig_17371
37	TcUz037	(ATG)5	209	CGAAGTCCGCGTTAGTGAA	GCCCTACGATCCGAACTAAA	Tan_OPT1_contig_2666
38	TcUz038	(ACC)5	230	TTTCGCTCTCATCAGTTCCA	ACCACGTACGCACCAAAAA	Tan_OPT1_contig_24814
39	TcUz039	(GAT)5	230	GCGAGTTTTGTTCCGATAG	TTTTCGATTCCGATCAATCAC	Tan_OPT1_contig_31285
40	TcUz040	(GAA)6	247	TGGATCCGTCAA AATCGATCT	CCTGATCCCGTACATATAATCA	Tan_OPT1_contig_39

(5) Testiranje novorazvijenih biljega na polimorfizam

(6) Selekcija najprikladnijih biljega za korištenje u analizi populacijske genetike dalmatinskog buhača



Istraživanje provedeno u sklopu projekta:

Genetska osnova insekticidnog potencijala dalmatinskog buhača (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip.)



Projekt je u cjelovitosti financiran od strane Hrvatske zaklade za znanost

