

Israeli germplasm: phenotyping and genotyping of native grapevines (*Vitis vinifera* L.)

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Summary

In this study 32 grapevine accessions from Israel were phenotyped according to O.I.V. descriptors and phyllometry, furthermore genotyped with seven microsatellite loci for the first time for further clarification of their potential biodiversity and phylogenetic grapevine analysis. The results according to the SSR microsatellites suggest that the majority of the studied Israeli grapevine accessions, compared to the international reference varieties, show considerably different genotypes - nine accessions showed a unique (individual) genotype, further three accessions represented a first group, four accessions represent a second group and eight groups contained two different accessions. The significant differences among the studied grapevine accessions were observed also in most of the 17 phyllometric measurements of significant leaf characteristics, especially in the leaf area, vein length (L1, L2, L3 and L4), depth of lateral sinuses (OS, OI), petiol length (Lp) and vein angles (β , β'). The observed grapevine characteristics according to the O.I.V. descriptors suggested that more than three-quarters of the studied accessions showed characteristics of table grapes.

Key words: microsatellite markers; phyllometry; phylogenetic; grapevine.

Introduction

In ancient times the territory of modern Israel undoubtedly played an important role in trade market, where merchants exchanged various products and traded their crops from East to West, the North and South. Numerous archaeological findings dating back to this era and ancient scripts as well, such as the Bible itself, suggest that the production of grapes and wine were important agricultural branches in the Middle East (McGOVERN *et al.* 1996). The grapevine *Vitis vinifera* L. supposedly originates from the Near- and Middle East around the Caucasus from where its domestication and later organized vine cultivation practices apparently arose as well. In the last centuries many cultural, political, religious, economic and ecological factors have affected a grapevine gene pool in Israel. Only few individual vines, which in the past used to grow in different parts of Israel, were rescued and replanted in a gene

collection near Haifa (North-East of Israel). KLEIN *et al.* (2008) studied some grapevine varieties planted in Indigenous Fruit Trees Rescue Gardens of Sataf (Jerusalem) and demonstrated that the presumably Israeli endemic vines are genetically close to the Greek gene pool.

The studied 32 vines have supposedly different genotypes, are unnamed, labelled only with encrypted codes or designated with the name of the village or location, where they were collected. This collection undoubtedly presents an important gene pool for and in Israel, while also from a grapevine phylogenetic point of view, this collection is of great importance, especially in the context of the ancient geographic extension of grapevines from the Near- and Middle East to Europe and the Mediterranean pool.

All vines planted in the gene pool in Israel (North-East from Haifa) were for the first time genotyped and phenotyped for further clarification of their potential biodiversity. The obtained data may contribute to the phylogenetic studies regarding grapevine spreading in the Near- and Middle East but also in Europe.

Material and Methods

In the preliminary study 32 vines from Israel were sampled, genotyped, while their genotypes were promptly compared to six grapevine varieties used as references. The genomic DNA was isolated from young grapevine leaves by using the modified CTAB method (KUMP and JAVORNIK 1996). The DNA concentration was measured by fluorimeter (Amersham Biosciences DyNAQuant 200) and samples were stored in TE buffer at -20 °C. Samples were genotyped at seven microsatellite loci VVln16, VVMD25, VVMD27, VVMD28, VVMD5, VVMD7 and VVS2 under PCR conditions performed by SCHUELKE (2000). Amplified alleles were separated by capillary electrophoresis ABI Prism 3730xl DNA analyser and sized with GeneMapper software version 4.0 (Applied Biosystems). UPGMA dendrogram of studied Israeli grapevine accessions based on Nei's (1972) standard genetic distance.

The vines were also phenotyped according to the O.I.V. descriptors codes 001 – 005, 011 – 017, 051, 053 – 056, 067 – 087, 093 – 094, 151, 202 – 209, 220 – 225, 238, 351 – 354, 502 and 503 (O.I.V. 2001) and phyllometry (PELENGIĆ and RUSJAN 2010), where at least 10 observations or measurements per individual characteristic were done. Considering the phyllometric measurements, the

Tree diagram has been plotted using the Unweighted pair-group average method (UPGMA) with the Squared Euclidean distance in Statgraphics 5.0 programme (Statistical Graphics Corp.).

Results and Discussions

Genetic characterization: The results of allele lengths obtained for seven microsatellite markers in studied accessions and reference varieties are given in the Table. According to the SSR microsatellites, the majority of the vines from Israel, compared to the reference varieties, showed quite different genotypes that are grouped separately as shown in the dendrogram (data available upon request). Nine genotypes from Israel showed a com-

pletely different genotype from others, further three accessions represent the first group, four accessions represent a second group and eight groups contain two different accessions. The obtained results showed that accessions 522-23, 558-59 and 710-211 showed quite similar genotype; therefore they are at least in close relationship. The results suggest and further confirm the thesis of KLEIN *et al.* (2008) that the Israeli endemic varieties are genetically quite different compared to varieties mostly cultivated in France, Italy, Austria, Slovenia and Croatia.

Ampelographic characterization: According to the 17 phyllometric measurements of significant leaf characteristics, the dendrogram was constructed (Figure). The phyllometry has shown that there are many morphological differences among studied accessions, especially in leaf area, vein length (L1, L2, L3 and L4), depth of lateral

Table

Microsatellite profiles of the 32 grapevine accessions from Israeli genepool in comparison with reference grapevine varieties. Size of the alleles for the seven microsatellite loci in the different accessions

N	Accession	VVln16		VVMD25		VVMD27		VVMD28		VVMD5		VVS2		VVMD7	
1	105-166	157	161	224	240	178	180	241	255			143	143	249	267
2	520-21	151	155	240	244	178	178	227	241	236	238	131	141	249	263
3	521-22	149	157	238	254	178	178	227	229			131	141	253	253
4	522-23	151	155	240	244	180	182	239	255	234	234	133	151	263	267
5	523-24	151	155	238	248	182	184	241	243	230	234	123	133	249	249
6	525-26	155	155	238	244			239	255	226	226	121	129	241	245
7	556-57	149	161	238	244	184	188	227	241	236	236	125	133	245	247
8	556-57	157	161	238	244	178	180	241	255			141	151	261	261
9	558-59	151	155	240	244	180	182	239	255	234	234	133	151	255	263
10	587-88	157	161	224	240	178	178	227	241			129	149	255	263
11	592-93	149	157	232	248	180	226	229	243	236	236	139	151		
12	609-110	151	155	238	248	180	240	243	247	226	236	129	139	251	251
13	641-142	149	161	238	254	180	182	239	255	236	238	139	143	259	269
14	642-143	157	161	238	240	178	178	239	241	234	234	131	149	263	263
15	643-144	161	161	244	248	182	182					147	151	249	253
16	644-145	149	161	240	244	178	178	227	229			141	149	249	249
17	645-146	149	161	238	244	216	230	219	233			143	153		
18	646-147	149	149	238	248	214	226	217	221	224	230	125	131	249	249
19	708-209	149	161	232	248	180	180	229	243	238	238	149	151	263	269
20	709-210	149	157	240	240	180	184	227	241	232	234	125	133	267	267
21	710-211	151	155	240	244	180	182	239	255	234	234	189	195	263	267
22	711-212											131	139		
23	712-213	151	155	244	248	184	188	239	255	226	234	131	141	263	267
24	713-214	149	157	240	244	226	226	229	243			149	157	259	263
25	776-277	151	155	224	240	178	178	227	241	232	234	129	149	239	251
26	812-313	151	155	240	244	180	184	239	255	232	234	133	151	247	247
27	93-154	151	155	224	240	178	180	227	243	232	232	125	131	233	241
28	94-155	151	155	238	254	178	216	219	221	226	236	131	141	233	249
29	95-156	149	157	238	248	178	224	227	241	232	232	141	151	233	251
30	Alelul	151	155	238	248	178	180	227	229	232	236	139	141	233	233
31	Keslev	149	149	238	254	180	182	255	255	236	238	151	155	243	249
32	Odem	151	155	240	244			255	255	234	234	133	151	263	267
Reference															
33	Barbera	149	157	238	254	186	190	231	255	224	224	131	133	249	253
34	Cabernet Sauvignon	151	151	238	248	174	186	231	233	230	238	137	149	239	239
35	Chardonnay	149	149	238	254	178	186	217	227	232	236	135	141	239	243
36	Pinot Noir	149	157	238	248	182	186	217	233	226	236	135	149	239	243
37	Sultanine	151	155	238	248	178	190	217	243	232	232	143	149	239	253
38	Touriga Nacional	149	149	248	254	178	186	231	265	224	234	141	149	239	239

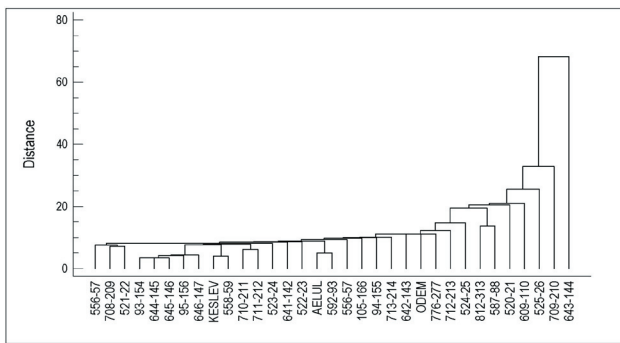


Figure: Dendrogram constructed from the 17 phyllometric measurements data distance matrix using UPGMA clustering method; representing similarities in leaf morphology among the Israeli studied accessions.

sinuses (OS, OI), petiol length (Lp) and vein angles (β , β'). The largest mature leaves were observed at accessions 520-21 (157.7 ± 6.3 mm) and 812-313 (152.3 ± 2.4 mm) but the smallest at 524-25 (97.0 ± 1.9 mm) and 776-277 (108.7 ± 2.5 mm). Most accessions have leaves with equal length of petiole compared to length of middle vein (L). The biggest angles between nerves measured from the petiole sinus to the first ramifications of vein (α , β , γ) were measured at accessions 609-110 ($58.1 \pm 1.5^\circ$, $80.8 \pm 2.4^\circ$, $59.6 \pm 2.0^\circ$, respectively) and 94-155 ($54.7 \pm 1.8^\circ$, $57.7 \pm 1.6^\circ$, $64.9 \pm 1.1^\circ$, respectively), but the smallest at 556-57 ($48.5 \pm 2.5^\circ$, $51.4 \pm 1.8^\circ$, $54.3 \pm 1.2^\circ$, respectively); the biggest angles between nerves from petiole sinus to apex of vein (α' , β' , τ) have accessions 525-26 ($46.4 \pm 2.4^\circ$, $54.7 \pm 2.8^\circ$, $62.3 \pm 2.1^\circ$, respectively) and Keslev ($51.5 \pm 1.9^\circ$, $56.8 \pm 1.9^\circ$, $51.4 \pm 1.2^\circ$, respectively), but the smallest at Odem ($41.5 \pm 1.7^\circ$, $47.7 \pm 1.9^\circ$, $45.5 \pm 2.0^\circ$, respectively).

According to the O.I.V. descriptor 001 all studied accessions have fully open shoot tips. At 8 accessions none or very low, at 16 medium and at 2 highest (710-211, 558-59) densities of prostrate hairs on the shoot tips (O.I.V. 004) were observed. However, the presence of erect hairs on shoot tips (O.I.V. 005), compared to the prostrate hairs, at studied accessions is rarer, while only at 6 accessions the low or non or very low density were determined. The evaluation of a colour of upper side of blade (O.I.V. 051) showed that 11 accessions had green, 13 accessions copper-reddish and 3 accessions bronze coloured 4th blade on a shoot. Regarding a blade shape (O.I.V. 067), 14 acces-

sions had wedge, 10 accessions circular and 8 accessions pentagonal shaped mature leaves. O.I.V. descriptor 223 suggested that the 10 vines have berries of a wide ellipsoid shape, 9 vines are narrow ellipsoid, 7 globose, 3 from obtuse ovoid to ovoid, 1 obloid, 1 obovoid and 1 cylindrical in shape. Most of the vines have white coloured berries, of which only five vines have a low berry weight (O.I.V. 503) just up to about 1 g; others from medium to high weight (5-7 g). The most frequent bunch shape was conical (wide width 15-17 cm; very length about 240 mm and more), followed by cylindrical (narrow width about 80 mm; very length about 240 mm and more) (O.I.V. 208) (all data available at the author). More than three-quarters of the studied varieties show characteristics of table grapes.

The preliminary results suggested that vines cultivated in grapevine gene pool collection near Haifa showed high genetic and phenotypic biodiversity and are therefore of high interests for phylogenetic studies.

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