

Ampelographic and genetic characterization of an initial Israeli grapevine germplasm collection

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Summary

The Holy Land has a long history of winemaking, widely mentioned in ancient scripts. The Muslim occupation of this region, starting in the 7th century initiated a wine consumption prohibition, resulting in the abandonment of most wine varieties. The renewing of Israeli wine industry in the 19th century was based on international cultivars. Between the years 2011-2014 we conducted a country-wide survey towards the collection and assessment of an Israeli grapevine germplasm collection, including for the first time *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi accessions. The collected population consists of 148 accessions which revealed 61 different genetic profiles when analysed at 9 SSR loci. Ten genotypes consistently matched the profiles of some Israeli and Palestinian accessions reported in previous studies. The phylogenetic analysis showed how the *V. vinifera* subsp. *sylvestris* accessions, all collected in the north of Israel around water sources, are well distinguished from the *V. vinifera* subsp. *sativa* group. Based on ampelographic evaluation we concluded that most *V. vinifera* L. subsp. *sativa* accessions have characteristics of the proles Orientalis.

We believe that by our efforts of collecting and assessing these populations, an array of new-old grapevine varieties suited for quality wine production would be offered, reconnecting the Israeli wine industry to its historical origins.

Key words: Indigenous varieties; *Vitis vinifera* L. subsp. *sylvestris*; microsatellite markers.

Introduction

The Holy Land has a long history of winemaking, widely mentioned in ancient scripts. The high quality of the wines produced was known in the ancient world. The Muslim occupation of this region, starting in the 7th century initiated a long period of wine consumption prohibition, resulting in the eventual deterioration of local grapevine growing practice, and the abandonment of most wine varieties (AMAR 2000). Thus, the renewing of Israeli wine industry in the end of the 19th century was based solely on

international cultivars. Two ampelographic descriptions of local traditional varieties were published in detail (WEITZ 1931; HOCHBERG 1954), followed many years later by KLEIN *et al.* (2008) who genetically analyzed some of those varieties. Between the years 2011-2014 we conducted for the first time a country-wide thorough survey towards the collection and assessment of an Israeli grapevine germplasm collection. The survey includes the collection of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi populations, considered the putative ancestor of the cultivated form, and representing the only endemic taxon of the *Vitaceae* in Europe and the Maghreb (HEYWOOD AND ZOHARY 1995). The survey concentrated in the collection of historically known varieties (~ 35 table grapes varieties), and addition of new varieties by the wide survey done all over Israel. Most importantly, we strive to identify varieties suited for quality wine production in order to reestablish the use of indigenous Israeli grape varieties in the wine industry.

Material and Methods

DNA of 148 accessions was extracted from young leaf tissue collected in the field, and analyzed at 9 SSR loci (VrZAG62, VrZAG79, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32 and VVS2) chosen from the European *Vitis* Database (MAUL *et al.* 2012), as described previously (EMANUELLI *et al.* 2013). Genomic DNA from the common cultivar Pinot noir was included in the analysis procedure in order to normalize SSR allele sizes and compare molecular genotypes among different studies and databases. The genotypic data were used to evaluate indices of intrapopulation genetic diversity through GenAlix 6.5. Moreover, a phylogenetic analysis was conducted using "Darwin 5" software. Ampelographic descriptors were analyzed for the unique profiles using standard descriptors of the OIV (MUNOZ *et al.* 2011).

Results and Discussion

We scanned wide areas in Israel, from the Lebanon border in the north, through the central mountains, sand dunes of the central and southern coasts, down to the Egyptian border in the south Negev desert. The survey was done

Table

SSR allele size at nine microsatellite loci, in 27 representative Israeli grapevine accessions of unique genotypes. Berry shape, color and length descriptors by OIV standard are indicated

Accession name	Berry shape	Berry color	Berry length	VVMD25	VVMD28	VVMD32	VVS2	VVMD5	VVMD7	VVMD27	VRZAG62	VRZAG79
Sorek m.s.	Broad ellipsoid	Blue black	Short	246	250	259	150	232	245	177	191	245
Nitzan 3	Broad ellipsoid	Blue black	Medium	240	250	nd	138	nd	247	173	191	203
Gesher Hardof*	Broad ellipsoid	Blue black	Short	240	240	249	140	226	249	177	193	245
Gesher Kane*	Broad ellipsoid	Blue black	Short	242	246	245	132	nd	249	177	193	245
Gesher Tahton*	Broad ellipsoid	Blue black	Short	240	250	249	132	nd	247	177	193	203
Ilanya Hemdatya	Broad ellipsoid	Blue black	Short	238	270	217	138	226	231	185	209	191
Chanot Orcha	Broad ellipsoid	Blue black	long	240	256	237	140	232	239	183	191	187
Ramtania Mish	Narrow ellipsoid	Blue black	Medium	246	250	259	148	232	243	179	183	199
Ramtania Teena	Broad ellipsoid	Green yellow	Medium	242	246	245	148	226	247	183	191	203
Jandali Gush Etzion	Narrow ellipsoid	Green yellow	long	242	242	259	132	236	240	183	199	247
Asba El Arus	Broad ellipsoid	Green yellow	Medium	250	250	247	150	238	247	179	187	203
Bitumi Turan	Narrow ellipsoid	Blue black	long	246	256	245	132	238	247	183	191	185
Chdari	Narrow ellipsoid	White	Medium	242	246	245	148	226	247	177	191	203
Dumiat	Broad ellipsoid	Green yellow	Medium	242	242	245	128	246	239	177	191	195
Maa' tar	Unknown	Unknown	Unknown	246	256	237	140	226	233	177	191	203
Marawani	Broad ellipsoid	Green yellow	Medium	240	246	237	132	226	239	191	187	199
Baluti	Broad ellipsoid	Blue black	long	242	246	245	140	236	233	177	191	203
Zeytuni	Unknown	Unknown	Unknown	246	256	245	130	238	246	177	191	187
Rumi	Broad ellipsoid	Dark red violet	Medium	246	256	259	150	238	240	179	183	187
Shami	Globose	Red	Medium	240	250	245	140	234	246	177	191	203
Medawar	Obloid	Green yellow	Short	240	246	259	128	226	243	177	191	239
Dabuki Masarik	Narrow ellipsoid	Green yellow	long	242	246	259	132	236	247	177	181	233
Dabuki Arub	Narrow ellipsoid	Green yellow	long	246	250	259	140	226	247	177	183	247
Darwishi	Broad ellipsoid	Green yellow	Medium	240	242	259	128	236	247	177	191	239
Sikusi	Broad ellipsoid	Rose	Medium	240	242	245	132	236	233	181	203	247
Hilwani	Broad ellipsoid	Rose	Medium	240	250	251	144	240	239	191	191	203
Tamar Chulata	Narrow ellipsoid	Green yellow	long	250	256	235	130	224	239	183	185	243
Pinot noir	Globose	Blue black	Short	240	250	219	134	226	239	183	187	239

* *V. vinifera* subsp. *syriensis* accessions.

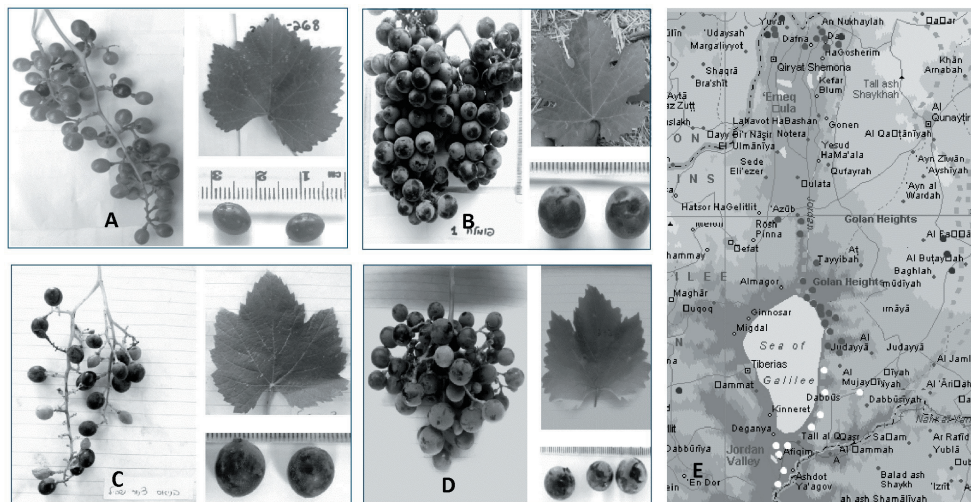


Figure: High polymorphism in bunches and leaves among *V. vinifera* subsp. *sylvestris* accessions in Israel: **A**: acc. no. 268, **B**: acc. no. 1, **C**: acc. no. 275, **D**: acc. no. 273; **E**: Map of nationwide grape sampling in Israel. Black dots: *V. vinifera* subsp. *sativa*. Grey dots: *V. vinifera* subsp. *sylvestris*. White dots: sampled areas without *vinifera* findings.

in specific areas suspected to contain grapevine populations, using information given by local authentic informers such as tour guides, local Jewish, Arab and Druze farmers and a wide literature survey. The population consists of 148 accessions, digitally mapped (Figure) and physically sampled for DNA extraction. The unique population was later planted in a collection vineyard in the city of Ariel, with 6 plants per accession.

Initial genetic analysis of the collection: Of the 148 samples considered so far, 61 different genetic profiles were found by the analysis of 9 SSR loci which were further compared to the SSR profiles reported in the European *Vitis* Database (www.eu-vitis.de) and with SSR profiles reported in literature (Table). Ten genotypes consistently matched the profiles of some Israeli and Palestinian accessions previously described (KLEIN *et al.* 2008, BASHEER-SALIMIA *et al.* 2014). Accessions 'Baluti', 'Asba el Arus', 'Shami', 'Hilwani', 'Dabuki Masarik' correspond to varieties described by KLEIN *et al.* (2008) with the exact names. Accessions 'Sorek m.s.', 'Jandali G. 'Etzion', 'Marawani', 'Dabuki Arub' correspond to the varieties 'Aswad-Baladi', 'Jandali', 'Darawishi' and 'Marawi-Taweel' described by BASHEER-SALIMIA *et al.* (2014) respectively. The genetic diversity analysis revealed levels of expected and observed heterozygosity ($H_E = 0.73$; $H_O = 0.79$) comparable to those observed in previous works (EMANUELLI *et al.* 2013). Furthermore, the phylogenetic analysis showed how the *V. vinifera* subsp. *sylvestris* accessions are well distinguished from the *V. vinifera* subsp. *sativa* group.

Ampelographic analysis: The 61 unique profiles were subjected to ampelographic analysis. Accessions found to have wine-wise interesting characteristics, such as high sugar accumulation, balanced acid, good anthocyanin and polyphenol contents (black) or good aroma profiles (white) were hand-picked in the wild origin for microvinification and further wine evaluation (not shown). Based on ampelographic evaluation (Table 1), we concluded that most *V. vinifera* L. subsp. *sativa* (DC) Hegi accessions have characteristics of the *proles Orientalis* group (LEVADOUX

1956). A second group is consisted of *V. vinifera* L. subsp. *sylvestris* (Gmelin) Hegi accessions, showing classic characteristics such as female flower, highly polymorphic leaf structure, and small bunch with small berry (Figure) and high polyphenols content. All of the *sylvestris* group accessions were collected in the north of Israel around water sources. This is a first report of an ampelographic and molecular analysis of a *sylvestris* population in Israel.

Conclusions

The wilds in Israel contain wide genetically unique populations of both *V. vinifera* subsp. *sativa* and *sylvestris* forms. We believe that by our efforts of collecting and assessing these populations, an array of new-old grapevine varieties suited for quality wine production would be offered to the local wineries, presenting an opportunity of reconnecting the Israeli wine industry to its historical ancient origins.

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