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Description of the *Vitis vinifera* L. phenotypic variability in eno-carpological traits by a Euro-Asiatic collaborative network among ampelographic collections

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Summary

The grapevine intra-specific variability captured an increasing interest during the last decades, as demonstrated by the number of recently funded European projects focused on the grapevine biodiversity preservation. However, nowadays, crop plants are mainly characterized by genotyping methods. The present work summarizes the phenotype data collected among 20 ampelographic collections spread over 15 countries, covering most of the viticultural areas in the Euro-Asiatic region: from Portugal to Armenia and from Cyprus to Luxembourg. Together with agro-climatic characterization of the experimental site, over two years about 2,400 accessions were described. A common experimental protocol mainly focused on the carpological and oenological traits was followed, obtaining a general overview of the distribution of the considered phenotypic traits in the cultivated *Vitis vinifera* species. The most replicated cultivars were selected and, for the subset of these reference cultivars, their behavior in the different environmental conditions over sites and years was described by ANOVA methods.

K e y w o r d s : phenotyping; cultivars; morphology; phenolics; grape quality.

Introduction

Modern viticulture is mainly based on the use of a small number of well-known and widespread international cultivars (Anderson 2013). On the other hand, a large number of old autochthonous cultivars still exists, often represented by few specimens maintained in only one collection. Since the beginning of the new millennium, the

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European community invested major funding aiming at the characterization of these genetic resources and the maintenance of their biodiversity (funded projects: GenRes081, GrapeGen06, GrapeNet). Along these projects, efforts have been devoted to the characterization of the most common morphological, phenological and biochemical descriptors, keeping in mind the importance of genetic variability for (i) quality production improvement (Rustioni *et al.* 2014b), (ii) adaptation to different/changing climatic conditions (Rustioni *et al.* 2014a, Tóth-Lencsés *et al.* 2015), and (iii) resistance towards diseases (Bitsadze *et al.* 2015, Toffolatti *et al.* 2016). In addition, a major result of these researches consisted in the correct cultivar identification of several thousands of accessions with the use of molecular fingerprint (European Vitis database: http://www.eu-vitis. de/index.php; Laucou *et al.* 2011).

Recently, the plant genotyping methods underwent a rapid improvement - the entire grapevine genome was sequenced and released since 2007 (Jaillon *et al.* 2007). These improvements in genotyping methods were much faster than phenotyping technologies. In this context, phenotyping became a bottleneck in plant research (Fiorani and Schurr 2013). Expensive phenotyping platforms have been developed to deeply characterize some specific phenotypic traits, however the high cost of the equipment restricts access to a short list of elite institutes and genetic resources. An additional problem for phenotyping in highly centralized platforms is represented by the need to move plant materials among countries, and the related need to cope with the complexity of quarantine protocols (Faltus *et al.* 2015).

On the other hand, a huge variability exists among the autochthonous *Vitis vinifera* cultivars grown around the world, most of which is still poorly known. East European, Caucasian, and Central Asian countries represent a main source of interesting biodiversity (Failla 2015). In addition, when phenotyping is addressed to monitoring plant response to different environments and growing conditions, a large network of field-based phenotyping activities in various locations focused on the same grape varieties, may allow consistent answers on environment-genotype interaction. Several studies have been done to investigate the impact of the environmental conditions on the genotype expression. For example, recent researches were focused on the plant plasticity in grapevines (Paim Pinto *et al.* 2016, Bianchi *et al.* 2018, Dal Santo *et al.* 2018).

Based on these considerations, the present project aimed at testing the potentiality of a "democratic" approach to phenotyping, based on simple and low-technology methods which could involve a high number of institutions including these of the less technologically developed countries, but rich in biodiversity. A large-base low-cost collaborative phenotyping effort can in particular be useful for pre-screening less known varieties, helping researchers to decide which ones are worth a deeper consideration, either with more specialized phenotyping or directly in breeding programs.

The easy to handle protocols (Rustioni *et al.* 2014a) proposed in the framework of the COST Action FA1003 GrapeNet (East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding) found a broad consensus among researchers. Publications based on these shared protocols are already available concerning grape resources held in local collections such as in: Abashidze *et al.* 2015, Aroutiounian *et al.* 2015, Cornea *et al.* 2015, Goryslavets *et al.* 2015, Maghradze *et al.* 2015a and b, Margaryan *et al*. 2015, Popescu *et al.* 2015, Tóth-Lencsés *et al.* 2015, Ujmajuridze *et al.* 2015. However, a concise overview of all the information collected over the many participating European grape collections is still missing.

This paper aims at presenting all the eno-carpological data collected in the framework of the Euro-Asiatic collaborative phenotyping COST Action FA1003 network in 2012 and 2013. Obtained results will allow a general description of *Vitis vinifera* L. *sativa* sub-species over different phenotypic traits and their variability. Future cultivar descriptions could refer to the information reported here as basis for comparison. Comparisons of these measures across different traits will also allow to highlight correlations among variables, confirming or debunking viticultural beliefs, on the basis of a massive dataset elaboration.

Finally, considering a limited number of "reference" cultivars (*i.e.* the same varieties monitored in different collection sites), the variation of traits across cultivars, years and sites has been studied. In this part of the work we intended to answer the following questions: Could the characterization of one cultivar in only one site be extended to other conditions, and therefore be considered the true behavior profile of the cultivar? Which characteristics can be considered relatively stable over different sites and field conditions, and which are so much dependent on the environment that they can only be assessed using measures repeated over sites and years? Quantifying the variability of several traits will inform users of the necessary steps for cultivar evaluation, phenotyping and pre-breeding.

Material and Methods

Plant material and experimental sites: During 2012 and 2013, accessions grown in 20 ampelographic collections (PRT051, ESP080, ESP217, LUX008, CHE001, ITA360, DEU098, ITA035, HRV041, HUN007, GRC014, ROM045, ROM06, MDA004, CYP001, UKR050, GEO015, ARM011-M, GEO038, ARM011-T) spread among 15 countries in Europe and Asia (details concerning the experimental sites are reported in suppl. Table SI1) were characterized by using the common protocols described in Rustioni *et al.* (2014b). Participants were asked to select healthy plants, and, among them, to exclude vines exposed to abnormal abiotic or biotic stress. These protocols were designed to be low cost and easy to apply, with the main objective to enlarge the participation network to all the interested researchers. The protocols were also organized to concatenate the data acquisition, with the objective to generate new derived variables. The obtained information concerns different aspects of the *Vitis vinifera* L. phenotype with particular attention to the traits of interest for productive purposes. More in details, we collected

data concerning: bunch and berry weight, berry shape and dimension, skin-seed-pulp mass repartition, seed number and weight, sugar and acid contents, anthocyanin and phenolic concentrations and distribution among seeds and skins. The shared protocol is available in suppl. material SI2.

Participants were allowed to restrict the protocols to the traits of major importance for their scopes and, thus, also incomplete and partial descriptions were welcomed.

Data elaboration. Agrometeorological characterization: In order to provide an agrometeorological characterization of the collection sites, daily meteorological data of maximum and minimum temperature and precipitation were obtained from the free network of USA NOAA-GSOD (https://data.noaa.gov/dataset/dataset/global-surface-summary-of-the-day-gsod). The analysis was focused on the current warm phase of European climate that started in 1988 (Mariani *et al.* 2012). Daily data for the collection sites were obtained by spatial interpolation (weighted average with weight inversely proportional to squared distances). In the case of temperature, data were homogenized for elevation adopting a lapse rate of -0.5 °C/100 m. No relation with elevation was considered for rainfall. Minimum distance among sites and NOAA-GSOD network stations are presented in suppl Table SI1. Data of seasons 2012 and 2013 were compared to reference average values calculated over the period 1988-2015. Köppen Geiger climate classification was also adopted in order to differentiate the sites, provided by the WORLD MAPS OF KÖPPEN-GEIGER CLIMATE CLASSIFICATION website (http://koeppen-geiger.vu-wien.ac.at/) and made by Rubel and Kottek (2010).

Six agrometeorological indices were selected: Winkler (Amerine and Winkler 1944); Huglin (Huglin 1986); NHH (Mariani *et al.* 2012); LHH (Mariani *et al.* 2012); HHH (Mariani *et al.* 2012); Water (Cola *et al.* 2014). Further details are reported in suppl. Table SI3. Indices were calculated for each site for seasons 2012, 2013 and for the reference period 1988-2015 in order to evaluate environmental resources and limitations for grape growth.

The anomaly of the season compared to the reference period was analyzed in terms of standard deviation: light anomaly happened when the difference between season and reference period was between 1 and 2 standard deviations (* in the Tables), strong anomaly when the difference was over 2 standard deviations (** in the Tables).

Cultivar characterization and correlation among traits: The entire dataset, collected over more than 2400 accessions in 20 field collections and 15 countries, was used to characterize the *Vitis vinifera* cultivars variability and the correlations among the studied traits. Statistical analyses were obtained by using SPSS statistical software (version PASW Statistics 24, SPSS, Inc. Chicago, IL). The cultivar pool was characterized by frequency histograms and descriptive statistics (minimum; maximum; average value; median; standard deviation; quartiles and percentiles). The trait distributions were described by the Kolmogorov-Smirnov test, kurtosis and skewness. Correlations among traits were evaluated by Pearson coefficients.

Analysis of variance of measures repeated over sites and years, for the subset of reference cultivars: For this analysis, we used 19 cultivars ('Babeasca neagra', 'Cabernet Sauvignon', 'Chardonnay', 'Chasselas blanc', 'Coarna alba', 'Coarna neagra', 'Cramposie', 'Feteasca alba', 'Feteasca neagra', 'Gordin', 'Grasa de cotnari', 'Malvasia', 'Pinot noir', 'Plavaie', 'Riesling weiss', 'Rkatsiteli', 'Tamaioasa romaneasca', 'Tata caprei', 'Zemoasa'), on which traits were measured repeatedly, using the same standard mentioned protocol across sites and years. The list of traits considered for this analysis are: berry color (OIV descriptor); sugar content (Brix); titratable acidity (g·L^{-1} tartaric acid); berry weight (mg); % skin (w/w); number of seeds/berry; weight of 1 seed (mg); anthocyanins (mg·kg^{-1} of grapes); anthocyanins (mg·g^{-1} of skin); skin phenolics (mg·kg^{-1} of grapes); skin phenolics (mg·g^{-1} of skin); seed phenolics (mg·kg^{-1} of grapes); skin phenolics (%); total phenolics (mg·kg^{-1} of grape).

The initial raw dataset was first cleaned of the most obvious mistakes: missing data were declared for the most extreme abnormal outliers, several misnames and typing problems.

The main model of analysis of variance (ANOVA model with GLM in SAS software (2008), SAS Institute Inc. 2008) was built so to study the cultivar, the site and the year effects, and their interactions, on the variability of each trait. Since we had also access to agrometeorological indices, we tried to use them as covariate in this model.

Variables with skewed distribution (mostly, anthocyanins and phenolic measures) were transformed using square root, which in our case performed better than log transformation (not shown).

For biochemical compounds with a known relation with berry color, such as anthocyanins and phenolics, we used for the analysis only the "deep colored" cultivars (including cultivars with red, black, blue black, dark red and violet berry color).

In a first exploratory analysis, we observed that different agrometeorological indices were highly correlated to both site and year, creating a redundancy in the model, in the end the study of cultivar x site and cultivar x year interactions making less reliable. Our approach consisted then in comparing three different models, the first two studying climatic indices effects (model A and B below), and the last one (C) without climatic effects but with categorical site and year information.

We first used the GLMSELECT procedure in SAS to determine which was the climatic index, among the four considered, best correlated to the measured traits:

Model (A): y = site + year + cultivar + Winkler + NHH + Precipitation Index + Water Stress + error term

Once the model above determined the most important climatic effect, we studied its effect on the variance of y, both as single effect and as interaction: Model (B): y = cultivar + selected_climatic_index + cultivar * selected_climatic_index + error. Finally, we used a model with only categorical site and year information, without climatic indices: Model (C): y = site + year + cultivar + site * year + site * cultivar + cultivar * year + error term.

We compared (i) the variance explained by, and (ii) the correlation to the measured trait of the three models to select the most efficient one. The model-adjusted means were finally used to compare performances across cultivars, years and sites.

Results

A g r o m e t e o r o l o g i c a l   c h a r a c t e r i z a t i o n   o f   t h e   a m p e l o g r a p h i c   c o l l e c t i o n s : Tab. 1 shows the main meteorological features of the collections sites, comparing seasons 2012 and 2013 within the reference period 1988-2015. Variations and anomalies from the reference period are also shown. The same approach was used to analyze agrometeorological indices (Tabs 2 and 3).

C u l t i v a r s   c h a r a c t e r i z a t i o n   a n d   c o r - r e l a t i o n s   a m o n g   t r a i t s .   C u l t i v a r s   c h a r - a c t e r i z a t i o n : Considering the entire sample list (about 2,400 observations, depending on the specific trait), the frequency of the considered traits generally followed a Gaussian-like distribution (suggesting a quite homogenous population) (suppl. Figure SI4), despite the one-sample Kolmogorov-Smirnov test highlighted some degree of skewness and kurtosis in all the traits of interest (Tab. 4). Only the seedless grapes were clearly differentiated from the main group (suppl. Figure SI4). Probably, white grapes would also be differentiated for the anthocyanin absence, however, in this work, only pigmented grapes were considered concerning the anthocyanin related traits. Nevertheless, the presence of pink and, in general, low colored cultivars did not produce multimodal distributions (suppl. Figure SI4). In general, the kurtosis indicated a prevalence of platykurtic distributions of the data. Concerning the skewness, only two traits (number of seeds/berry and percentage of seed phenolics) had higher probability density in the left side of the function (Tab. 4). The distribution of the considered traits within the *Vitis vinifera* cultivars are characterized in Tab. 4. Beside the average, median, minimum and maximum values, it is possible to find the percentiles (deciles and quartiles) for each trait.

C o r r e l a t i o n s   a m o n g   t r a i t s : This dataset allowed to highlight a number of correlations among the studied variables (suppl. Table SI5). The bunch weight appeared directly correlated to the berry dimension (considering both weight and volume) and with the berry elongation. Heavier bunches generally had less sugars, lower titratable acidity, and lower accumulation of phenolic compounds, including anthocyanins.

Berry weight increased in parallel with both diameters (width and length), with a major contribution of the length (elongated berries are generally heavier). The increase in berry weight generally results in a decrease in sugar concentration, unaffecting the acidity. Obviously, bigger berries showed a major increase of the pulp weight contribution (variation of the volume/surface ratio), negatively correlating with the skin and seed percentage (w/w), despite the positive correlation with both skin and seed weights.

The incidence of seed weight on berry weight (% seed) was significantly correlated to the total phenolics (mg·kg^{-1} of grapes), however this concentration does not depend on the number of seeds per berry, and it is inversely correlated to the weight of one seed. Furthermore, also the skin weight appeared inversely correlated to the total phenolic content (mg·kg^{-1} of grapes). All those data suggest a ma-

Table 1

Average yearly temperature and precipitation of the collections sites. Seasons 2012 and 2013 are compared with 1988-2015 Normal, percentage variation from normal is presented between brackets. Light anomalies are represented by * while strong anomalies by **

	K-G class	Yearly average minimum temperature			Yearly average maximum temperature			Yearly total rainfall		
		1988-2015 AVG	2012	2013	1988-2015 AVG	2012	2013	1988-2015 AVG	2012	2013
ARM011-M	Dfa	**7.0**	9 (28)	6.8 (-2)	**18.1**	17.6 (-3)	17.2 (-5)	**357**	547 (53)	292 (-18)
ARM011-T	Dfb	**4.4**	4.5 (3)	4 (-8)	**14.5**	15.2 (5)	15.3 (6)	**705**	658 (-7)	669 (-5)
CHE001	Cfb	**6.4**	6.3 (-1)	6.2 (-4)	**15.3**	15.5 (1)	14.6 (-4)	**1009**	1135 (12)	1116 (11)
CYP001	Csa	**11.3**	11.9 (5)	11.7 (4)	**20.8**	21.2 (2)	21.4 (3)	**421**	621 (48)	177 (-58)
DEU098	Cfb	**6.4**	5.2 (-18)	5.4 (-16)	**15.1**	15.3 (2)	14.4 (-5)	**699**	611 (-13)	741 (6)
ESP080	Bsk	**8.3**	8.2 (-1)	8.2 (-1)	**20.8**	20.8 (0)	20.4 (-2)	**424**	286 (-33)	349 (18)
ESP217	Csc	**8.6**	8.8 (3)	8.2 (-4)	**19.9**	20.5 (3)	19.3 (-3)	**447**	334 (-25)	499 (12)
GEO015	Cfb	**7.8**	7.9 (2)	7.6 (-2)	**18.0**	18.6 (3)	18.2 (1)	**783**	954 (22)	620 (-21)
GEO038	Cfa	**7.2**	7 (-2)	6.5 (-10)	**17.5**	18.3 (5)	17.7 (1)	**738**	560 (-24)	404 (-45)
GRC014	Csa	**10.5**	10.6 (1)	10.8 (3)	**20.6**	21.5 (4)	21.8 (6)	**488**	363 (-26)	311 (-36)
HRV041	Cfb	**5.5**	5.3 (-5)	5.6 (2)	**15.8**	16.9 (7)	15.6 (-2)	**849**	660 (-22)	929 (9)
HUN007	Cfb	**6.0**	6 (0)	6.3 (5)	**15.9**	17.1 (7)	15.7 (-1)	**716**	572 (-20)	785 (10)
ITA035	Cfa	**9.5**	9.7 (2)	9.5 (0)	**18.1**	18.4 (2)	17.7 (-2)	**1012**	856 (-15)	974 (-4)
ITA360	Cfa	**10.9**	11.2 (4)	10.9 (0)	**18.8**	19.3 (3)	18.6 (-1)	**849**	687 (-19)	881 (-2)
LUX008	Cfb	**6.6**	6.3 (-4)	6.2 (-6)	**14.6**	14.7 (0)	13.8 (-6)	**783**	861 (10)	674 (-14)
MDA004	Cfa	**5.7**	6 (4)	6.5 (13)	**14.8**	16.1 (8)	15.3 (3)	**594**	612 (3)	622 (5)
PRT051	Csa	**13.0**	12 (-8)	12.4 (-5)	**20.9**	21 (0)	21.1 (1)	**759**	921 (21)	985 (30)
ROM045	Cfa	**6.8**	7.2 (7)	7.6 (12)	**16.9**	18.2 (8)	17.7 (5)	**509**	451 (-11)	570 (12)
ROM06	Cfa	**5.9**	5.6 (-4)	6.2 (6)	**17.2**	18.2 (6)	17.8 (4)	**575**	692 (20)	584 (2)
UKR050	Cfa	**7.9**	8 (2)	9.1 (15)	**17.0**	18.3 (8)	17.8 (3)	**740**	629 (-15)	829 (12)

T a b l e   2

Agrometeorological indices for the collection sites. Thermal resources (Winkler, Huglin and NHH). Light anomalies are represented by * while strong anomalies by **

Sites	WINK			HUGH			NHH		
	1988-2015 AVG	2012	2013	1988-2015 AVG	2012	2013	1988-2015 AVG	2012	2013
ARM011-M	2111	2499 *	2041	2604	2836	2574	2245	2839 *	2373
ARM011-T	1220	1423 *	1121	1642	1931 *	1624	1526	1733	1444
CHE001	1285	1347	1296	1721	1766	1669	1572	1669	1532
CYP001	2026	2215 *	2096	2267	2426 *	2390*	2982	3224 *	3166
DEU098	1257	1172	1169	1711	1681	1605	1519	1466	1340 *
ESP080	1996	2072	2036	2580	2638	2563	2164	2206	2073
ESP217	1841	1968	1716	2367	2498	2186*	2144	2261	2010
GEO015	1830	2116 *	1717	2294	2623 *	2274	2181	2479 **	2175
GEO038	1964	2239 *	1865	2425	2706 *	2391	2272	2607 **	2335
GRC014	2363	2648 **	2495	2794	3062 *	2982 *	2698	2778	2847 *
HRV041	1426	1576 *	1457	1958	2210 *	1977	1729	1793	1624 *
HUN007	1462	1629 *	1505	1972	2225 *	1986	1772	1861	1689
ITA035	1919	2021	1933	2308	2395	2289	2390	2527 *	2304
ITA360	2095	2204	2127	2428	2517	2431	2645	2811 *	2586
LUX008	1190	1157	1186	1602	1556	1523	1426	1414	1376
MDA004	1512	1985 **	1597	1985	2521 **	2078	1843	2204 **	1991
PRT051	2137	2090	2191	2375	2390	2487	3072	2858	2759 *
ROM045	1762	2202 **	1917 *	2275	2725 **	2459 *	2087	2364 **	2267*
ROM06	1740	2109 **	1819	2327	2720 **	2464	2020	2229 **	2117
UKR050	1847	2344 **	1953	2298	2800 **	2418	1881	2262 **	1987*

T a b l e   3

Agrometeorological indices for the collection sites. Thermal stress (LHH and HHH) and water stress. Light anomalies are represented by * while strong anomalies by **

Sites	LHH			HHH			Water stress index		
	1988-2015 AVG	2012	2013	1988-2015 AVG	2012	2013	1988-2015 AVG	2012	2013
ARM011-M	1835	1644	1741	255	317	270	186	126	163
ARM011-T	1928	2143 *	2075 *	31	49	3 *	58	45	46
CHE001	2143	2205	2061	36	39	47	3	0	0
CYP001	3121	2994 *	3243 *	23	63 **	27	148	128	177 *
DEU098	2108	1989	1989	46	37	60	28	28	23
ESP080	2244	2193	2012*	233	273 *	244	133	164	146
ESP217	2435	2373	2344	174	228 *	174	124	181 *	111
GEO015	1983	2013	2031	142	179	97*	72	115	164*
GEO038	1966	2096*	2091*	178	190	116	62	32	79
GRC014	2186	2074	2232	278	360 *	325 *	103	131	164 *
HRV041	2031	1934	1905	82	150 *	90	10	38 **	20
HUN007	2089	1983	2006	81	144 *	88	29	64 *	30
ITA035	2318	2336	2341	111	146	134	40	63	67 *
ITA360	2407	2438	2378	135	184	175	40	68 *	38
LUX008	2147	2179	2032	33	26	48	14	0	21
MDA004	1961	1944	2061	78	185 **	66	43	85 *	7
PRT051	3717	3353 *	3387 *	93	131	189 **	94	73	85
ROM045	2029	1952	2040	139	260 **	154	100	132	61
ROM06	1932	1942	1896	158	237 *	168	92	87	56
UKR050	1895	1919	1924	144	233 *	172	43	80*	21

jor role of the pulp weight proportion with respect to the phenolic rich seeds and skins tissues in the determination of the total phenolic content available during winemaking (mg·kg⁻¹ of grapes). As a consequence, bigger berries have lower phenolic and anthocyanin concentrations (mg·kg⁻¹ of grape), despite the higher synthesis in the berry (mg·berry⁻¹). A parallel trend is observable concerning anthocyanins (mg·kg⁻¹ of grapes) with a significant negative corre-

T a b l e 4

Statistical characterization of the distribution of the studied traits

Variable	Sample number	Average	Median	Skewness	Kurtosis	Minimum	Maximum	10	20	25	30	40	50	60	70	75	80	90	Kolmogorov–Smirnov statistic	Asymptotic significance (two tails)
Berry length (mm)	22383	15.02	15.00	1	3	5	37	11	12	13	13	14	15	15	16	17	17	19	0.117	0.000
Berry width (mm)	22385	14.18	14.00	0.5	0.6	6	29	11	12	12	13	13	14	15	15	16	16	18	0.102	0.000
Length/width	22383	1.062	1.000	2.3	18.9	0.5	3.6	0.9	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.1	1.1	1.2	0.225	0.000
Bunch weight (g)	5737	247.76	220.00	1.7	5.5	10	1362	95	128	143	157	190	220	252	294	319	351	434	0.092	0.000
Sugar content (Brix)	2162	20.8	21.0	0.2	0.5	10.0	35.0	17.0	18.0	19.0	19.0	20.0	21.0	21.0	22.0	23.0	23.0	24.0	0.071	0.000
Titratable acidity (g·L⁻¹ tartaric acid)	2161	6.3	6.0	1.5	5.6	0.8	22.7	3.3	4.3	4.7	5.0	5.5	6.0	6.6	7.1	7.4	7.8	9.1	0.085	0.000
Berry weight (g)	2404	2.4	2.2	2.0	6.9	0.6	10.1	1.3	1.5	1.6	1.7	1.9	2.2	2.4	2.7	2.8	3.0	3.5	0.111	0.000
% Skin (w/w)	2368	17.0	15.0	1.1	1.2	3.0	54.0	8.0	10.0	11.0	12.0	14.0	15.0	17.0	20.0	21.0	23.0	29.0	0.114	0.000
% Seed (w/w)	2355	4.0	4.0	1.2	4.8	0.0	17.0	2.0	3.0	3.0	3.0	3.0	4.0	4.0	5.0	5.0	5.0	6.0	0.159	0.000
1 Skin weight (g)	2369	0.4	0.3	1.6	4.4	0.1	1.9	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.5	0.7	0.188	0.000
Number of seeds·berry⁻¹	2321	2.1	2.1	-0.2	0.7	0.0	4.3	1.4	1.6	1.7	1.8	2.0	2.1	2.2	2.4	2.5	2.6	2.9	0.043	0.000
Weight of 1 seed (mg)	2293	41.0	40.0	1.5	7.6	10.0	160.0	30.0	30.0	30.0	30.0	40.0	40.0	40.0	50.0	50.0	50.0	60.0	0.215	0.000
Anthocyanins (mg·kg⁻¹ of grapes)	1141	710.1	550.0	1.8	5.0	50.0	5350.0	100	150	200	300	400	550	650	850	1000	1100	1600	0.162	0.000
Anthocyanins (mg·berry⁻¹)	1138	1.4	1.0	1.8	3.9	0.1	8.5	0.2	0.4	0.5	0.6	0.8	1.0	1.3	1.7	1.9	2.1	3.0	0.153	0.000
Anthocyanins (mg·g⁻¹ of skin)	1138	4.7	3.2	2.9	13.0	0.1	45.0	0.7	1.2	1.5	1.8	2.4	3.2	4.2	5.4	6.1	7.1	10.3	0.184	0.000
Skin phenolic (mg·kg⁻¹ of grapes)	1739	1375.8	1090.0	1.6	3.2	90.0	6590.0	450	600	680	750	910	1090	1320	1620	1800	2020	2720	0.132	0.000
Skin phenolic (mg·berry⁻¹)	1739	2.8	2.4	1.5	3.3	0.2	12.0	1.1	1.4	1.6	1.7	2.0	2.4	2.8	3.3	3.6	3.9	5.1	0.113	0.000
Skin phenolic (mg·g⁻¹ of skin)	1735	9.1	7.3	2.4	9.6	0.3	61.4	2.5	3.9	4.5	5.0	6.1	7.3	8.8	10.6	11.9	13.2	17.5	0.130	0.000
Seed phenolic (mg·kg⁻¹ of grapes)	1724	337.0	210.0	3.9	26.7	10.0	4180.0	50	90	100	120	160	210	280	370	430	510	785	0.205	0.000
seed phenolic (mg·berry⁻¹)	1692	0.7	0.5	2.1	7.2	0.1	5.4	0.1	0.2	0.2	0.3	0.4	0.5	0.6	0.8	0.9	1.0	1.5	0.176	0.000
Seed phenolic (mg·g⁻¹ of seed)	1704	8.7	6.0	2.8	12.9	1.0	98.0	1.0	2.0	3.0	3.0	4.0	6.0	7.0	9.0	11.0	13.0	20.0	0.203	0.000
Seed phenolic (μg·seed⁻¹)	1723	338.4	220.0	3.5	27.5	10.0	5390	54	90	110	120	170	220	290	380	440	510	796	0.184	0.000
Skin phenolics (%)	1734	79.9	84.0	-0.9	0.1	22.0	100.0	57.0	66.0	70.0	74.0	79.0	84.0	88.0	91.0	92.0	93.0	96.0	0.124	0.000
Seed phenolics (%)	1734	20.1	16.0	0.9	0.1	0.0	78.0	4.0	7.0	8.0	9.0	12.0	16.0	21.0	26.0	30.0	34.0	43.0	0.124	0.000
Total phenolics (mg·kg⁻¹ of grape)	1735	1708.7	1450.0	1.9	6.1	100.0	9550.0	600	800	900	1000	1150	1450	1700	2000	2200	2400	3170	0.123	0.000
Total phenolics (mg·berry⁻¹)	1737	3.4	3.0	1.4	2.6	0.3	12.3	1.5	1.9	2.1	2.3	2.7	3.0	3.5	4.0	4.3	4.7	5.9	0.099	0.000

lation with berry weight. Anthocyanin and phenolic (the ones arising from skins) contents strongly correlate among them, and with the sugar content. The correlation between berry weight and the number of seeds was very weak, while a strong correlation was observed with seed weight and seed percentage (w/w). Both the number and weight of seeds did not correlate with sugar concentration. Finally, our data indicate a total independency of sugar content and acidity.

Analysis of the phenotyping components of reference cultivars: Studying the subset of 19 reference cultivars repeatedly measured over sites and years, the model (A) revealed that climatic indices were high and significantly related to most of the measured traits (suppl. Table SI6). The most important climatic indices were Water Stress, Winkler and NHH. The Precipitation Index was never selected by the GLMSELECT model as the most important one. In supplementary material (suppl. Figure SI7), it is possible to find the simple linear regression of the phenotypic traits with the best climatic index as selected by the GLMSELECT procedure, for all cultivars or for only colored cultivars, respectively. The correlation of the models based on climatic indices (A and B) with the measured traits were generally quite high. The model using all climatic indices together (A) was always better than the model using only the best climatic index (B).

However, the model with site and year information without climatic indices (C) was also always better than model (A). The percentage of variance explained by the model was large and highly significant (suppl. Table SI8; the F-value is the ratio between the variance explained by the model and the unexplained variance, or error). The correlation of the model to the measured trait was between $r^2 = 0.69$ and 0.94, which can be considered also as very high. For some traits, like titratable acidity (g·L^{-1} tartaric acid), berry weight (mg) and total phenolics (mg·kg^{-1} of grape), the model performed better than for other traits such as number of seeds/berry and weight of 1 seed (mg).

Within the chosen model (C), the partition of variance showed that the cultivar effect (measured by the F-value, SI8) was always highly significant, and in most cases, it was also the most important effect among all, as for example for sugar content and berry weight (but not concerning titratable acidity, in which the most important effect was the site). However, the interaction terms of the model (site * cultivar, cultivar * year, and site * year) were also highly significant, except in one case (for weight of one seed (mg), the site x year interaction was not significant).

Given the highly significant cultivar effect, the boxplots of the mean and variance of each cultivar, for each trait, are available in suppl. Figure SI9. An example of interaction between cultivar and site effects is graphically represented in suppl. Figure. SI10. The cultivar 'Grasa de Cotnari' produced low sugar contents in the MDA004 collection and comparatively high contents in the ROM06 collection, while an opposite trend was observed for 'Cabernet Sauvignon'. Inverted performance ranking was also evident between 'Cabernet Sauvignon' and 'Chardonnay' across sites, or for several cultivars across years even if to a lower extent in the latter case.

Discussion

Agrometeorological characterization of the ampelographic collections: The distribution of collection sites provides a good coverage of the different European environments suitable for grapevine cultivation as confirmed by the Köppen-Geiger climate types (Bsk, Cfa, Cfb, Csa, Csc, Dfa and Dfb) represented and also by the range Winkler classes (from 1 to 5). Generally, the analysis of the seasons 2012 and 2013 for the collection sites highlight very few anomalies for temperature and precipitation.

In 2012, ARM011-M was characterized by a light positive anomaly in yearly minimum temperature, while negative anomalies characterized DEU098 (light) and PRT051 (strong). With reference to maximum yearly temperature, light positive anomalies were detected for GRC014, HRV041, HUN007, MDA004, ROM045, ROM06 and UKR050. The only precipitation anomaly was found in HRV041 (light negative).

In 2013 light positive anomalies characterized the yearly minimum temperatures of DEU098, MDA004, PRT051 and ROM045 while a light negative anomaly was found in GEO015. Only the two sites of CYP001 and GRC014 showed a positive light anomaly in maximum yearly temperatures, while negative light anomalies were detected in CHE001, LUX008. A light negative anomaly of yearly precipitation characterized 2013 in CYP001, while all the other sites had normal levels.

Focusing on resources and limitations for grapevine development, in 2012 the picture given by Winkler and Huglin indices is similar. Positive anomalies of the Winkler index were found in ARM011-M, ARM011-T, CYP001, GEO015, GEO038, GRC014, HRV041, HUN007, MDA004, ROM045, ROM06 and UKR050. The same sites showed positive anomalies of the Huglin index, with the only exception of ARM011-M. The analysis of Normal Heat Hours shows a partially different picture of positive anomalies (ARM011-M, CYP001, GEO015, GEO038, ITA035, ITA360, MDA004, ROM045, ROM06 and UKR050). This difference is due to the different approach to thermal resources given by NHH on one side and Winkler and Huglin Indices on the other. In fact, while Winkler and Huglin indices increase as temperature raises, NHH are characterized by an upper limitation in order to take into account stress given by high temperature (HHH). Regarding thermal stress, positive anomalies of high temperature stress were found in CYP001, ESP080, ESP217, GRC014, HRV041, HUN007, MDA004, ROM045, ROM06 and UKR050. ARM011-T and GEO038 showed light positive anomalies in low temperature stress while negative anomalies were found for CYP001 and PRT051. The high level of high temperature stress is linked to the positive anomalies in water stress, detected for ESP217, HRV041, HUN007, ITA360, MDA004 and UKR050.

In 2013, the anomalies of thermal resources were very limited with ROM045 showing positive anomaly for both Winkler and Huglin indices and CYP001, ESP217 and GRC014 only for Huglin. Positive anomalies of NHH were found for GRC014 and ROM045 and negative anomalies for DEU098, HRV041 and PRT051. Regarding

low temperature stress, positive anomalies characterized ARM011-T, CYP001 and GEO038. ESP080 and PRT051 showed negative anomalies. Considering high temperature stress positive anomalies were found only in GRC014 and PRT051, while negative ones in ARM011-T and GEO015. Positive water stress anomalies were finally detected in CYP001, GEO015, GRC014 and ITA035.

Vitis vinifera cultivar characterization and correlations among traits. Cultivar characterization: At the whole subspecies level, a multimodal distribution was expected, at least concerning the berry length/width ratio, representing the three eco-geographical variety groups (proles occidentalis, pontica and orientalis) proposed by Negrul (1946) as main subspecific taxa of domesticated *Vitis vinifera*. Nevertheless, our data suggest the presence of a main population characterized by round berries, with few outlier cultivars characterized by extremely elongated fruits (the 90 percentiles of the length/width ratio presents a rise in this ratio of only 1.2, while the maximum record was 3.6). Negrul (1946) also observed an increase in the bunch weight moving towards the East. In our dataset, considering the bunch weight, it is possible to observe shoulders in the right side of the histogram, however, the availability of cultivars with higher bunch dimension could also be due to human selections of productive grapes, not necessarily related to the original eco-geographic distribution.

Mattivi *et al.* (2006) proposed the use of anthocyanin profiles for chemotaxonomy and, considering pink grapes, the physiological dysfunctions of the pigment biosynthetic pathway are well demonstrated (Rustioni *et al.* 2016; Ferrera *et al.* 2017). Nevertheless, the anthocyanin content distribution did not clearly differentiate specific sub-populations (e.g.: pink, red and black berried grapes).

In general, we suppose that, comparing only homogeneous and defined groups of individuals, differences could appear clearly, and could be used for cultivar classification. Nevertheless, when looking at the whole subspecies variability, the distribution results are continuous, highlighting the presence of intermediate individuals among possible groups. This evidence is in accordance with the uniformity of the subspecies here described and underline the importance of using quantitative and continuous scales for phenotypic trait characterizations.

Vitis vinifera species are divided in two subspecies: sativa and sylvestris. It is worth to notice that the present work is focused on cultivar accessions, belonging to the sativa subspecies. *Vitis vinifera sylvestris* have been recently described in a multifaceted research (Ocete *et al.* 2011). Authors observed an interesting distribution of the number of seeds per berry: *sylvestris* subspecies showed lower number of seeds when compared to grapevine cultivars (*sativa* subspecies). This is probably due to the dioecious character of Vitis vinifera sylvestris and the consequent lower rate of pollination in the wild condition in respect to the *sativa* subspecies, that is generally hermaphrodite and self-fertile.

Correlations among traits: The correlations found among bunch and berry weight, diameters and sugars are in agreement with the expected major contribution of table grapes among cultivars with bigger bunches. Quantitative characteristics of table grapes measured in Australia are available in Wei *et al.* (2002) and the average values, compared to our dataset, are: berry weight (g) 3.3 (80-90 percentiles); berry width (mm) 16.5 (75-90 percentiles); berry length (mm) 19.8 (over the 90 percentiles); acidity (g·L^{-1}) 4.4 (20-25 percentiles). Only sugar concentration (Brix) 22.9 (70-75 percentiles) appears higher than expected, probably due to the Australian site specific climatic conditions.

Concerning phenolics, our data highlighted a major role of the dilution effect of the pulp with respect to the solely accumulation in synthetizing tissues. As observed by Roby *et al.* (2004), the degree of skin solute dilution upon crushing during winemaking, may not be a simple function of berry volume, however at subspecies level a major contribution of the berry geometry is undeniable. Similar data are available in literature: Ojeda *et al.* (2002) described a grape phenolic accumulation as an indirect and positive response to water deprivation due to berry size reduction. According to Ojeda *et al.* (2002), it is worth to notice a significant and positive direct effect of berry size on physiological phenolic accumulation in berries (mg·berry^{-1}).

The strong correlation among anthocyanins and skin phenolics was expected, due to the common biosynthetic pathway (Boss *et al.* 1996). Moreover, anthocyanins are a part of total phenolics. However, anthocyanins are accumulated only after veraison; thus, this correlation suggests the presence of common regulatory factors which promote the cultivar phenolic biosynthesis during all the berry development, resulting in a general increase in phenolic molecules (pigmented and non-pigmented) during both pre- and post-veraison. In general, skin phenolics are positively correlated with the grape sugar concentration, which represents so the carbon and energy source as a regulatory signal for their synthesis (Vitrac *et al.* 2000).

Within the same cultivar, big berries are described to contain a higher number of seeds, due to the effect of seeds on growth regulator supply (Ollat *et al.* 2002, Roby *et al.* 2004, Walker *et al.* 2005). Nevertheless, this correlation is very weak considering different genotypes. Roby *et al.* (2004) suggested a major role of total seed mass, which is dependent on seed number and weight, and our data point out a strong correlation among berry weight and seed weight, as well as with seed percentage (w/w). The not-significant correlations between sugar accumulation and seed number and weight suggest that seeds do not significantly affect the sink-source balance.

Finally, a strong correlation between sugar content and acidity was expected, due to the well-known ripening trends of sugar accumulation accompanied by acid dilution/degradation (Hardy 1968). Nevertheless, our data indicate a total independency among these variables in a multi-cultivar frame. This means that, the variability among cultivars (at a given sugar concentration, some of them has low and others high acidity) is largely higher than the variability in the ripening status at the sampling time. Thus, despite the climatic differences among the ampelographic collections involved in the data records, the grape ripening status did not strongly affect the cultivar evaluation.

Analysis of the phenotyping components of reference cultivars: This part of our study showed that climatic effects were well correlated with cultivar performance for most of the traits. This is a first indication that we measured with efficiency. Information about the interactions between grape performances and climate is also an important element for helping viticulture to adapt to climate change. However, the model with site and year categorical information performed better than the model with climatic indices. Probably this is because the site and year model (C), in addition to the local climate, also accounts for other local conditions such as soil, training method, micro-organisms, etc.

In spite of preliminary data filtering, occasionally some cultivars displayed a variance significantly larger than others (for example, the cultivar 'Grasa de Cotnari' for the sugar content and berry weight traits, suppl. Figure SI9). Part of this difference can be intrinsic to the plasticity of a cultivar, but we can not exclude that data filtering (for outliers, anomalies, eventual accession misnaming and clonal variability) left behind some imprecision or errors, either of the field measure or due to data manipulation. In cases like this, we recommend adding one or more years of measurement. All effects of the statistical model (C) were highly significant, and the cultivar effect was often the most significant one. On the one hand, this result confirmed that measures were globally well done and comparable across the whole experiment; on the other hand, it showed that the genetic contribution to the standing phenotype is often important in grape, confirming previous findings (LIU *et al.* 2007, LIANG *et al.* 2009, CORREA *et al.* 2014).

However, interaction effects were also highly significant, in particular the cultivar x site, the cultivar x year and the site x year effects. This means that in order to describe or characterize one old autochthonous – unknown – variety, the phenotyping in only one site is a good initiative, but it must be completed by phenotyping in other sites and in more than one year.

Finally, the outcome of this work highlighted the usefulness of using a standard protocol across a network of field stations, allowing to repeat measures over years and sites, and a joint analysis with a coherently built statistical model. This finding may support the organization of a permanent network of field collections that could work together over many years for pertinent cultivar characterization and promotion towards breeders and viticulturists.

Conclusions

In conclusion, this work addressed the study of the main phenotypic traits of grapevines grown in ampelographic collections spread among different countries, characterized by different pedo-climatic conditions. The participation of a large network of researchers allowed the description of the phenotypic variability among the *Vitis vinifera* cultivars grown in the Euro-Asiatic context. Furthermore, we demonstrated that analyzing multi-site field data with a sound statistical model provides useful information about reliability of the measures, confidence inter-

vals and variability. This initiative may be used, on larger grape panels as an efficient and useful tool to add value to local grape collections and to promote conservation initiatives.

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