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First results of the European grapevine collections' collaborative network: validation of a standard eno-carpological phenotyping method

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Summary

A standard protocol for phenotyping structural and compositional aspects of the grape berry has been adopted by 18 East and West European germplasm collections during one season by testing a total of 469 accessions, including reference cultivars as well as local and minor germplasm accessions of specific interest. The protocol consists in the collection of triplicates for 26 phenotypic traits, from biological samples, each formed by 10 berries collected from 9 representative bunches from every analyzed accessions. The protocol concatenates the data from measurements and acquisitions, with the objective to generate new derived variables, which are expressed with different units (%; content per kg of grapes, per berry, per g of tissue). For each variable, the Least Significant Differences (LSD), to contrast a pair of single accession mean values, and the Confidence Intervals (CI), to estimate each single accession mean value, were computed. The application of the protocol revealed satisfactory results with high accuracy and efficiency in estimation of phenotypic traits of each accession. The whole data set will be useful for researchers, breeders and viticulturists in yield evaluation of grapevine cultivars, as well as in comparative analyses of environment-variety interaction.

K e y w o r d s : fruit quality, germplasm, phenotyping, polyphenols, *Vitis vinifera.*

Introduction

The COST Action FA1003 – Grapenet: East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding – aims to improve knowledge of grapevine genetic diversity, which is considered essential for the long-term conservation and sustainable use of this important crop species. The Action intends to strengthen scientific competence across Europe, through the organization of voluntary networks, bridging the gap that exists between the East and West European scientific

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communities working on grapevine genetics, breeding and preservation. It intends at completing research knowledge on grapevine genetic resources including minor cultivars and less studied accessions from Eastern Europe (Maghradze *et al.* 2012), and at the same time empower the scientists from East European countries to participate, develop and share the results of innovative approaches of modern genetics. Among a number of Action's specific objectives, the development of phenotyping methodologies to be applied in the germplasm collections has been considered of top importance (Rustioni *et al.* 2013).

Phenotyping can be defined as precise and comprehensive analysis of phenotypic traits, in which single components of the phenotype are observed and described. Phenotyping is required for a range of research applications, including genetic associations studies and cultivar evaluation. *Via* phenotyping, individual genotypes at the same developmental stage are compared, in uniform growing and physiological conditions, during several years and under different environments. In this perspective the adoption of standard methods for sampling, measurements, and analysis procedures, is crucial (Volk 2010).

A complete analysis of grapevine genetic resources from each country was a good opportunity to improve the knowledge of the grapevine genetic diversity, to share information and make an efficient network between the West and East European scientific communities working on grapevine genetics.

Correct identification, characterization, and evaluation are essential elements for establishing a core collection and imposes to adopt a unique methodology applied at the European level.

The development and the validation of phenotyping protocols standardized at the pan-European level have several advantages: i) a comparative evaluation of the phenotype expressions across germplasm collections from different countries; ii) the exploration of genetic diversity across a large panel of grapevine genotypes, independently of quarantine limitations; iii) the constitution of shared core-collections on which many researchers can work collectively. At the end, the common phenotyping protocol opens the road to modern tools as genetic association studies and marker assisted selection, of great help for breeders.

According to these premises, in the framework of the "Grapenet" collaborative network, it was decided to focus the attention on phenotyping for the structural and compositional aspects of the grape berry, as a key factor of the fruit and wine quality. This project involves a large number of institutions, and the established protocol had to be defined using easy, fast and low-cost methods. Easy, to be adopted without specific laboratory equipment and highly trained operators; fast, to allow to record the highest possible number of accessions at the proper phenological phase, *i.e.* during the ripening season; low-cost, at least in term of consumables and equipment needs, to consent to the highest number of partners to join to this action task.

Central aspects of the work and prerequisites for participation in phenotyping were uploading of i) passport data, together with the internationally recognized variety number (VIVC number; www.vivc.de) and ii) SSR fingerprinting profiles in the European *Vitis* Database (www.eu-vitis.de; Maul *et al.* 2012). These aspects are indispensable for both trueness to type assessment and retracement which accessions were evaluated, thus ensuring reliable phenotype data.

With this background, a protocol has been proposed to be voluntarily applied by researchers involved in the COST Action FA1003 network across Europe. Phenotyping analysis concerned grape quality description, in relation to qualitative expectations for both wine and table grapes production. They involved bunch and berry morphology as well as grape bio-chemical composition. A key aspect of the protocol consists in the analysis of 26 phenotypic traits, rigorously in the same biological samples. In this way the study of the possible morphological and physiological correlations among the expression of different traits (e.g. sugar levels *vs.* berry size or skin width *vs.* anthocyanins accumulation per berry) can be examined on a consistent sampling procedure. Another distinctive aspect of this protocol for the characterization of the grapevines genetic resources, in respect to other protocols adopted so far, which mainly refer to the OIV (2009), is the adoption of only quantitative and continuous variables, excluding any qualitative descriptors. In this way all the phenotyping records can be elaborated by parametric statistical procedures, as requested by common procedures for genotype x environment interaction modeling and association genetic studies.

The protocol was launched in June 2012 and tested by 18 project partners. The main concern for the application of the protocol is its ability to estimate the phenotypic values of the single accessions, in relation to the different traits, as well as its ability to put in evidence significant differences among the accessions, in relation to the single phenotypic traits. Both aspects depend on the intrinsic variability of the studied trait, on the accuracy of the sampling procedures and on the number of analyzed biological replications. In this paper, this first evaluation of the protocol performance is presented and discussed.

Material and Methods

The 18 East and West European collections which collaborated to the project, collecting data during the 2012 harvest season, are reported in Tab. 1 and localized in the map of the Figure.

During the 2012 season, 469 accessions were studied, including both references cultivars as well as local and minor germplasm accessions of specific interest. Two levels of control were introduced: reference cultivars and clonal intra-site variation. To allow the comparison among collections and the estimation of environmental variance effects on morphological features of grapevine genotypes, widespread reference cultivars present in several collections were chosen to be considered as replicates. Thus, 36 varieties were replicated and phenotyped in at least two germplasm collections: 'Cabernet Sauvignon' (8 replications); 'Chasselas' and 'Pinot Noir' (6 replications); 'Chardonnay' (5 replications), 'Riesling Weiss' (4 replications); 'Rkat-

Table 1

List of institutions participating to the phenotyping network

FAO Institute code	
ARM011	Academy of Viticulture and Wine-making, Armenia
CHE001	Agroscope, Protection des végétaux grandes cultures & vigne / Viticulture & œnologie, Nyon, Switzerland
CYP001	Agricultural Research Institute, Ministry of Agriculture Natural Resources and Environment, Nicosia, Cyprus
DEU098	Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany
ESP080	Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Alcalá de Henares, Madrid, Spain
ESP217	Instituto de Ciencias de la Vid y del Vino (ICVV), Logroño, Spain
GEO038	AGRO - The National Center for Grapevine and Fruit Tree Planting Material Propagation. Village Jighaura, Mtskheta, Georgia
GEO015	Institute of Horticulture, Viticulture and Oenology. Agrarian University of Georgia. Tbilisi, Georgia
GRC014	Aristotle University of Thessaloniki, Faculty of Agriculture 54124 Thessaloniki, Greece
HRV041	University of Zagreb, Faculty of Agriculture, Zagreb, Croatia
HUN007	University of Pannonia, Georgikon Faculty, Department of Horticulture, Keszthely, Hungary
ITA035	Università degli studi di Milano, Dipartimento di Scienze Agrarie ed Ambientali, Milano, Italy
ITA360	Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Grugliasco, Torino, Italy
MDA004	Research and Practical Institute for Horticulture and Food Technologies, Chisinau, Republic of Moldova
PRT051	Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Dois Portos, Portugal
ROM045	Research and Development Station for Viticulture and Oenology, Dragasani-Valcea, Romania
ROM06	University of Agronomic Sciences and Veterinary Medicine, Faculty of Horticulture, Bucharest, Romania
UKR050	National Institute of Vine&Wine "Magarach", Yalta , Crimea, Ukraine

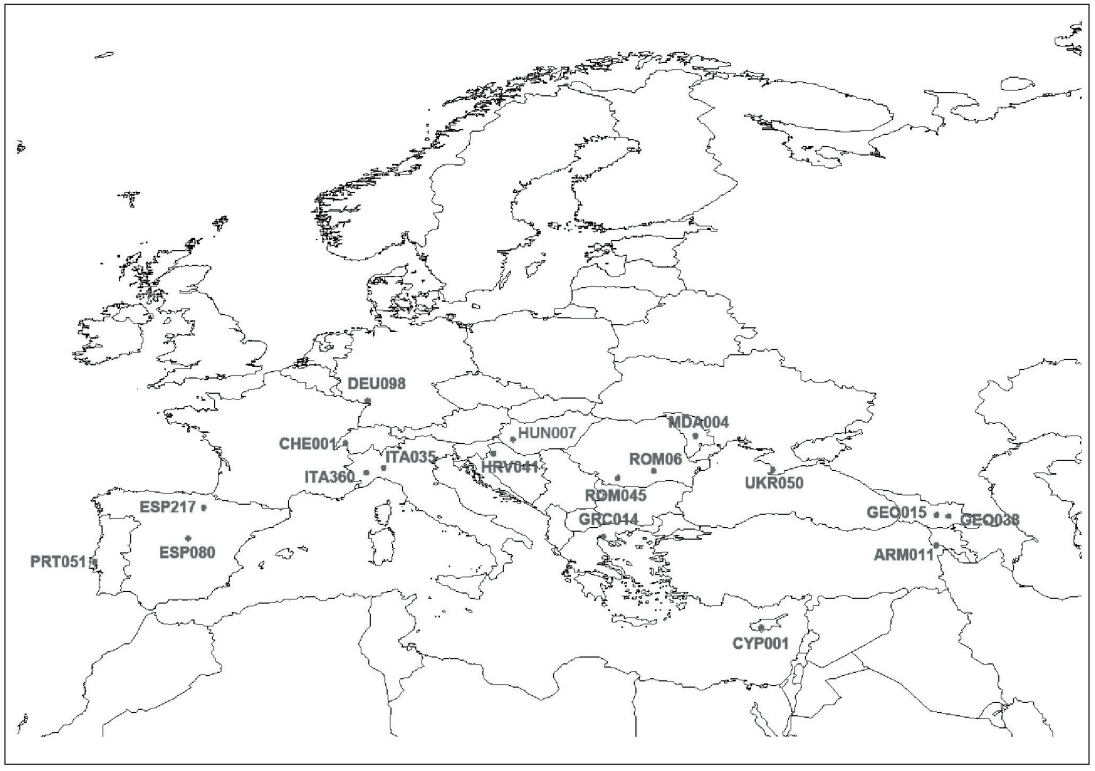

Figure: Map of the localization of sites participating to the phenotyping network.

siteli', 'Saperavi', 'Sultanina' and 'Feteasca Alba' (3 replications); 27 other cultivars were duplicated. On the other hand, to allow the estimation of intra-site variation, several clones were phenotyped within a site. For example, partner ITA035, included in its experimental plan twenty-one clonal lines of 'Moscato di Scanzo'.

For each studied accession, 3 replications (kept separated along all the analysis) of 3 bunches each were collected. After determining the bunch weight, 10 berries were selected from each replication to be weighed (berry weight) and measured by a caliper (berry length and width). The seeds were then separated, weighed, counted, and total phenols extracted in 20 mL of an ethanol:water:hydrochloric acid (70:29:1) solution overnight. Also the berry skins were separated, weighed and extracted following the same procedure. After filtration, total phenolics in the extracts were analyzed according to a modified protocol from Di Stefano *et al.* (1989): 2.5 mL of water were put in a 10 mL

flask and added of 0.5 mL of diluted extract and 0.5 mL of Folin Ciocalteu reagent. After 3-5 minutes 2 mL of 10 % Na_2CO_3 were added and the flask was filled up to 10 mL with water. After 90 min the absorbance at 700 nm was read at a spectrophotometer (compared with a blank made in the same way, but with water instead of the tissue extract). The total polyphenols were expressed as catechin ($mg \cdot L^{-1}$) concentration and calculated applying the formula "catechin ($mg \cdot L^{-1}$) = 186.5 x E_{700} x d" (E_{700} = absorbance at 700 nm; d = dilution). Pigmented grape skin extracts were also analyzed concerning the anthocyanin contents after dilution. The absorbance values at 540 nm were converted in concentration values of malvidin-3-*O*-glucoside through multiplying the absorbance by the coefficient 16.17 and by the dilution factor (Nagel and Wulf 1979). Sugar content (measured by refractometer) and titratable acidity (obtained by titration of the juice with NaOH 0.1 N until pH 7.0) were measured on the juice obtained from the rest of harvested bunches.

The protocol was organized to concatenate the data acquisition, with the objective to generate new derived variables, such as: berry length/width (to have an index of the berry shape); number of seeds per berry; anthocyanins or phenolic content expressed with different units (%; content per kg of grapes, per berry, per g of tissue). In this way, variables with a more direct enological meaning (e.g. sugar or anthocyanins per berry weight), and variables with physiological (e.g. variables expressed on skin weight) and developmental (skin vs. berry weight or number of seeds per berry) implications were considered.

Meteorological site characterization: Each site included in the network has been characterized from the meteorological point of view in terms of thermal resources. Two different thermal indexes have been computed: growing degree days (GDD) and normal heat hours (NHH) (Mariani *et al.* 2013). They are based on two different approaches to account for thermal variation. NHH, based on estimation of cardinal (min. 12 and max. 35 °C respectively) and optimal (25 °C) temperatures, has a more physiological meaning, helping to overcome the systematic overestimation of the effects of high temperatures given by the widely adopted GDD method (Mariani *et al.* 2012). GDD and NHH yearly sums have been calculated for each site for season 2012 and compared to average values calculated on the 2003-2012 period. Daily temperatures for each site have been obtained by means of geostatistical techniques (a weighted mean, with weight inversely proportional to squared distance, applied to data previously homogenized for slope and height) applied to the time series of the European Climate Assessment and Dataset - ECA&D (Klein Tank *et al.* 2002).

Statistical analysis: A preliminary data exploration, of each data set from the different collections, to detect possible outliers has been conducted by visual inspection of the frequency histograms. Outliers were defined and set aside by considering i) possible errors of data transcriptions and ii) results clearly out of the physiological expected range, considering current literature and available data on accession behavior.

Data depurated from the outliers were then statistically processed with a General Linear Model (GLM) considering as only source of variability the "site x accession" combinations, according the following model: $y_{asi} = \mu + \alpha_{as} + \varepsilon_{asi}$ where y_{asi} = value of Y variable for the "i" replication of the "as" (accession x site) combination; μ = general mean values for Y variable; α_{as} = mean value of variable Y for the "as" (accession x site) combination; ε_{asi} = error for the "i" replication of the "as" (accession x site) combination; variance components were then estimated by random model method.

For each variable, the Least Significance Differences (LSD) to contrast a pair of single accession mean values and the Confidence Intervals (CI) for each single accession mean value, were then computed on the residual variability. $LSD = t_{0.05} * (V_{res.} * 2 / n)^{1/2}$ where $t_{0.05}$ = Student's t per P = 5 %; $V_{res.}$ = residual variance; n = number of values comprised in the compared means. $CI = t_{0.05} * s / n^{1/2}$ where $t_{0.05}$ = Student's t per P = 5 %; s = standard deviation estimated on the basis of the expected residual variance component; n = number of values comprised in the single means.

The percentages of residual variance, in respect to the total variance, have been computed after the assessment of the estimation of the variance components, namely "site x accession" and "error".

The same statistical procedure was then followed for each collection data set, applying the model: $y^s_{ai} = \mu^s + \alpha^s_a + \varepsilon^s_{ai}$ where y^s_{ai} = value of Y variable for the "i" replication of the "a" accession in the "s" site; μ^s = general mean values for Y variable in the "s" site; α^s_a = mean value of variable Y for the "a" accession in the "s" site; ε^s_{ai} = error for the "i" replication of the "a" accession in the "s" site.

Results and Discussion

The high participation of partners in the first year of data sampling allowed us to obtain a satisfactory number of cases, and their analysis proved to be very efficient for method validation.

The network allowed to cover a wide geographical range, in term of longitude, from Georgia to Portugal, and latitude, from Cyprus to Germany (Tab. 2 and Figure). Sites represented also a wide array of thermal conditions, from about 1300 (Germany) up to more than 2500 GDD (Armenia), which resulted not strictly related to the latitudes as a consequence of a different location elevation (7-870 m asl). The 2012 thermal resources were generally higher in comparison to the 2003-2012 period (Tab. 2).

First results are summarized in Tabs 3 and 4. The number of data collected ranged as following: from 3500 up to 12500 for carpological data (e.g. bunch weight and berry size); from 1200 to 1400 for variables related to berry tissue component and technological maturity; between 730 and 1000 for variables related to polyphenols analysis. The mean, minimum and maximum values represent the quantitative evaluations of the range for each variable among the *Vitis vinifera* L. populations. They show the central po-

Table 2

Localization from East to West and thermal indexes of the sites participating to the phenotyping network

FAO Institute code	Altitude	Longitude	Latitude	Annual heat summations			
				2012	2003-2012	2012	2003-2012
	m asl	Degree		Growing degree day (GDD)		Normal heating hours (NHH)	
GEO038	586	44.77	41.91	2213	1961	2630	2302
GEO015	616	44.00	41.99	2050	1719	2466	2130
ARM011	870	43.98	40.07	2538	2094	2801	2381
UKR050	28	33.65	44.85	2257	1851	2579	2235
CYP001	630	32.92	34.87	2181	2048	3059	2909
MDA004	201	28.77	46.97	1866	1518	2162	1913
ROM06	80	26.12	44.78	2053	1751	2381	2158
ROM045	192	23.87	44.32	2208	1787	2454	2169
GRC014	7	22.95	40.62	2622	2393	2771	2715
HUN007	110	17.24	46.75	1709	1539	2001	1898
HRV041	260	16.00	45.86	1759	1599	2110	1981
ITA035	144	9.08	44.97	1963	1953	2485	2431
DEU098	195	8.05	49.22	1312	1359	1717	1748
ITA360	220	7.99	44.65	2053	2008	2600	2500
CHE001	457	6.66	46.51	1433	1464	1852	1837
ESP217	343	-2.17	42.17	1964	1936	2296	2236
ESP080	604	-3.28	40.52	1999	2009	2280	2233
PRT051	110	-9.18	39.03	2045	2130	2899	2994

Table 3

Phenotyping descriptors: statistical indexes tested to evaluate their general performances

Variables	n. of cases	Min.	Max.	Mean	CI (+/-)		IAV (%)	Group
					absolute value	% of the mean		
Bunch weight (g)	3546	9.81	1042.28	222.43	45.01	20	27.83	4
Berry length (mm)	12508	5.45	29.40	14.29	0.47	3	21.46	3
Berry width (mm)	12507	5.76	24.00	13.75	0.45	3	27.52	3
Berry length/width	12508	0.60	2.00	1.04	0.023	2	34.93	3
Berry weight (mg)	1401	563.0	10069.0	2214.7	316	14	5.93	1
Skin weight (mg)	1371	45.00	1151.78	317.43	65.06	20	12.79	2
Number of seeds/berry	1320	0.00	4.30	2.16	0.37	17	28.26	4
Seed weight (mg)	1297	4.55	129.23	39.60	5.85	15	17.59	3
Skin (% w/w)	1368	3.19	41.49	15.31	2.88	19	15.37	2
Seed (% w/w)	1365	0.00	16.60	4.14	0.91	22	14.40	2
Sugar content (Brix)	1263	12.00	32.50	20.95	1.41	7	16.06	3
Titratable acidity ($g \cdot L^{-1}$ tartaric acid)	1231	1.00	15.80	5.66	0.68	12	7.42	1
Sugar/acidity (Brix / $g \cdot L^{-1}$ tartaric acid)	1231	1.09	23.00	4.36	0.80	18	10.35	2
Anthocyanin content ($mg \cdot kg^{-1}$ grapes)	730	8.40	5340.82	699.46	170.64	24	5.46	2
Anthocyanin content ($mg \cdot berry^{-1}$)	730	0.02	8.52	1.27	0.30	24	5.50	2
Anthocyanin content ($mg \cdot g^{-1}$ skin)	727	0.07	45.03	4.79	1.81	38	10.31	2
Skin polyphenols ($mg \cdot kg^{-1}$ grapes)	970	171.91	6591.87	1584.21	391.16	25	9.80	2
Skin polyphenols ($mg \cdot berry^{-1}$)	970	0.25	11.95	2.92	0.64	22	10.34	2
Skin polyphenols ($mg \cdot g^{-1}$ skin)	965	0.01	61.39	10.59	2.98	28	11.18	2
Seed polyphenols ($mg \cdot kg^{-1}$ grapes)	965	2.45	4175.12	399.51	153.05	38	7.54	2
Seed polyphenols ($mg \cdot berry^{-1}$)	962	0.01	4.34	0.69	0.22	32	8.93	2
Seed polyphenols ($mg \cdot g^{-1}$ seed)	953	0.05	66.81	9.24	3.97	43	13.25	2
Seed polyphenols ($\mu g \cdot seed^{-1}$)	959	2.26	2285.12	341.17	122.87	36	10.07	2
Skin polyphenols (% of the total)	963	23.42	100.00	79.71	5.33	7	8.85	1
Total phenolic content ($mg \cdot kg^{-1}$ grape)	966	304.60	9527.84	1980.88	424.41	21	7.90	2
Total phenolic content ($mg \cdot berry^{-1}$)	966	0.55	12.24	3.62	0.69	19	10.25	2

Table 4

Collection performances of the phenotyping descriptors as LSD (P=5%) for the single accession mean values

Variables	Collection																		
	All	ARM011	CHE001	CYP001	DEU098	ESP080	ESP217	GEO015	GEO038	GRC014	HRV041	HUN007	ITA035	ITA360	MDA004	PRT051	ROM045	ROM06	UKR050
No. of accessions	**469**	47	4	10	11	13	19	26	14	93	20	7	97	18	35	7	13	13	22
Bunch weight (g)	**63.65**	11.21	68.83	57.95	64.24	100.21	43.19	51.97	55.27	67.42	54.21	nr	69.72	47.81	69.18	71.41	37.68	23.21	56.58
Berry length (mm)	**0.66**	0.70	0.65	0.58	0.60	0.58	0.42	0.61	0.49	0.69	0.84	nr	0.63	0.81	0.79	0.61	0.87	0.46	0.60
Berry width (mm)	**0.63**	0.75	0.74	0.52	0.62	0.56	0.40	0.60	0.46	0.66	0.90	nr	0.60	0.78	0.67	0.58	0.66	0.50	0.55
Berry length/width	**0.03**	0.02	0.04	0.03	0.03	0.02	0.02	0.03	0.04	0.04	0.04	nr	0.03	0.02	0.04	0.02	0.04	0.02	0.02
Berry weight (mg)	**446.8**	720.0	427.9	431.3	377.4	183.9	140.1	346.8	297.9	426.7	122.5	320.3	419.2	304.0	465.7	534.2	395.1	300.3	637.0
Skin weight (mg)	**92.00**	93.2	72.0	nr	64.0	52.1	38.4	116.7	98.5	91.1	16.3	79.2	96.5	64.2	126.4	102.1	80.5	95.1	97.9
Number of seeds/berry	**0.53**	0.48	0.64	nr	0.29	0.36	0.55	0.42	0.42	0.56	0.35	0.27	0.55	nr	0.59	0.37	0.42	0.53	0.79
Seed weight (mg)	**8.27**	7.20	6.63	nr	3.77	2.67	3.33	6.36	9.03	8.35	8.52	6.07	8.64	nr	12.40	5.28	5.14	6.44	10.79
Skin (% w/w)	**4.07**	2.66	4.14	nr	2.41	2.73	2.11	4.22	4.00	5.18	1.11	4.30	3.67	2.72	6.18	1.99	2.26	5.75	3.52
Seed (% w/w)	**1.29**	0.72	1.04	nr	0.53	0.75	1.11	0.75	0.94	1.33	1.36	0.74	1.57	0.00	2.01	0.71	0.54	1.08	1.60
Sugar content (Brix)	**1.99**	nr*	1.11	1.76	1.23	1.48	1.84	2.19	1.19	1.88	1.04	1.36	2.47	1.26	2.53	1.37	1.79	1.64	1.84
Titratable acidity (g·L^{-1} tartaric acid)	**0.96**	nr	0.70	nr	1.04	0.52	0.30	0.84	0.59	0.73	0.47	1.45	1.24	0.44	1.12	0.72	0.78	0.81	1.38
Sugar/acidity (Brix / g·L^{-1} tartaric acid)	**1.12**	nr	0.40	nr	0.23	0.57	0.30	0.67	0.37	1.65	0.56	0.73	0.91	0.04	1.44	0.42	2.00	0.88	0.88
Anthocyanin content (mg·kg^{-1} grapes)	**241.33**	75.5	81.1	nr	9.9	387.7	10.3	204.0	365.2	274.8	175.8	164.4	255.3	nr	147.3	nr	242.9	140.7	379.1
Anthocyanin content (mg·berry^{-1})	**0.42**	0.32	0.11	nr	0.01	0.42	0.02	0.51	0.84	0.31	0.17	0.19	0.48	nr	0.18	nr	0.65	0.14	0.48
Anthocyanin content (mg·g^{-1} skin)	**2.55**	1.14	0.41	nr	0.12	2.24	0.18	1.23	1.54	4.32	1.53	1.30	1.80	nr	0.96	nr	4.88	0.64	2.53
Skin polyphenols (mg·kg^{-1} grapes)	**553.19**	188.7	312.5	nr	84.1	506.9	316.6	273.5	535.8	488.2	1213.6	323.5	686.4	nr	594.2	nr	785.4	221.8	813.7
Skin polyphenols (mg·berry^{-1})	**0.90**	0.46	0.35	nr	0.15	0.44	0.63	0.68	1.02	0.72	1.25	0.29	1.25	nr	0.65	nr	1.27	0.18	0.78
Skin polyphenols (mg·g^{-1} skin)	**4.21**	2.60	1.92	nr	0.91	3.18	1.79	1.82	3.44	5.62	8.45	1.01	4.57	nr	2.78	nr	5.71	1.48	5.34
Seed polyphenols (mg·kg^{-1} grapes)	**216.45**	120.7	300.5	nr	94.1	343.5	121.5	80.6	121.3	244.0	514.2	505.5	175.8	nr	35.2	nr	133.0	187.1	439.8
Seed polyphenols (mg·berry^{-1})	**0.32**	0.29	0.66	nr	0.18	0.48	0.07	0.17	0.27	0.28	0.54	0.72	0.28	nr	0.04	nr	0.29	0.39	0.66
Seed polyphenols (mg·g^{-1} seed)	**5.62**	3.46	14.11	nr	3.00	7.02	3.97	2.11	2.62	7.48	9.56	7.58	4.33	nr	0.50	nr	4.44	4.21	12.55
Seed polyphenols (µg·seed^{-1})	**173.77**	153.7	371.3	nr	138.6	296.5	118.1	100.9	141.0	147.4	231.3	253.8	122.5	nr	28.8	nr	224.9	168.4	536.3
Skin polyphenols (% of the total)	**7.54**	6.32	9.57	nr	4.61	8.99	2.85	3.67	4.77	9.66	13.45	12.56	7.60	nr	3.13	nr	3.56	6.47	7.95
Total phenolic content (mg·kg^{-1} grape)	**600.21**	217.3	501.7	nr	153.4	691.0	337.7	310.0	610.8	616.8	877.3	662.8	738.2	nr	611.8	nr	820.3	304.7	926.1
Total phenolic content (mg·berry^{-1})	**0.98**	0.49	0.74	nr	0.26	0.69	0.63	0.75	1.19	0.85	1.03	0.77	1.32	nr	0.64	nr	1.27	0.45	1.04

* nr = trait not recorded

sition and the extreme limits of the distribution ranges of the collected variables. The higher the number of cases and wider the range of data variation, the more reliable will be the judgment on the performance of the protocol. Due to the novelty of this population approach, in the grapevine germplasm characterization, reference data are not available. However in the author's experience, these statistics attest for a very ample exploration of the *Vitis vinifera* phenotypic variability.

For the evaluation of method performance, Tab. 3 should be observed. A low Confidence Interval (CI), expressed as percentage of the mean, indicates that the method is highly efficient in the estimation of the mean value of a single accession, due to the low variability between the replications of the same accession. In parallel, it has to be examined the percentage of variance due to the Intra-Accession Variability (IAV) that makes an estimation of the intra-accession variability in comparison to the between-accession variability.

An arbitrary and convenient threshold of 15 % for both CI and IAV, was defined. It represents an expression of accurate work performed in each research center and also a good indicator of the most sensible descriptors in correlation to genotype and environment. In this way, variables may be classified in four groups (Tab. 3).

1. Both CI and IAV low: the method well estimated the mean value of the single accession and discriminated the different accessions.
2. High CI and low IAV: in spite of the high variability among replications (CI), a low IAV indicates that the range of variation between the different accessions is appreciably higher than the one related to the replications of the same accession. Thus, the detected variability between the replications is acceptable.
3. Low CI and high IAV: the high IAV indicates that the trait is really variable, even so, the low CI, obtained thanks to the high number of replications, allows the accession's characterization.
4. High CI and high IAV: these traits are really variable within accessions. More careful sampling and a higher number of replications are necessary to characterize them.

It is remarkable to note that only two variables are grouped in the fourth group: the bunch weight and the number of seeds per berry. Both these traits are known to be really variable in relation to the environmental conditions.

In Tab. 4, the single collection performances are reported for all the variables in terms of Least Significant Difference (LSD). This statistic represents the threshold value that permits to separate two accessions in relation to their phenotypic values for the considered trait. In general terms, it should be stressed that the LSD statistics obtained by the single research center have to be evaluated individually, due to their dependence to the specific characteristics of the selected accessions; in fact a correlation among the mean values and the ranges of the tested accessions with the LSD statistics is expected. This is for example the case of the LSD obtained for the berry weight in Armenia (720.0 mg) which tested mainly large berried grapes, in comparisons to the value obtained in Georgia (346.8 mg) which included medium berried grapes.

Nevertheless, comparing the LSD calculated separately in each collection to those computed for the totality of the data, it is possible to obtain a comparative indicator of the accuracy of the application of the protocol in the single collection. Mostly, the LSD values of the single collection were lower or close to the general LSD value. This underlines that all the collections produced data of comparable quality, attesting that the samplings, measurements and analyses were correctly and carefully performed in all the research centers.

As general comment, data quality can be improved by more careful sampling and measurements (so to lower the number of excluded outliers), and by having a higher number of replications. In the following years of the project, new measurements will be carried out on the same genotypes at the same sites, so that a new component "year effect" will be added to the model, strengthening its validity.

Conclusions

The application of a common protocol between different collections represents a further step to explore the phenotypic diversity among grapevine varieties. The first test of the protocols proposed in the framework of Cost Action FA1003 produced satisfactory results in terms of accuracy and efficiency in the estimation of the phenotypic traits of the single accessions, in relation to their practical evaluation as well as for comparative purposes. The practical evaluation refers to the enological or direct eating quality, while the comparative evaluation is fundamental for the association genetic approaches.

According to this, the protocol has been confirmed for a second year test. Some adaptations have been added to take into consideration the sampling procedure for bunches that showed a too high intra-accession variability.

Documentation of the collected data in the European *Vitis* Database (EVDB; www.eu-vitis.de) will increase the public access to the knowledge of eno-carpological aptitudes of described accessions.

The further development of standard methods for comparative accession phenotyping will be of value for both viticulture practice and research, including i) direct use of minor or neglected varieties for their newly discovered enological and qualitative useful traits, ii) selection of appropriate grape variety for breeding programs, iii) genetic association studies and identification of candidate genes, and iv) studies of interactions between genotype and environment.

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References

Di Stefano, R.; Cravero, M. C.; Gentilini, N.; 1989: Metodi per lo studio dei polifenoli dei vini. L'Enotecnico Maggio, 83-89.

Klein Tank, A. M. G.; Wijngaard, J. B.; Können, G. P.; Böhm, R.; Demaree, G.; Gocheva, A.; Mileta, M.; Pashiardis, S.; Hejkrlik, L.; Kern-Hansen, C.; Heino, R.; Bessemoulin, P.; Muller-Westermeier, G.; Tzanakou, M.; Szalai, S.; Pálsdóttir, T.; Fitzgerald, D.; Rubin, S.; Capaldo, M.; Maugeri, M.; Leitass, A.; Bukantis, A.; Aberfeld, R.; Van Engelen, A. F. V.; Forland, E.; Mietus, M.; Coelho, F.; Mares, C.; Razuvaev, V.; Nieplova, E.; Cegnar, T.; Antonio Löpez, J.; Dahlström, B.; Moberg, A.; Kirchhofer, W.; Ceylan, A.; Pachaliuk, O.; Alexander, L. V.; Petrovic, P.; 2002: Daily dataset of 20th-century surface air temperature and precipitation series for the European Climate Assessment. Int. J. Climatol. **22**, 1441-1453.

Maghradze, D.; Rustioni, L.; Scienza, A.; Turok, J.; Failla, O.; 2012: Caucasus and Northern Black Sea Region Ampelography. Vitis, Special Issue, JKI - Julius Kühn-Institut, Siebeldingen, Germany.

Mariani, L.; Parisi, S. G.; Cola, G.; Failla, O.; 2012: Climate change in Europe and effects on thermal resources for crops. Int. J. Biometeorol. **56**, 1123-1134.

Mariani, L.; Alilla, R.; Cola, G.; Dal Monte, G.; Epifani, C.; Puppi, G.; Failla, O.; 2013: IPHEN - a real-time network for phenological monitoring and modeling in Italy. Int. J. Biometeorol. **57**, 881-893.

Maul, E.; Sudharma, K. N.; Kecke, S.; Marx, G.; Müller, C.; Audeguin, L.; Boselli, M.; Boursiquot, J. M.; Bucchetti, B.; Cabello, F.; Carraro, R.; Crespan, M.; de Andrés, M. T.; Eiras Dias, J.; Ekhvaia, J.; Gaforio, L.; Gardiman, M.; Grando, S.; Gyropoulos, D.; Janduova, O.; Kiss, E.; Kontic, J.; Kozma, P.; Lacombe, T.; Laucou, V.; Legrand, D.; Maghradze, D.; Marinoni, D.; Maletic, E.; Moreira, F.; Muñoz-Organero, G.; Nakhutsrishvili, G.; Pejic, I.; Peterlunghер, E.; Pistoli, D.; Pospisilova, D.; Preiner, D.; Raimondi, S.; Regner, F.; Savin, G.; 2012: The European *Vitis* Database (www.eu-vitis.de) – a technical innovation through an online uploading and interactive modification system. Vitis **51**, 79-85.

Nagel, C. W.; Wulf, L. W.; 1979: Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. Am. J. Enol. Vitic. **20**, 111-116.

OIV; 2009: OIV Descriptor List for Grape Varieties and *Vitis* Species (2nd ed.). O. I. V. (Office Int. Vigne Vin), Paris, France.

Rustioni, L.; Basilico, R.; Fiori, S.; Leoni, S.; Leoni, A.; Maghradze, D.; Failla, O.; 2013: Grape colour phenotyping: development of a method based on the reflectance spectrum. Phytochem. Anal. **24**, 453-459.

Volk, G. M.; 2010: Advantages for the use of standardized phenotyping in databases. HortScience **45**, 1310-1313.

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