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RESEARCH ARTICLE

Key Descriptors for Sugars and Acids to Evaluate Vitis sylvestris Grapevines

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Abstract

Sugars and organic acids in grape berries are crucial quality traits to evaluate the market use and consumer's preference. The composition of glucose, fructose, sucrose, tartaric, malic and citric acid were determined in grapes from wild and domesticated *V. vinifera* growing under Mediterranean conditions of the north of Tunisia. Sugars and organic acids were enzymatically determined using a SAFAS FLX-Xenius microplate equipped with a SAFAS automatic injector. Wild grape berries are distinguished by a typical small berry, round shaped and blue-black colored with large seeds. Glucose, fructose and tartaric acid are the main sugars and acid identified in both subspecies. All genotypes were hexose accumulators (α *ratio* > 0.81). Sucrose was present in trace amount (<0.1 g/100g FW). Genotypic variance was significant for all berry traits (p <0.05). Wild vines develop berries rich in acids (67% tartrate and 24% malate), while domesticated vines are high sugar genotypes (58% glucose and 41% fructose). The sole analysis of total soluble solids (TSS) or titratable acidity (TA) may not reveal the essence of sugar or acid in grape berries. The glucose/fructose ratio, tartaric and malic acid contents are key descriptors for biochemical evaluation of wild genotypes. If considering that the selection of parents should be based on organic acids content and composition rather than sugars in a grape breeding program, Tunisian *V. sylvestris* resources should represent an interesting gene pool for viticulturists to cope with drought and dry-hot climate prevailing currently in the south side of the Mediterranean basin.

Keywords: *Vitis sylvestris*; Genetic Resources; Gluc/Fruc Ratio; Malic Acid; Tartaric Acid; Mediterranean Environment

List of abbreviations: IPGRI (The International Plant Genetic Resources Institute); **UPOV** (The International Union for the Protection of New Varieties of Plants); **OIV** (International Organization of Vine and Wine); **TSS** (Total soluble solids); **TA** (Titratable acidity); **FW** (Fresh weight); **ANOVA** (Analysis of variance); **SD** (Standard deviation); **SPSS** (Statistical Package for the Social Sciences)

Introduction

The distribution area of the wild grapevine (*Vitis vinifera* subsp. *sylvestris*) is constantly declining all over the world [1]. *Vitis sylvestris* is a heliophilous liana growing generally along riverbanks and in alluvial and colluvial deciduous and semi-deciduous forest [1,2]. Genetically, wild vine is considered the ancestor of the cultivated vines or at least as being the origin of many domesticated vines [1,3]. Currently, vines found in natural habitats are considered a mixture of wild forms, naturalized cultivated forms and hybrids derived from spontaneous hybridizations because the free gene flow between the two forms [4-6].

In Tunisia, wild grapevine *V. vinifera sylvestris* have been reported in forest sites, along the seashores, isolated or in groupings, in the northwest side [7,8,2]. Wild ecotypes, marked by a large polymorphism and great variability [7,9], are seriously threatened due to the actual context of climate change. At present, the conservation of wild forms close to cultivated plants seems essential to maintain genetic variability and to prevent genetic erosion. Resistance to some virus (*Grapevine Fan Leaf Virus* GFLV, *Grapevine Leafroll-associated Virus* GRLaV) and the good adaptation to different bio-climates make wild grapevines a valuable gene reservoir. Genes encoding for such properties are useful in biotechnology for plant selection and breeding [1,10,11].

The level of sugars and organic acids are important factors in determining quality of grapes. Glucose and fructose are the main sugars present in grape berries at harvest while tartaric and malic acids are the major acids and constitute together until 90% of total organic acids [12-14]. Their combination contributes to the final taste of the fruit [15]. Studies on primary metabolites are limited to selected cultivars. Data concerning wild vines are sporadic. In Tunisia, ripening of table grapes occurs under sunny and hot weather conditions. Sunny weather conditions enhance the sugar production in the berry. Local cultivars were qualified as sugar balanced genotypes while, wild ecotypes were described as high glucose genotypes [16].

The ripening process indicates a continuous progress in the quality of the berries as the grapes mature, the evolution pattern proceeding rapidly during the early stage of ripening, slowing in its middle part, and almost ceasing as the grapes become very ripe [17]. The glucose/fructose ratio, made to determine the normal content of reducing sugars in grape varieties, was recently provided in a large spectrum of wild and domesticated grapevines growing in the north of Tunisia [16]. In general, the ratio is about 1 at ripeness. In unripe grapes glucose predominates, while in overripe grapes fructose becomes predominant [13].

The *sylvestris* subspecies, a promising germplasm for breeder and viticulturists, involves interesting genes which could be explored for cultivars selection and improvement. The present study aims to shed light on the sugar balance and acidic composition of Tunisian wild grapes in comparison with domesticated grapes. This study will help to lay out the biochemical foundation for accelerating trait improvement process of quality in local grapevines for future breeding and selection programs under the climate shift in the Mediterranean basin.

Materials and Methods

Plant material

Grape clusters were collected from vines (Cap Negro 5/2000, Cap Negro 6/2000 and Cap Negro III) growing spontaneously in forests and downside of hills and mountains in the north-west of Tunisia (Latitude 37.049235, Longitude 9.2406321, Altitude 131 m). Local cultivars 'Meski Rafraf', 'Boukhasla' and 'Gualb Sardouk' were selected as domesticated grape samples. Cultivar 'Meski Rafraf' is very well appreciated as a table grapevine bearing heavy berries with very sweet taste. The vintage is widely disseminated in the local market and used by rural inhabitants as a standard to compare and appraise other local or introduced varieties. Grape clusters from local cultivars were harvested from the north-east of Tunisia (Latitude: 37.1907937, Longitude: 10.1816494, Altitude: 90 m). The two sampling areas are 142 km distant. Grape clusters were collected 109 days after anthesis for wild ecotypes and 114 to 136 days after anthesis (full ripe stage) for domesticated vines during the two years 2014 and 2015. Because of the millerandage, wild grape clusters were harvested at total color change stage of at least 50% of the berries per cluster.

The berry: Morphometric features

Shape, size, colour, berry weight and cluster weight were determined to describe morphometric features of the berry for the two subspecies using the descriptors for grapevine [18]. All details are listed in Table1.

Sugar and Acid Analysis

Total soluble solids (TSS) were determined using a digital refractometer (OPTECH GmbH, Munchen, Germany) and expressed in percent (%) at 20 °C. Titratable acidity (TA), expressed as g of tartaric acid/l, was determined by titrating grape juice with 0.1 M NaOH. Grape clusters were scraped, and seeds were removed from the berry. For the following, the method used by Trad et al. [16] was applied. Glucose, fructose, sucrose, malic, citric and tartaric acids were enzymatically quantified with kits for food analysis (Boehringer Mannheim Co., Mannhein, Germany) using a SAFAS FLX-Xenius microplate (SAFAS Monaco) equipped with a SAFAS automatic injector. Data results were expressed in g/100g of fresh weight (FW) for sugars and in meq/100g FW for organic acids..

Statistics

The results displayed are means ($N = 3$) ± standard deviations (SD). One-way analysis of variance (ANOVA) was carried out for data analysis. Significance level of the differences were assessed by Duncan's multiple range test ($p < 0.05$) and means for groups in homogeneous subsets were displayed. Stacked bar charts of summaries of separate variables were layout for the different biochemical compounds. Radar chart was designed to highlight inheritance of sugars and acids between wild and domesticated grape samples. All statistics were performed using PC software package SPSS (IBM® SPSS® Statistics, version 20.0.0).

Results

Berry morphology

Wild berries were round shaped, blue-black colored, small size but with large seeds. Berry weight ranged between 2.3 g ('Cap Negro 5/2000') and 2.7 g ('Cap Negro III'). Local cultivars developed medium to large berries weighing between 5.7 g (cv. 'Gualb Sardouk') to 10.1 g (cv 'Meski Rafraf') sharply different in shape and external colour. Shape of the berry was round in the two cultivars 'Boukhasla' and 'Gualb Sardouk' and obtuse-ovate in cultivar 'Meski Rafraf'. Colour of the berry varied from green yellow (cv 'Meski Rafraf'), dark-red violet (cv 'Boukhasla') to blue-black (cv 'Gualb Sardouk') (Table 1). Phenotypic variance between wild

Ecotype	Label	Origin	Berry [a]			
			Size O-221	Shape O-223	Colour O-225	Weight (g)
V. vinifera						
Meski Rafraf	MKR	Rafraf	Large	Obtuse-ovate	Green yellow	10.1 ±0.2
Boukhasla	BKS	Rafraf	Medium	Round	Dark red-violet	7.0 ±0.3
Gualb Sardouk	GSR	Rafraf	Medium	Round	Blue-black	5.7 ±0.3
V. sylvestris						
Cap Negro 5/2000	CPN5/2000	Cap Negro	Very small	Round	Blue-black	2.3 ±0.8
Cap Negro 6/2000	CPN6/2000	Cap Negro	Very small	Round	Blue-black	2.4 ±0.9
Cap Negro III	CPNIII	Cap Negro	Very small	Round	Blue-black	2.7 ±0.4

[a]Descriptors for Grapevine[18]

Table 1: Origin and general description of the collected vine samples

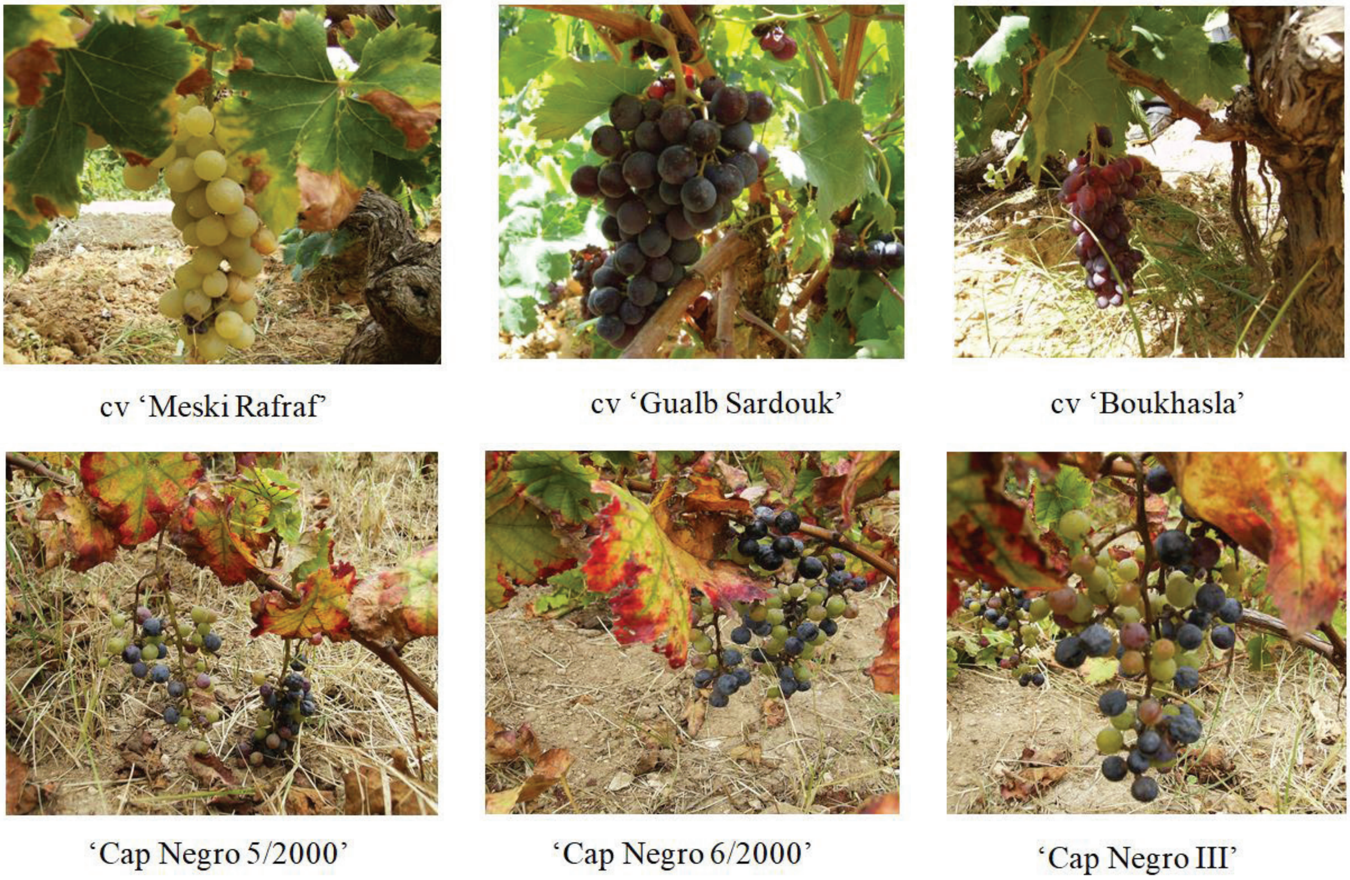

Figure 1: Grape clusters from wild and domesticated vines growing in the north of Tunisia (cv: cultivar).

Harvest time: full ripeness stage for domesticated grapes; total color change for at least 50% of the berries per cluster for wild grapes

and domesticated grapes was clearly identified (Figure 1).

	Domesticated grapevines				*Wild grapevines*			
	MKR	BKS	GSR	Means	CPN 5/2000	CPN 6/2000	CPN III	*Means*
Sugars								
TSS	22.7b ±2.5	15.9c ±0.8	17.0c ±0.3	18.5	27.6a ±1.0	26.5a ±0.7	27.8a ±0.7	27.3
Glucose	8.77a ±0.83	6.29b ±0.36	6.49b ±0.68	7.18	6.93b ±0.3	5.85b ±0.7	5.88b ±0.3	6.22
Fructose	7.16a ±0.27	2.92d ±0.37	5.28b ±0.33	5.12	1.70e ±0.3	0.52f ±0.2	3.85c ±0.7	2.02
Sucrose	*< 0.1*	*< 0.1*	*< 0.1*	-	*< 0.1*	*< 0.1*	*< 0.1*	-
Total sugars	16.03a	9.31bc	11.87b	12.40	8.73c	6.47d	9.83bc	8.34
α ratio	1.22de	2.15c	1.23de	1.53	4.16b	12.38a	1.57d	6.04
Acids								
TA	1.18b ±0.06	0.95b ±0.02	0.90b ±0.00	1.01	2.29a ±0.28	2.53a ±0.14	2.36a ±0.52	2.39
Tartrate	9.00bc ±1.72	9.03bc ±1.87	8.13c ±2.95	8.72	17.97a ±4.32	18.47a ±2.58	15.18ab ±5.83	17.21
Malate	1.11d ±0.28	3.83bc ±0.21	0.95d ±0.23	1.96	4.00bc ±0.49	8.95a ±0.29	5.40b ±0.12	6.12
Citrate	2.04b ±0.32	1.74bc ±0.34	1.40c ±0.13	1.73	1.41c ±0.35	1.82bc ±0.22	3.32a ±0.38	2.18
Total acids	12.15cd	14.60c	10.48d	12.41	23.38b	29.24a	23.90b	25.51
β ratio	8.11a	2.36c	8.55a	6.34	4.49b	2.06c	2.81c	3.12

Different letters within a row indicate significant difference between genotypes at $p < 0.05$

Table 2: Sugar and acid's content in wild and domesticated Vitis vinifera berry.

(TSS: total soluble solids (%), glucose, fructose and sucrose: g/100g fresh weight; α ratio: glucose to fructose;

TA: titratable acidity (g tartrate/l), tartaric, malic and citric acids: meq/100g FW, β ratio: tartaric to malic acid)

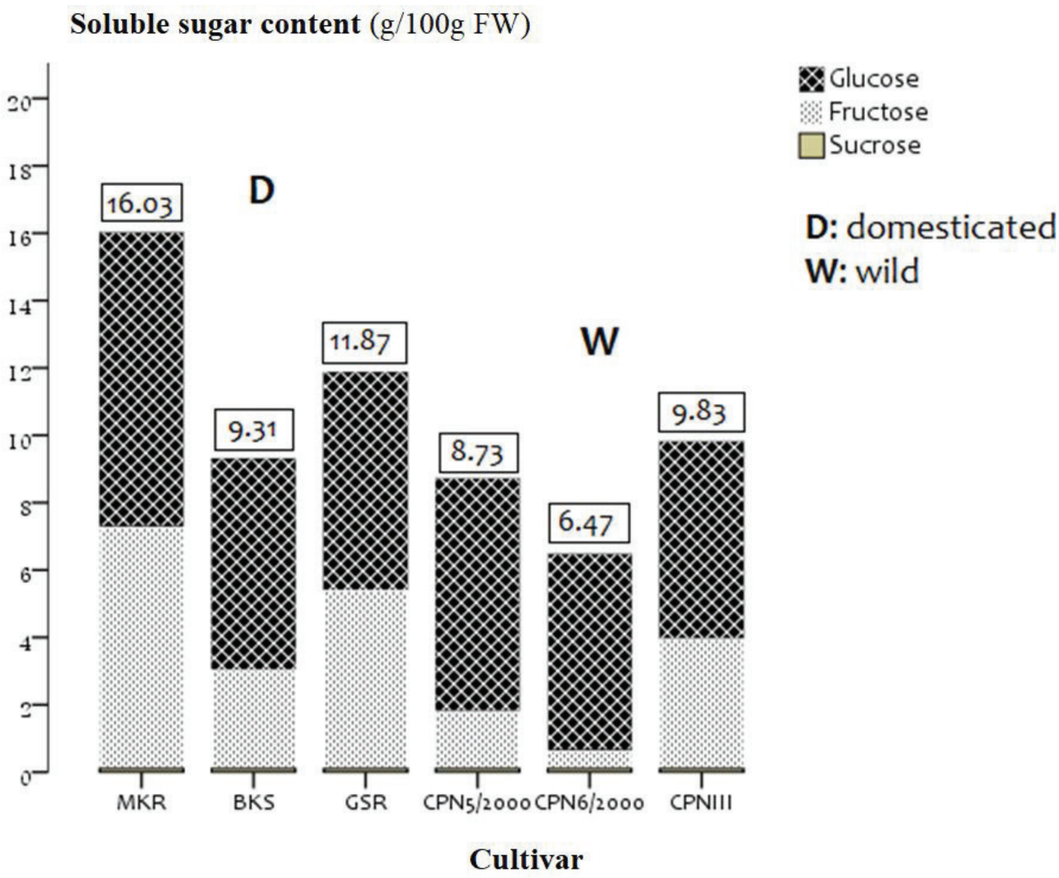

Figure 2: Sugar content (g/100g FW) of wild and domesticated grapes collected from the north of Tunisia

Sugars in wild grapes

In wild grapes, TSS values ranged from 26.5% ('Cap Negro 6/2000') to 27.8% ('Cap Negro III') compared to an average value of 18.5% in domesticated grape juice (Table 2). TSS indicates sweetness degree of the fruit and is closely related to consumer acceptance [19]. Total sugar content varied between 6.47 g/100g FW ('Cap Negro 6/2000') and 9.83 g/100g FW ('Cap Negro III') and was lower than average value found in grapes from local cultivars (12.4 g/100g FW). Glucose and fructose were the main sugars identified in both types (Figure 2). Trace amounts of sucrose were found in all genotypes (<0.1 g/100g FW). Concentration in glucose and fructose reached respectively 6.22 and 2.02 g/100g FW in wild berries against 7.18 and 5.12 g/100g FW in berries from local cultivars (Table 2). Groups from homogeneous subset highlights an effect of genotype for all sugar components except for sucrose (p <0.01). Wild grapevines were high soluble solids genotypes and were clustered in one homogeneous subset (Table 2). Among the three grape cultivars, 'Meski Rafraf' produces the sweetest berries when ripe.

α ratio (glucose/fructose)

The *α ratio* is a useful index to evaluate variations in sugar composition among grape accessions. It helps to monitor changes in sugar constituents during ripening and thus define whether a genotype is glucose or fructose accumulator. Analysis of wild grape samples showed a sharp variance in the glucose/fructose ratio ranging from 1.6 in Cap Negro III to 12.4 in Cap Negro 6/2000. In opposite, *α ratio* was more stable in berries from local cultivars with relatively equilibrated sugar balance (Table 2). The glucose/fructose ratio was four times higher in wild grapes compared to domesticated grapes (Table 2), and with *α ratio* > 0.81, both types are considered hexose (glucose and fructose) accumulators.

Acids in wild grapes

Wild grape juice was more acidic than domesticated grape juice. Average titratable acidity of the juice was 2.39 g tartrate/l in wild grapes against 1.01 g tartrate/l in domesticated grape samples (Table 2). Total acids, sum of the three organic acids, were twice higher in wild grape samples (25.51 meq/100g FW) compared to domesticated grapes (12.41 meq/100g FW). Tartaric acid largely predominates grape organic acid composition (Figure 3). Tartrate represents 68% and 70% of total acids found in the berry of wild and domesticated grapes respectively. In wild grapes, malic acid concentrations varied from 4.00 meq/100g FW (CPN5/2000) to 8.95 meq/100g FW (CPN6/2000). Levels of citric acid were comprised between 1.41 meq/100g FW (CPN5/2000) and 3.32 meq/100g FW (CPNIII). Differences in citrate content were highly significant between all studied grapes. However, the gap was more important within the wild genetic pool compared to local cultivars (Figure 3).

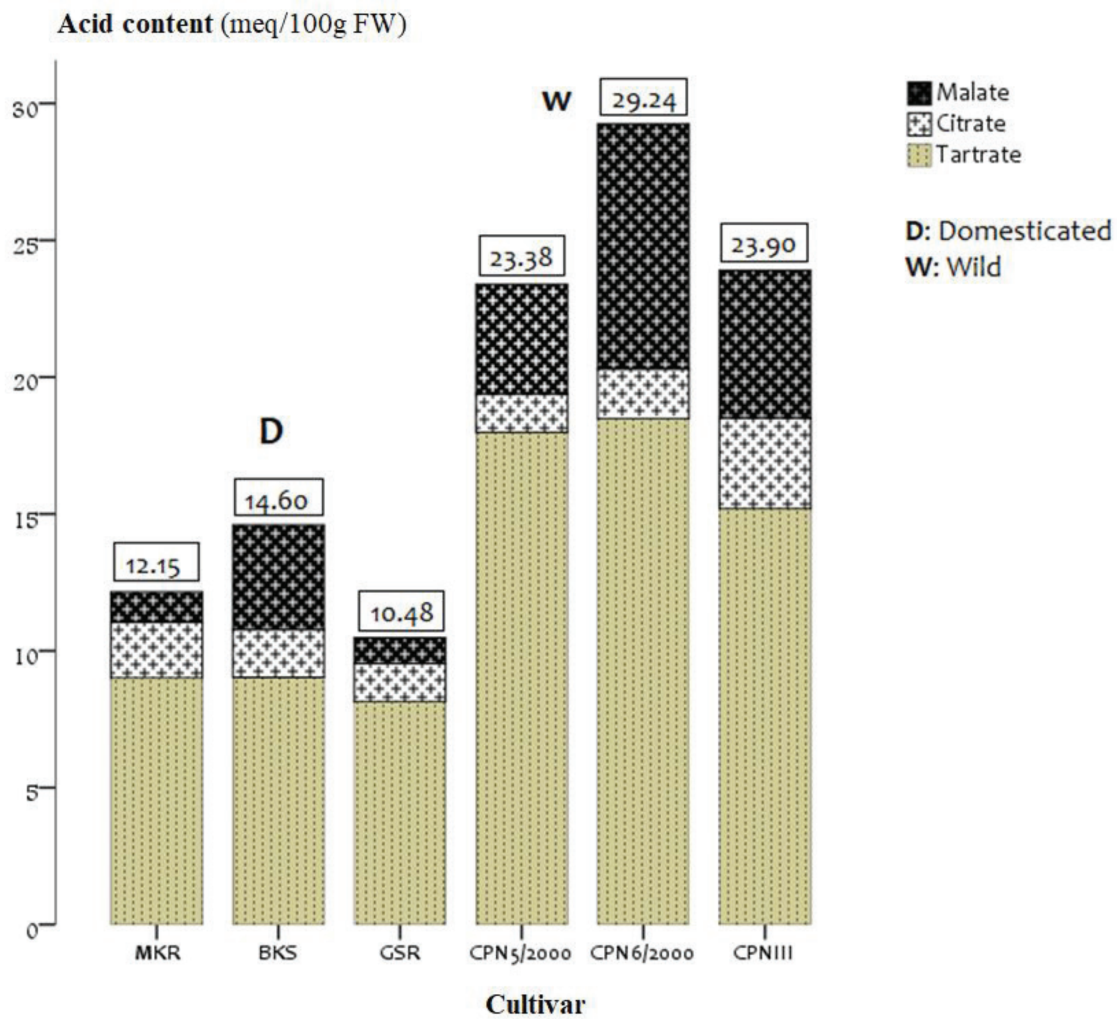

Figure 3: Organic acids composition (meq/100g FW) of wild and domesticated grapes collected from the north of Tunisia

β ratio (Tartrate/malate)

The *β ratio* reflects the organic acid composition of grape genotypes. The ratio of tartaric to malic acid can be used as an index to follow changes in organic acid composition during ripening. *β ratio* was around 3.1 in wild vines and 6.3 in domesticated vines. The highest value was recorded in cultivars 'Gualb Sardouk' and 'Meski Rafraf' with tartrate in the berry being eight-fold higher than malate (Table 2). A high *β ratio* is necessary for wine stability and high-quality wine production. This explains why cultivar like 'Meski Rafraf' could be used either for fresh consumption or wine-making. In the opposite, a table grape should have low *β ratio* for palatability [20], this was the case for cultivar 'Boukhasla'. In the wild grape ecotypes, *β ratio* was comprised between 2.1 and 4.5. The highest ratio was recorded for Cap Negro 5/2000 while the lowest ratio was found in berries from Cap Negro 6/2000.

Sugars and Acids Heritability

The distribution pattern of sugar and organic acid components was displayed in figure 4 for the two *Vitis* subspecies. High proportions of tartrate and malate characterize wild grapes compared to domesticated vines. It was clear from the figure 4 that inheritance of titratable acidity may not reveal the essence of organic acid heritability in wild grape berries and that inheritance of total acids is mainly influenced by both malic and tartaric acids. Heritability of tartaric acid was lower than that of malic acid.

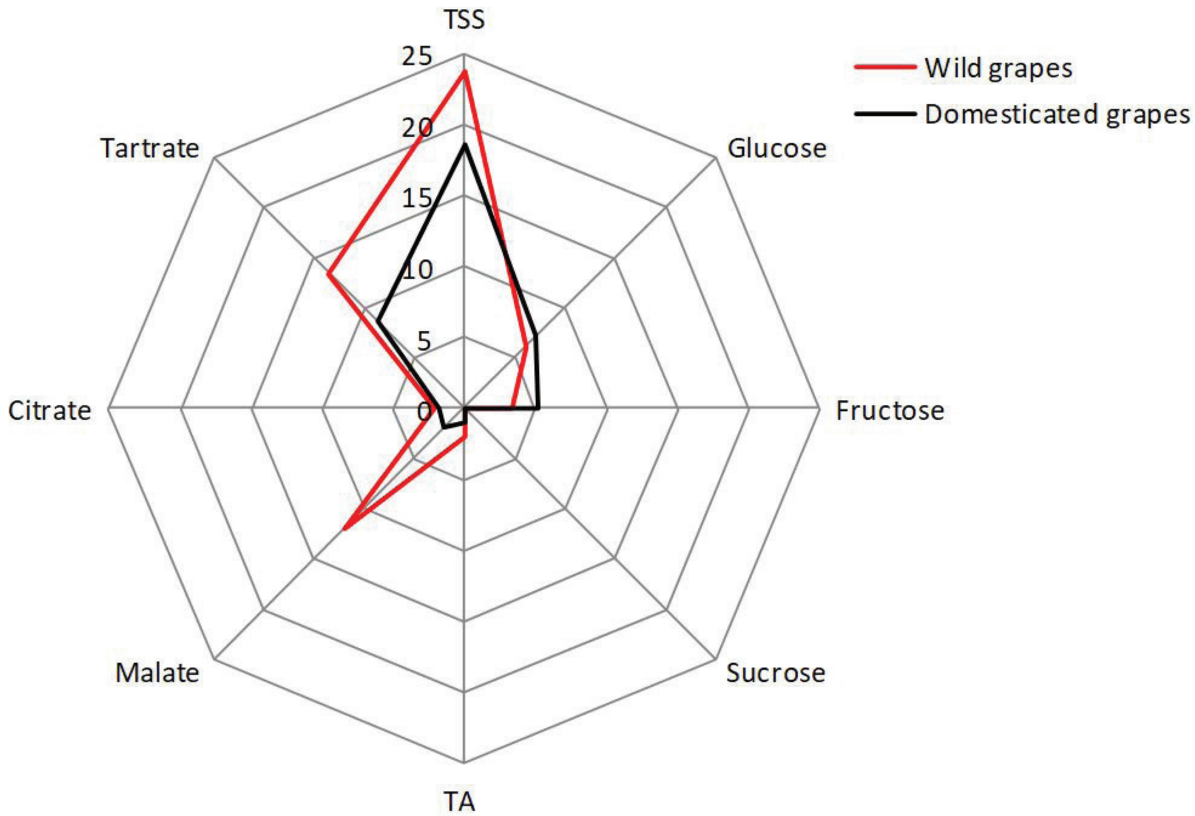

Figure 4: Radar chart showing the distribution pattern of sugar and acid components in the two *Vitis* subspecies

This was earlier demonstrated by Liu et al. [21]. The same conclusion could be made for soluble solids. Inheritance of total soluble solids cannot inform about heritability of reducing sugars in *V. vinifera* berries. It is established that the TSS measures not only sugar content but includes also the carbohydrates, organic acids, proteins, fats and minerals of the fruit. This explains why the TSS of wild grapes was significantly higher than domesticated grapes although total sugars of wild grapes were lower than domesticated grapes (Table 2).

Discussion

Grapes are a versatile crop typically adapted to the Mediterranean climate where clusters adequately exposed to sunlight ensure enough supply of carbohydrates. The accumulation of the two monosaccharide (glucose and fructose) starts early at *veraison* (inception of berry ripening) and continues throughout ripening [22]. The sugar content of the berry was higher in domesticated grapes compared to wild grapes, such data agree with those published earlier by Shiraishi [23]. Grape berries, identified as hexose accumulators, start the sugar accumulation 8 weeks post-flowering and continue until the fruit was ripe at 16 weeks [22]. Sugars are among leading factors affecting taste formation in fruits and are highly correlated with ripeness [14,24]. In general, expected sugar content for selected grapes is around 14.5 g/100g FW [25]. Domesticated berries reached an average of 12.4 g/100g FW, while wild berries produced a means of 8.3 g/100g FW. Except during the green berry stage while glucose may account for 85% of total sugars [26], glucose and fructose amounts are equal with increasing maturity [27]. In some cases, in the ripe fruit, fructose content might slightly exceed that of glucose [28]. Sucrose contributes to about 1% of total sugars. However, a few high-sucrose content cultivars have been characterized in *Vitis rotundifolia* and hybrids between *V. labrusca* and *V. vinifera* [29]. In grapevines, sucrose is the major transported sugar to berries via phloem; however, it is enzymatically hydrolyzed to glucose and fructose [30,31]. In the present study, sucrose was found in trace amounts for all ecotypes. The hexose- and sucrose-accumulating character in grape berries is thought to be related to regional and genetic differences [32]. Sucrose accumulator has not yet been found among *V. vinifera* cultivars [32]. This also applies for wild vines studied in the present work. Full ripeness stage is defined by sugar ratio fairly equal to 1 except for the green berry stage: more glucose than fructose is generally found in immature berries [33,27]. Clusters from wild vines may be considered unripe enough when picked. In the contrary, with an average sugar ratio of about 1.53, domesticated grape samples were evenly at the full ripe stage when picked. Liang et al. [34] demonstrated that the ratio of glucose to fructose is close to 1 by the second week of *veraison* through maturity. The glucose/fructose ratio of eight table grape varieties originated from California was comprised between 0.80 (Thompson seedless) and 1.12 (Red Malaga), while this ratio was ranged between 1.05 and 1.15 in 'Yinhong' grape and its eight mutants grown in China [35]. There is no large discrepancy with results found by Pavlousek and Kumsta [36] whose found similar proportions in 15 varieties cultivated in Czech Republic (0.92 < *α ratio* < 1.06). They also concluded that the Glucose/Fructose ratio remains stable and is not influenced by the year of harvest. However, it depends on the cultivar as demonstrated in our work for wild grapevines.

The composition of sugars in grape berries remains stable after ripening onset, while acids were qualified as sensitive to climate changes [29]. Grape organoleptic traits greatly depend on both content and composition of sugar and acids and these are important factors in the selection of a new cultivar [21]. In general, sugars contribute a very large proportion to the °Brix value in the ripening stage. However, the °Brix value does not always run parallel with the sugar content [23]. This was proved for Tunisian grapes with a net discrepancy registered between TSS and reducing sugars for both vine types. Tartaric and malic acids represent the most important organic acids encountered in grape berries as well in wild as in domesticated vines. The former displays the essential characteristics of a secondary product, the latter, on the other hand, is known to be a very active intermediate in grape metabolism and seems to play a significant role in anabolic reactions such as dark fixation of carbon dioxide as well as in the acid catabolizing processes of ripening [37]. Photosynthesis, which takes place in green berries, produces approximately 50% of all acids. A decrease in the content of organic acids starts at the beginning of ripening [38]. Malic acid accumulates steadily in the fruit after anthesis and fruit set and reaches a maximum shortly before the beginning of ripening. With the onset of berry softening, colour change and sugar accumulation, a period of rapid malate consumption is initiated. In wild vines, this acid consumption appears slower compared to domesticated vines and wild berries conserve significant amount of acids stored in their cellular tissues. Wild vines are by far high acidic genotypes. Organic acids enhance grape flavor and help to improve mouthfeel of table grapes [39,40]. Tartaric acid is stronger than malic acid and tastes sourer at the same concentrations [41]. Sourness was clearly appreciated in lots of wild Tunisian grapes. Malic acid tastes harsh and yields a green sensation. A high ratio of tartaric to malic acid generates more stability to wine while table grape should have a lower ratio of tartaric/malic acid for palatability [21]. The quantitative ratio between tartrate and malate is reported to vary considerably depending on the grape variety [37]. This was true for both *sylvestris* and *vinifera* grape populations. Wild as well domesticated vines predominantly store tartrate at ripening time. Although the low *β ratio* observed for wild vines compared to domesticated vines, berries from *V. sylvestris* are characterized by considerable amounts of organic acids at the last stage of fruit development.

The inheritance of sugars and organic acids between wild and domesticated vines is basically quantitative. Acidity was strongly additively inherited. Wild grapevines can be qualified as high acidic genotypes. The excessive tartness was correlated with low reducing sugar concentrations. Similar results were found in apple fruit: sugars were inherited quantitatively and acidity was strongly additively inherited [42]. It was admitted that with time, cross breeding and domestication lead to genotypes with low acid content which influence on the acceptability of grape berries. For a table grape breeding program, the cross parents should be moderate in both tartaric and malic acids. In the opposite, a high tartrate and a low malate cross parent is necessary for wine grape breeding program [21]. The rising warm conditions observed these last few years in the southern Mediterranean region needs new breeding reflections and alternatives to continue producing grapes sustainably. It was demonstrated that *V. sylvestris* material contains specific genes useful in genetic crossings with domesticated vines related to crop yield and quality of the berry [43]. El Oualkadi et al. [43] suggest that wild vines contain genes which facilitate the adaptation of grapes to their environment. Used as a rootstock, wild vine may widen the perspectives of *V. vinifera* crop cultivation. Li et al. [44] recently showed that the use of moderate rootstock could control vegetative growth and gain higher on quality of the berry. Tunisia, located in the southern Mediterranean seashore, is a hotspot for grape genetic biodiversity. Qualified as progenitor of domesticated grapevines, wild ecotypes are an important gene pool for viticulture. With the actual context of climate change, the need for new adapted cultivars grafted on more resilient rootstocks remains urgent. In addition to market demand and consumer acceptance, genetic research should move towards the establishment of new cultivars having strong adaptation to harsh growing conditions.

Conclusion

The fact that spontaneous *Vitis* resources are quite prospering in the north-west elevations of Tunisia confirm the authenticity and adaptive capacities of such plant material. The importance of adaptation remains evident since recent studies suggest that crop yield reduction will be severe in the coming decades under conditions of drought and extreme heat. Glucose/Fructose ratio, tartaric and malic acids are key descriptors for biochemical evaluation of wild grape germplasm. Wild vines are acidic genotypes largely developing tartaric and malic acid in their berries. Glucose largely predominate fructose with proportions exceeding 12-fold in some genotypes. With ampelometric and molecular tests already established on local vine resources, advanced works on interspecific crossing within *V. vinifera* should be carried out to provide alternatives for sustainable cultivation of grapes. The actual study should represent a comprehensive biochemical database of *V. vinifera* subsp. *sylvestris* for grape breeders and viticulturists.

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