FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

The Glucose-Fructose ratio of wild Tunisian grapes

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Abstract: Glucose and fructose make up the bulk of sugars in grapes at all stages of development of the berry. The present study was made to determine the glucose/fructose balance of 17 wild grapes *Vitis vinifera* subsps. *sylvestris* growing under Mediterranean conditions of Tunisia for future breeding programs and selection. Cultivar "Meski Rafraf" (MKR) was used as reference for evaluation of wild ecotypes. Grape samples were collected from the North-west of Tunisia during 2014 and 2015. Glucose and fructose were the main sugars described in wild berries. Sucrose was present in little amount (<0.1 g/100 g FW). Except for ecotype 33 (EC.33), glucose was higher than fructose in all accessions. EC.46 is high-glucose ecotype (9.69 g/100 g FW) and would yield less sweet and better-balanced musts. EC.10 and EC.13 are high-fructose ecotypes (6.49 and 6.45 g/100 g FW respectively) and would result in sweeter tasting musts at lower total sugar content. The glucose/fructose ratio of wild Tunisian grapes varied over a wide range, from at least 0.26 (EC.33) to 12.38 (Cap Negro 6/2000) with differences being highly significant (*p* < 0.01). Grapes from cultivar "Meski Rafraf" showed an average value of 1.22. This cultivar belongs to the same homogeneous subset composed by EC.01, EC.10, EC.13, EC.15, EC.26 and EC.33. The level of reducing sugars in grape berry should represent an ideal tool for harvest date prediction and for sensory evaluation of wild grapevines. This study will help to lay out the foundation for accelerating the progress of trait improvement of quality in local grapevine resources.

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PUBLIC INTEREST STATEMENT

With accentuation of the aridity in the southern shore of the Mediterranean, the urgency to save local grapevine resources remains undeniable. The substitution of many local cultivars by newly bred varieties is causing a rapid decline in the number of local genotypes. Domestication of grapevine should consider sugar content and composition of the berry. Taste and flavor of table grapes depend primarily on their initial sugar content. Several vines growing spontaneously in the forest and mountainous zones of the north of Tunisia were collected for sugar assessment of their berries. Berry sugar concentration is a relatively stable trait for a given cultivar, being less responsive to environmental conditions and viticulture practices. The glucose/fructose ratio, a useful index to evaluate sugar composition among grape accessions, showed high genetic variability in both sugar concentration and sugar balance within the *Vitis sylvestris* plant material.

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1. Introduction

The wild grapevine (*Vitis vinifera* subsp. *sylvestris*) is a heliophilous liana growing generally along river banks and in alluvial and colluvial deciduous and semi-deciduous forest (Arnold, Gillet, & Gobat, 1998; Levadoux, 1956). It is distributed in a wide area from Western Europe to the Trans-Caucasian zone and around the Mediterranean Basin, except the most southern infra-Mediterranean and non-Mediterranean zones (Arnold et al., 1998). Most botanists regard the wild ancestral grape *V. sylvestris* as the primitive form of the cultivated grape because of the close morphological resemblance and free gene flow between them (Heywood & Zohary, 1995). In Tunisia, wild grapevines are known as "Aneb El Jali" or "Hormos" (Harbi-Ben Slimane, Snoussi, Bouhlal, & Nahdi, 2010) and wild populations have been reported along the seashores, as well as in the northwest region of the country (Harbi-Ben Slimane, 2004; Levadoux, 1956). Spontaneous ecotypes were encountered in forest sites of northern side of the country in isolated forms or in groupings. These ecotypes show a remarkable polymorphism and a great morphological variability (Harbi-Ben Slimane et al., 2010). However, with the actual climatic context and intensive forest exploitation, their number remains very limited and spontaneous vines become threatened by various environmental and anthropogenic factors. *V. vinifera* subsp. *sylvestris,* is a unique and valuable genetic resource for the improvement of cultivated grapevines regarding their genetic tolerance to salinity (Raymond et al., 2008), their resistance to many virus diseases and their high adaptation potential to different soil types and climates (Arnold et al., 1998; Ocete, Del Tío, & Lara, 1995).

Grapevine breeding efforts focused on the stable yields, good quality of grapes but also on the adaptability to soil and to climatic conditions prevailing now worldwide and subsequently in the Mediterranean basin (Pavlousek & Kumsta, 2011). Grape quality is determined by primary (carbohydrates and organic acids) and secondary metabolites (phenolic compounds and aromatic substances) (Pavlousek & Kumsta, 2011; Rusjan, Korosec-Koruza, & Veberic, 2008). The contents and proportions of primary metabolites are decisive for the final quality of grapes and juice (Ali, Maltese, Hae Choi, & Verpoorte, 2010; Rusjan et al., 2008). Grape organoleptic quality greatly depends on both the content and composition of sugars and these are important factors in the selection of a new cultivar (Liu, Wu, Fan, Xu, & Li, 2007). Sugar concentration in grapes plays an important role in shaping berry sensory properties, determining alcohol concentration after fermentation, and providing precursors for synthesis of organic acids, phenolics, and aroma compounds (Dai et al., 2011). In cultivated grapes, berry ripening is accompanied by accumulation of hexose sugars (glucose and fructose) which contribute a large proportion to the soluble sugars in ripe berries (Coombe, 1992; Shiraishi, 1993). Mature grape berries contain one of the highest concentrations of sugar. This concentration may be higher than 1 M glucose and 1 M fructose (Coombe, 1976). Sugars accumulated in berry originate from sucrose that is imported from photosynthesizing leaves. Sucrose, produced by leaf photosynthesis, is thought to be transported by the phloem to the berry, where most of sucrose is enzymatically hydrolyzed into glucose and fructose (Hardy, 1968; Shiraishi, 2000). Thus, the sugars present in grapes are largely glucose and fructose. Fructose is much sweeter than glucose. The relative sweetness changes slightly with concentrations. On the other hand, most yeasts ferment glucose more rapidly than fructose but rapidly ferment both in mixtures (Amerine & Thoukis, 1958). These facts are of particular interest for viticulturists. In Tunisia, where production of table grapes or juice generally occurs under hot climate conditions, sugar production is too high for balanced grape juice. High-glucose varieties would yield less sweet and better balanced musts. Information on the glucose/fructose ratio existed but is limited to some varieties harvested from limited climatic regions. This sugar ratio is made to determine the normal glucose and fructose content of grape varieties. It was admitted that at maturity the ratio is about 1. In unripe grapes glucose predominates, while in overripe grapes fructose constitute the major sugar (Amerine & Thoukis, 1958).

The genetic diversity of the *sylvestris* in the northwest Tunisia was already evaluated by ampelometric tests (Harbi-Ben Slimane, 2004) and by nuclear and chloroplast molecular markers (Snoussi, Harbi-Ben Slimane, Ruiz-Garcia, Martinez-Zapater, & Arroyo Garcia, 2004; Zoghlami et al., 2013). Quality potential of wild populations is still little known and biochemical analysis of the berry could further lighten breeder and viticulturists about quality performances of these fragmented resources. The local cultivar "Meski Rafraf" (MKR), well appreciated for size, colour and taste of the berry, produces rich fruit in sugars with low acid taste. Defined as a table grapevine, the vintage is widely disseminated in the market and used as a reference to make comparison with other local or introduced varieties. The present study was made to evaluate Tunisian wild grape populations toward the quality of their berries, in particular their sugar content and hexose ratios searching for new adapted cultivars able to cope with the threats of aridity in the vine growing areas.

2. Experimental

2.1. Plant material

During the two seasons 2014 and 2015, lots of grapes were picked from about 17 vines growing spontaneously under Mediterranean conditions in the north-west of Tunisia (latitude: 37°15′ N; longitude: 9°26′ E; altitude: 202 m). *Sylvestris* ecotypes were often found along the continuous water streams and channels in the down side of hills and mountains generally in association with other plant species. Ecotype was defined as a variant in which the phenotypic differences are too few or too subtle to warrant being classified as a subspecies (Mayr, 1999). Grapes from wild ecotypes were collected from 6 locations (Table 1) with a minimum of 5 clusters per ecotype depending on the availability of clusters

Ecotype	Label	Origin	Berry*			TSS	Glucose	Fructose	Sucrose	Glucose–Fructose
			Size O-221	Shape O-223	Colour O-225					
30	EC.30	Tabarka	Small	Round	Dark red-violet	25.9 ± 2.0	7.93 ± 1.1cde	4.27 ± 0.9cd	<0.1	1.90 ± 0.4cd
46	EC.46	Sejnane	Very small	Round	Blue-black	27.5 ± 1.3	9.69 ± 0.9a	4.43 ± 0.7cd	<0.1	2.24 ± 0.6cd
3	EC.03	Nefza	Very small	Round	Blue-black	24.0 ± 0.6	6.83 ± 0.7 ef	2.91 ± 0.2e	<0.1	2.36 ± 0.4cd
45	EC.45	Sejnane	Very small	Round	Blue-black	27.8 ± 1.7	8.15 ± 0.9bcd	4.64 ± 0.3c	<0.1	1.77 ± 0.3cd
15	EC.15	Nefza	Very small	Round	Blue-black	24.5 ± 2.1	7.52 ± 0.3de	6.01 ± 0.3b	<0.1	1.25 ± 0.1d
26	EC.26	Ain Draham	Very small	Round	Dark red-violet	23.3 ± 2.2	6.10 ± 0.7f	4.67 ± 0.5c	<0.1	1.31 ± 0.0d
13	EC.13	Nefza	Very small	Round	Blue-black	24.8 ± 1.7	8.44 ± 0.5bcd	6.45 ± 0.4ab	<0.1	1.32 ± 0.2d
10	EC.10	Nefza	Very small	Round	Blue-black	25.1 ± 0.4	8.63 ± 0.4abcd	6.49 ± 0.7ab	<0.1	1.33 ± 0.1d
33	EC.33	Tabarka	Very small	Round	Blue-black	26.3 ± 0.9	0.45 ± 0.1g	1.68 ± 0.2f	<0.1	0.26 ± 0.1d
14	EC.14	Nefza	Very small	Round	Blue-black	25.4 ± 1.4	7.50 ± 0.4de	1.22 ± 0.4fgi	<0.1	6.60 ± 2.1b
9	EC.09	Nefza	Small	Round	Green yellow	24.7 ± 0.9	9.19 ± 0.3ab	1.38 ± 0.2fg	<0.1	6.77 ± 1.0b
1	EC.01	Nefza	Very small	Round	Blue-black	25.3 ± 1.2	0.62 ± 0.1g	0.68 ± 0.3gij	<0.1	1.04 ± 0.5d
35	EC.35	Tabarka	Very small	Round	Green yellow	25.8 ± 1.5	0.39 ± 0.3g	0.31 ± 0.1j	<0.1	1.57 ± 1.6cd
Cap Negro 5/2000	CPN5/2000	Cap negro	Very small	Round	Blue-black	27.6 ± 1.1	6.93 ± 0.3ef	1.70 ± 0.3f	<0.1	4.16 ± 0.6c
Cap Negro 6/2000	CPN6/2000	Cap negro	Very small	Round	Blue-black	26.5 ± 0.7	5.85 ± 0.7f	0.52 ± 0.2ij	<0.1	12.38 ± 5.1a
Cap Negro III	CPNIII	Cap negro	Very small	Round	Blue-black	27.8 ± 0.7	5.88 ± 0.3f	3.85 ± 0.7d	<0.1	1.57 ± 0.3cd
Ouchtata 16	OCT16	Ouchtata	Small	Round	Green yellow	23.7 ± 1.4	7.66 ± 0.9cde	0.99 ± 0.2fgij	<0.1	7.86 ± 1.2b
Meski Rafraf	MKR	Rafraf	Large	Obtuse-ovate	Green yellow	22.7 ± 2.5	8.77 ± 0.8abc	7.16 ± 0.3a	<0.1	1.22 ± 0.1d

Note: TSS: total soluble solids expressed in °Brix at 20°C.

*Descriptors for Grapevine (IPGRI, UPOV, OIV, 1997). TSS: total soluble solids expressed in °Brix at 20°C.

and the number of ripe berries per cluster. Given the heterogeneity in the fruit set and ripening process of the berry observed in the same cluster (Millerandage), some grapes were found unripe at the sampling date. The local cultivar "Meski Rafraf" was subject to the same sugar analysis and used as standard for wild ecotypes evaluation. Grape clusters were collected 107 days after anthesis for ecotypes originated from "Nefza" and "Ouchtata", 104 days after anthesis for ecotypes originated from "Tabarka" and "Ain Draham" and 109 days after anthesis for ecotypes originated from "Cap Negro" and "Sejnane". Clusters from the cultivar "Meski Rafraf" were harvested 136 days after anthesis. All these accessions are actually part of the national germplasm collection maintained in the vineyards of the Horticulture Laboratory in the National Institute of Agricultural Research (INRAT, Tunisia).

2.2. Sugar analysis

A preliminary description was given on the basis of morphological characters. Size, shape and colour of the berry were determined referring to the descriptors for grapevine (IPGRI, UPOV, & OIV, 1997). Grape clusters were scraped and seeds were removed from the berry. Total soluble solids (TSS) of the juice were determined with a digital refractometer (OPTECH GmbH, Munchen, Germany) and expressed in °Brix at 20°C. The pulp was then ground with liquid nitrogen using a CryoMill (Retsch MM301, Retsch GmbH, Haan, Germany). From each sample, 5 g of frozen grape pulp powder were mixed with 20 ml ultra pure water. Samples were ground using ultraturrax T25 equipment (Ika Labortechnik, Staufen, Germany) to obtain slurry. The mixture was homogenized and then centrifuged for 10 min at 4°C (9,000 rpm). Samples were then filtered and 250 µl of the supernatant was recovered. The extracts were kept at −20°C until analysis. Glucose, fructose and sucrose were enzymatically quantified with kits (D-Glucose and D-Glucose/D-fructose) for food analysis (Boehringer Mannheim Co., Mannhein, Germany) using a SAFAS FLX-Xenius microplate (SAFAS, Monaco) equipped with a SAFAS automatic injector and results were expressed in g/100 g fresh weight. The amount of NADPH formed during Hexokinase (HK) and glucose-6-phosphate dehydrogenase (G-6-P-DH) reactions is proportional to that of D-glucose and D-fructose. The measurements were carried out at 340 nm, maximum absorption of NADPH.

2.3. Glucose–fructose ratio determination

The Glucose–fructose ratio was calculated from periodical tests made during the ripening period on a minimum of 5 cluster samples of fruit depending on the availability of grapes in wild grapevines. The ratios were generated after determining the relative proportions of glucose and fructose being the prime source of experimental information about sugar content in wild grape accessions. The glucose/fructose ratio was determined referring to the works of Amerine (1954), Amerine and Thoukis (1958) and later Shiraishi (1993) who proposed glucose to fructose ratio as useful descriptor for the evaluation of sugar composition in grape berries. The general conclusion made by these authors has been that in unripe grapes, glucose predominates, that at maturity the ratio is about 1, and that in overripe grapes fructose constitutes the major sugar.

2.4. Statistics

Soluble and individual sugar statistics are means ($N = 3$) ± standard deviations (SD). Data were subject to one-way analysis of variance (ANOVA). Significant differences were assessed with Duncan's multiple range test ($p < 0.05$) and means for groups in homogeneous subsets were displayed. Principal component analysis (PCA) was carried out to discriminate between wild vine ecotypes on the basis of sugar content and sugar balance of the berry. Statistics were performed using PC software package SPSS (IBM® SPSS® Statistics, version 20.0.0).

3. Results and discussion

Wild vines develop small to very small berries. For most accessions, the berry was round, blue-black coloured with large seeds (Figure 1). Three ecotypes produced light coloured berries. Cultivar "Meski Rafraf" is characterized by a large berry, obtuse-ovate shaped with a green-yellow skin colour which turns to yellow at over-ripeness. Compared to the domesticated "Meski Rafraf", there is a clear phenotypic variance between wild and cultivated grapes. Geographical origin of these spontaneous populations (humid locations of the coastline) could help to define their origin and confirm the

Figure 1. Grape cluster from wild "Cap Negro 6/2000". Origin: Cap Negro, harvest time: 23 August.

hypothesis which argues in favour of the existence of genetic grapevine exchanges and migrations among Mediterranean basin (Bouby & Marinval, 2001; Terral et al., 2010).

3.1. Sugars in wild grapes

Total soluble solids (TSS) ranged from 23.3 to 27.8% in wild grapes. The lowest content was recorded in EC.26 berries while the highest value was determined in berries from EC.45 and CPNIII grapevines. All ecotypes yielded grapes with high soluble solids content with variance being no significant between samples. TSS values defined in wild grapes were higher than that determined in berries collected from cultivar "Meski Rafraf" (Table 1). Among 26 species of *Vitis* including species from North America and Middle East regions, Kliewer (1967) reported a wider range of variation in TSS at maturity, ranging from 13.7 Brix (*V. champini*) to 31.5 Brix (*V. riparia* from Wyoming). Considered an important predictor of consumer acceptability, TSS is closely related to consumer acceptance (Crisosto, Bremer, Ferguson, & Crisosto, 2010). As it informs about sweetness of the fruit, TSS is highly correlated with ripeness in most fruit.

The sugars present in wild grapes were largely glucose and fructose (Table 1). Concentrations in glucose ranged between 0.39 and 9.69 g/100 g FW. EC.46 produced the richest berries in glucose while grape berries from EC.35 had the lowest content. Concentrations in fructose were comprised between 0.31 and 6.49 g/100 g FW. Grapes from EC.10, EC.13 and EC.15 had the highest values. In cultivar "Meski rafraf", concentrations in glucose and fructose reached respectively 8.77 and 7.16 g/100 g FW. Sucrose was present at trace amounts in all accessions (<0.1 g/100 g FW). As shown in Table 1 by groups from cluster analysis, differences between all accessions were high significant ($p < 0.01$). Except for Ecotype 33, glucose was the predominant sugar determined in wild grapes. Ecotypes EC.46, EC.09, EC.10 were among high glucose ecotypes bearing fruits with high glucose proportions.

Other works dealing with commercial "Red Globe" and "Thompson Seedless" varieties showed 8.06 and 8.71g/100 g FW glucose and, 8.74 and 8.05g/100 g FW fructose respectively (Munoz-Robredo, Robledo, Manriquez, Molina, & Defilippi, 2011). "Chasselas red" and "Muscat Hamburg", cultivated in the sub-Mediterranean region of Slovenia showed average values of 7.43g/100 g FW glucose and 7.70g/100 g FW fructose (Rusjan et al., 2008). Mature grape samples from five Turkish varieties cultivated in Van province showed average concentrations of 12.05 and 11.34g/100 ml

glucose and fructose respectively (Sensoy, 2015). Such results are in agreement with values recorded for cultivar "Meski Rafraf" (8.77 and 7.16g/100 g FW glucose and fructose respectively). However for the wild vines there is a large array of concentrations in glucose and fructose between spontaneous genotypes. Two among wild genotypes (EC.09 and EC.46) developed more glucose in their berries than cultivar "Meski Rafraf". On the other hand, concentrations in fructose were lower in spontaneous grapes than that developed by "MKR" berries. This was clearly appreciated by the low sweet taste of the wild berries. Sugars are among leading factors affecting taste formation in fruits and are highly correlated with ripeness (Sensoy, 2015; Trad, LeBourvellec, Gaaliche, Renard, & Mars, 2014). Except during the green berry stage, glucose and fructose amounts are equal with increasing maturity (Kliewer, 1966). 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* (Liu, Wu, Fan, Li, & Li, 2006).

The differences between wild genotypes might be the consequence of genetic factors as weather conditions are the same for the different collection sites. Some authors suggest that wild vines contain genes which facilitate the adaptation of grape to its environment (El Oualkadi et al., 2011). The reduction of diversity attributable to domestication and breeding appears to be weak on a genome-wide scale; a few notable changes in morphology have emerged since grape domestication, including larger berry sizes, higher sugar content and a wide range of berry colours (Olmo, 1995). *V. sylvestris* material contains specific genes useful in genetic crossings with domesticated vines for improvement programs related to quality and production of the local resources (El Oualkadi et al., 2011).

3.2. The glucose–fructose ratio

Investigations on spontaneous grape samples of the 2014 and 2015 seasons showed that five have about equal proportions of both sugars, 11 having more glucose than fructose and only one with more fructose than glucose. Determination of the glucose/fructose ratio separated wild grape populations into 5 homogeneous subsets (Table 2).

Table 2. Means for groups in homogeneous subsets based on the glucose–fructose ratio performed by *Duncan*'s multiple range test* (*p* < 0.05)

	a	b	c	d
CPN6/2000	12.38			
OCT16		7.86		
EC.09		6.77		
EC.14		6.60		
CPN5/2000			4.16	
EC.03			2.36	2.36
EC.46			2.24	2.24
EC.30			1.90	1.90
EC.45			1.77	1.77
EC.35			1.57	1.57
CPNIII			1.57	1.57
EC.10				1.33
EC.13				1.32
EC.26				1.31
EC.15				1.25
MKR				1.22
EC.01				1.04
EC.33				0.26

*Post Hoc test using mean harmonic sample size *N* = 3.

Only EC.33 produced berries with more fructose than glucose (0.45 g glucose and 1.68 g fructose/g FW). The first three groups are composed by ecotypes yielding grapes with high-glucose content CPN6/2000, OCT16, EC.09, EC.14 and CPN5/2000 (glucose/fructose ratio > 4). Six ecotypes showed almost equal proportions of both sugars and were associated in one homogeneous subset. In this group we could identify the cultivar "Meski Rafraf" with sugar ratio of 1.22. It was adopted that full ripeness stage is defined by sugar ratio fairly equal to 1 except for the green berry stage (Kliewer, 1966). Grapes from ecotypes EC.01, EC.10, EC.13, EC.15 and EC.26 with respectively: 1.04, 1.33, 1.32, 1.25 and 1.31 sugar ratio should be classified as ripe material at harvest time. Immature berries contain more glucose than fructose (Kliewer, 1965, 1966). This was the case for 11 among the 17 accessions considered unripe enough when picked. Hydrolysis of starch reserves into glucose, conversion of fructose into glucose, or preferential metabolism of fructose could account for the differences between glucose and fructose amounts during the green berry stage (Kliewer, 1966).

Liang et al. (2011) 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 (Fu, Wu, Liu, Wang, & Yang, 2015). There is no large discrepancy with results found by Pavlousek and Kumsta (2011) whose found similar proportions in 15 varieties cultivated in Czech Republic (0.92 < Glucose/Fructose < 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 here for wild vines. In the present study, the gap in sugar ratio seems very important and could represent an additional mark of genetic diversity in wild populations. Shiraishi (1993) proposed that the glucose/(glucose + fructose) ratio could be a useful index to evaluate variations in sugar composition among grape accessions. The weekly change in sugar composition of grape cultivars was easily monitored by this ratio. He reported no significant changes in the sugar ratio for 14 days prior to maturity indicating that glucose to (glucose + fructose) is likely to be stable near to maturity.

The glucose–fructose ratio is reported to vary from 0.47 to 1.12, with only two species containing more glucose than fructose (*V. champinii* and *V. doaniana*). Most wine grapes from *V. vinifera* have a glucose–fructose ratio of 1 at maturity (Kliewer, 1967). Shiraishi, Fujishima, and Chijiwa (2010) showed that sugar composition and sugar balance are influencing parameters on the genotypic variance. With a sugar ratio ≥ 0.81, Tunisian wild grapevines are classified among hexose accumulators which accumulate fructose, glucose and trace amounts of sucrose (Shiraishi et al., 2010). The variance observed between ecotypes relies more on the genotype than on the region as grape clusters developed under the same bioclimatic conditions.

3.3. Principal component analysis

PCA indicated that the first two PCs accounted for about 95% of total variance among sugar content and sugar ratio (Figure 2). PC1 (55%) was highly connected with fructose content. Glucose and glucose/fructose ratio were important contributor to the variance for PC2 (40%). In a scatter plot of the score values of all genotypes projected to the PC1 and PC2 plane three groups of accessions tend to cluster based on the sugar content and sugar balance of the berry.

The first group, comprising the cultivar "Meski Rafraf", was defined by high fructose content. Fructose confers the sweet taste to the berry. Grapes from cultivar "Meski Rafaf" develop a very sweet taste when ripe. EC.10, EC.13 and EC.15 were also characterized by high amounts of fructose in the fruit. These ecotypes yield dark blue coloured berries and should be recommended as table grapes in future breeding programs. The second group was represented by EC.09, EC.14, OCT16 and

Figure 2. PCA showing the variability between wild vine ecotypes on the basis of the berry sugar content.
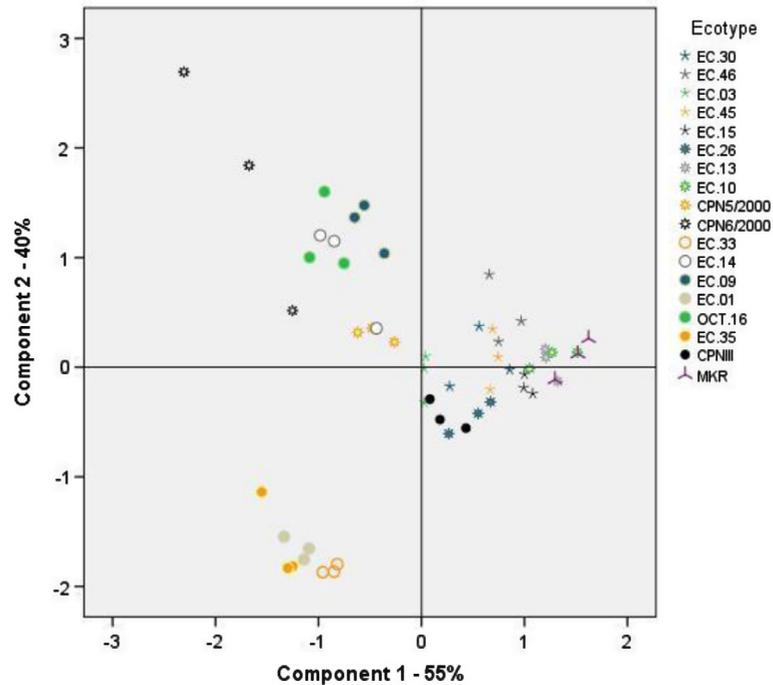
CPN6/2000 and was linked with glucose content. These ecotypes are high-glucose grapevines and would yield high fermentable musts. Such genotypes could be intended for wine making. Among cultivars of *V. vinifera*, wine grapes were found in general to have more sugars than table grapes (Liu et al., 2006). "Meski Rafraf" was the genotype with high sugar content (sum of glucose and fructose) in comparison to the wild vines. Among wild populations, EC.10, EC.13 and EC.46 produced berries with high sugar content.

Domestication of grapevine should take into account sugar content of the berry as a determining chemical component for the postharvest destination (table grape use, juice making, wine making …). Biochemical resemblances between spontaneous and domesticated grapevines concerned 3 among the 17 wild genotypes studied in this work. Further consideration should be paid to this invaluable plant material to hasten selection and conservation activities for sustainable viticulture. More actions should be undertaken to preserve the spontaneous *Vitis* heritage, as a result, to enrich the diversity among the local national germplasm.

4. Conclusions

The study related to local resources of spontaneous grapevines pointed out a large genetic diversity on the basis of sugar content and sugar balance of the berry. Wild ecotypes developed grapes with high soluble solids, a key component for evaluating fruit quality both for processing and fresh market use. Wild grape ecotypes are hexose accumulators containing large proportions of glucose and fructose and trace amounts of sucrose. Amongst the wild accessions studied in this work, eleven developed more glucose than fructose in the berry with sugar ratio more than 6 for 4 among there. Five ecotypes (EC.01, EC.10, EC.13, EC.15 and EC.26) yielded grapes with balanced sugar musts (Glucose/fructose ratio ≈ 1). These vines originated mostly from "Nefza" were closely associated to the local "Meski Rafraf" cultivar. Such plant material should be further considered for possible breeding programs and selection. The results disclosed in the present work could develop a comprehensive database of primary metabolites in the *Vitis* subsp. *sylvestris* for grape breeders and contribute to complete ampelometric and molecular tests established on local vine resources for an accurate inventory.

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