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Genetic and Morphological Characterization of Grapevines in Hebron District

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DEDICATION

To the spirit of my dear mother

Acknowledgement

Thanks God for helping me.

I would like to express my sincere appreciation to my advisors Dr. Ayed Salama and Dr. Jamil Harb for their guidance, encouragement, and continuous support for this study.

Many thanks to my friends Arwa Mujahed and Saleh Al Seikh for their help and trust.

Thanks a lot to my father, my husband, and my brothers for their help, advice, and encouragement.

Great thanks to my mother (God rest her soul), for her continued support from the beginning**.**

For all……

Thank you

Mirvat

List of Contents

List of Tables

List of Figures

Abstract

Morphological and genetic studies were carried out on grape (*Vitis vinifera*) accessions in Hebron district (Dura, Hebron, Halhul, and Al Arrub). The morphological study was designed to characterize and describe some ampelographic characters for woody shoots, leaves, and clusters for five grapevines cultivars (Zane, Dapougy, Betuny, Shame, and Halawany) grown in the same field in Halhul.

The five cultivars were showed a high variability in many ampelographic characters, such as color of upper side of blade, shape of blade, degree of opening overlapping of petiole sinus, berry shape, berry skin color, distribution of anthocyanin coloration on prostrate hairs of young shoot tip, and density of prostrate hairs on tip of young shoot. Also there were common ampelographic characters between the five cultivars such as structure of surface of woody shoot, teeth in petiole sinus of mature leaf, and petiole sinus limited by veins in mature leaf. The highest approximate similarity percentage was 69.7% between Zane and Dapougy cultivars, and 63.6% between Shame and Dapougy cultivars. The lowest similarity values were 48.5% between Betuny and Zane, Halawany and Zane, and Shame and Zane combinations of cultivars.

Genetic characterization was carried out on 28 grape accessions from Dura, Hebron, Halhul, and Al Arrub by using 8 SSR markers (VVMD27, SCU05VV, SCU08VV, SCU11VV, SCU15VV, VMC8A7, VRZAG62, and VRZAG79). High genetic diversity is evident between unlike grape accessions, whereas high similarity is evident between identical grape accessions, with the greatest shared allele distance (d) detected is 1.00,

and the smallest is 0.2; the polymorphic percent of 100% indicates the high performance and accuracy of SSR markers.

High similarity between Zane and Dapougy in the morphological characters also related with rapprochement between these cultivars at the molecular level, as it is clear in the dendrogram. Taking into account that there are no big differences in various environmental factors between the four locations (Dura, Hebron, Halhul, and Al Arrub), the genetic characterization proves to be accurate to distinguish between the assessed 28 grape accessions.

Based on the obtained results, there is an urgent need in reconsidering of the naming of some grape accessions. Moreover, fingerprinting techniques should be utilized for characterization of all grapevine varieties in Hebron district by competent scientific institution, since grape is a very important economic crop in Hebron district.

Introduction

A grape is considered as one of the most important and widely distributed fruit crops in the world. It is believed that grape cultivation began 6,000–8,000 years ago in the Near_East, and later distributed to Mediterranean, and Central Europe (Bassermann, 1923; Kirchheimer, 1938). Concerning its nutritional value, grapes are very nutritious and may be even of medical value. In this respect, Phoenicians spreaded the cultivation of grapes to Palestine, Syria, Jordan, North Africa and Spain (Jabar et al., 1989; Saidi et al., 1984).

The long domestication of grape led to huge diversity in its genotypes; Alleweld et al. (1990) estimated that the number of grape cultivars worldwide is around 14,000, although with numerous synonyms and occasional use of the same or similar names for genetically different cultivars. This huge diversity is attributed partially to the ease of vegetative propagation, which favored widespread diffusion of many cultivars to diverse regions of the world (Dion, 1977; Fregoni, 1991). As a consequence, some cultivars may now have up to 100 synonyms, and numerous homonyms also exist. Because accurate identification of accessions is a basic requirement for the rational management and use of germplasm, the clarification of synonymy, homonymy, and misnaming is a significant problem in the 130 grapevine collections that exist worldwide (Dettweiler et al. 2000). In this sense, the validity of various grape species is questioned frequently, since most of the grape species can be inter-crossed to give vigorous and fertile F1 hybrids (Hancock, 1992).Consequently, the documentation, preservation and utilization of grape germplasm is problematic. Accordingly, scientists try to alleviate this situation by developing techniques for grape genotypes and species

characterization based on various morphological, biochemical and molecular criteria, including protein and/or DNA polymorphisms (Grando et al., 1996; Lamikanra, 1993; Subden et al., 1987; Thomas and Scott, 1993).

This study was applied in Hebron District in Palestine, where grape accessions from four locations (Al Arrub, Dura, Halhul, and Hebron) were studied. Taking into account that Hebron is the first in cultivation of grape in Palestine, there are many grape varieties grown in this region, with significant differences in color, taste, morphology, and shape between these varieties. Moreover, there are some grapes accessions have the same name (synonyms) but differ in terms of genetic, and vice versa. For this, morphological and genetic characteristic study for grape accessions in these four locations in Hebron was applied to get morphological and genetic descriptions for these accessions and to make comparison between them. In addition, the study will provide information about their genetic and morphological relatedness.

The morphological observations were carried out both at the field and in the laboratory, and led to elucidating of significant differences between grapevine cultivars (Santiago *et al*., 2005). In addition, the identification and mapping of DNA polymorphisms in many cultivated plants has been done using microsatellite, and random amplified polymorphism DNA (RAPD) (Luo and He, 2001; Weising et al., 2005).

Objectives

The aims of this study are:

1. Morphological description of five grape cultivars grown at Hebron district (Zane, Betuny, Halawany, Dapougy, Shame).

2. To determine the genetic variability and cultivars relatedness for available grape accessions through utilizing SSR fingerprinting technique.

Chapter One

Literature Review

1.1 Grape

Grapevine (*Vitis vinifera* L.) is one of the oldest and most important crops in the world (Karatas et al., 2007), and there are around 60 known grape species, primarily in the Northern Hemisphere (Winkler et al., 1974). The importance of grapevines is related to its products, mainly wine, table grapes, and dried fruits; the total number of grapevine cultivars in ampelographic collections is estimated to be more than 10,000 (Pelsy, 2010).However, other *Vitis* species are also important in grape improvement. In this sense, (*V. riparia* Michx.) from North America has served as a commercial rootstock due to its resistance to phylloxera (*Daktulosphaira vitifoliae* Fitch). Further, *(V. amurensis* Rupr.) of East Asian origin, is used widely for breeding cold-tolerant hybrids (Person and Goheen, 1990). Accordingly, various grapevine species are attractive for genomic research; the small genome size of 475-500 Mb (diploid; 19 chromosomes) makes such work easier than other plants (Thomas et al., 1993; Lodhi and Reisch, 1995).

The species *Vitis vinifera* is the only grape species that is used extensively in the global wine industry, and it is the only species of the genus that is indigenous to Eurasia. Accordingly, the two forms still co-exist: the cultivated form, namely the *V. viniferasub* sp. *vinifera* (or *sativa*) and the wild form, namely *V. vinifera* subsp. *silvestris* (or *sylvestris*); both are treated as two separate subspecies. It is worth to mention here that this historical separation into subspecies is based primarily on morphological differences (Zohary, 1995).

Taking into account that grapes are temperate climatic plants, grape cultivation in Palestine can be traced back to the earliest recorded history. Temperature is one of the most important factor in determining grape success, in particular the physiological growth of root, which requires moderate temperatures. In this sense, temperatures above 30Cº may harm vegetative and reproductive growth. On the other side, two to three months of winter temperatures between -1 and 10Cº are needed for breaking bud dormancy. Another important factor is the rainfall, and amounts between 400-500 mm are required. In addition, deep soil with proper water holding capacity is also needed for normal growth and development of grape vines. However, grapes may be cultivated in different types of soil, although shallow, poorly drained, and salty soils should be avoided, grapes may tolerate moderately saline soils (2000 ppm) (Kleif et al., 1991). Soil pH may affect the growth of vines, and pH requirements vary according to cultivars, with *V. vinifera* cultivars require 6.5-7.0 pH range.

1.2 Effect of environmental factors on plant characteristics

As mentioned above, various environmental factors highly influence the growth and development of grape vines. Among these factors are temperature, soil, rainfall, solar radiation, and wind (Saatchi et al., 2007). Taking into account that plants exhibit a variety of responses to abiotic stresses that enable them to grow, develop, and survive adverse conditions (Knight and Knight, 2001). It is of great value to assess connection of genetic makeup and plant growth and development. In this

sense, plants have evolved highly sophisticated and varied methods to enable them to survive environmental changes (Ferguson, 2004). Among these survival mechanisms are structural traits, which reflect the adaptation strategies of plant to the environmental stresses; these traits include leaf size, leaf shape, and specific leaf area. Accordingly, the relationship between plant forms and environmental factors play central roles in plant ecology and in the study of convergent evolution (Mooney, 1977). In this respect, leaf traits are considered as the major ones that are particularly important in carbon assimilation, water relations and energy balance. It was proven that leaf size and specific leaf area (SLA) decline along the gradients of decreasing moisture and nutrients availability (Hamann, 1979; Cunningham et al., 1999; Fonseca et al., 2000). Moreover, such patterns are consistent with scientists understanding of the functional roles of these traits. As an example SLA is negatively correlated with the assimilation rates and life span of plants, and usually lower in leaves of evergreens (Reich et al., 1997). In this sense, lower SLA, which reflects thicker leaves, contributes to higher protection from desiccation (Mooney and Dunn, 1970). Moreover, small leaf size is usually coupled with a reduced boundary layer resistance, which may help in maintaining favorable leaf temperatures and consequently higher photosynthetic water-use efficiency, in particular under high solar radiation and low water availability (Parkhurst and Loucks, 1972).

The adaptive value of functional traits reflects usually the widespread correspondence between phenotypic variations and various environmental conditions, which also reflects the adaptedness of such plants. As an example, as water availability increases the leaf size commonly increases, which can be seen among individuals (Sultan and Bazzaz, 1993), species (Cunningham et al., 1999), and even communities (Dolph and Dilcher,

1980; Fonseca et al., 2000). In this sense, these patterns are the reflections of various interacting processes that include phenotypic plasticity, (Sultan, 1987), ecological sorting (Weiher and Keddy, 1995) and adaptation by natural selection (Cody and Mooney, 1978).

1.3 Plant morphology and genetic variability

Plant morphology includes both internal and external forms from the molecular level to organism level (Sattler, 1978), and is highly related to the genetic makeup of the plant, although environmental factors play major role in defining the final form of it.

Accordingly, the ability of an individual to tolerate stresses through morphological adjustments is a major feature that determines the survival of any species, and hence the ecological breadth of that species (Bazzaz, 1996; Sultan et al., 1998). As an example, plants tend to have a reduced total dry mass and leaf number, when suffer nutrient stress in standing water (Zhang, 1996; Crossley et al., 2002). Moreover, plants increased the allocation of assimilates to root and stem, and reduce it to leaves (Gedroc et al., 1996) and reduce both leaflets number per leaf and leaf area (SLA) (Li et al., 1999). These changes reflect adaptations to nutrient-poor habitats. The significance of such morphological adaptations is to reduce damage risk.

However, following changes in environmental conditions, there will be changes in morphological characteristics so that plants can cope with the environmental changes. The most familiar morphological characteristics are those at the level of entire shoots, including live crown ratio, crown

shape, and multilayer distributions of foliage (Horn 1971; Kramer and Kozlowski, 1979). In addition, morphological traits of stems include length of internode, node density, total leaf area, and the density of flowers, fruits, leaves and buds (Bonser and Aarssen, 1994; Wilson, 1995).

Referring to the above mentioned adaptations, which can be either due to genotypic differentiation or phenotypic plasticity, the genotypic differentiation among plant populations is common (Heslop-Harrison, 1964; Langlet, 1971).However, genotypic differentiation alone is not sufficient, and large morphological differences among populations from different environments is partially due to phenotypic plasticity (Williams and Black, 1993), and plasticity is one of the choices in heterogeneous environments (Sultan, 1992). Moreover, with slow growing plants in stressful environments the physiological rather than morphological plastic responses are common (Grime, 1979; Hutchings and de Kroon, 1994).

1.3.1 Grape morphology and genetic diversity

Standard ampelographic descriptors are used usually to differentiate various grapevine cultivars, and ampelogrpahy is based on visual observation of certain traits. In addition, with ampelometry a method that relies on precise measurement of various phenotypic characteristics was developed, which is mainly based on leaf traits.

In this sense, the Office International de la Vigne et du Vin (OIV) published standard descriptors that are useful for the ampelography and comparison studies (OIV, 1984). However, the morphology alone is not

enough to distinguish between close varieties and other systems are needed (Ortiz et al., 2004).

Taking into account that ampelographic descriptions enable us to identify cultivars, it is crucial to consider the development stage of the plants, in addition to their health status and the prevailing environmental conditions (Cipriani et al., 1994). This is important to avoid misunderstandings, since the expression of morphological characters depends highly on the developmental stage of the plant (sample) and environmental conditions (Sefc et al., 2001).

Concerning previous studies with grape cultivars that belong to *Vitis vinifera* and *Vitis riparia* species, researchers confirmed that distinct macroscopic differences exist between them (Huglin, 1986; Galet, 2000). The great variability in leaf shape, in particular in the form of leaf sinuses aid highly in differentiating these cultivars (Galet, 2000; Martinez *et al*., 2005). It is worth to mention that variations in leaf characteristics reflect variations also in the histological structure. As an example, some grape cultivars that have only reclining threadlike trichomes of different length (Martinez *et al*., 2005), whereas others have erect trichomes like small transparent spines (Galet, 1956), and it is believed that such variation may render some cultivars more resistant to pathogens or environmental stresses.

Genetic diversity is the basis for survival and adaptation. The variation in morphological characteristics is largely a reflection of genetic variation among cultivars. In this sense, the evaluation of genetic diversity using morphological characters is very limited (Afghan *et al*., 2005), and there

is a need for modern techniques that can measure the genetic relationships, and exclude the influence of environmental factors and phenotype properties (Hussain, 2010).

Accordingly, the assessment of genetic diversity at the molecular level is routinely performed using laboratory-based techniques such as allozyme or DNA fingerprinting that measure directly the levels of variation. In this sense, genetic diversity may be gauged using biochemical and morphological characterization (Mondini et al., 2009).

Consequently, modern studies employed techniques like RFLPs, RAPDs, AFLPs or SSRs to differentiate between grape cultivars. (Faria et al., 2000). In addition, several genetic linkage maps are already published for grapes and the older ones were conducted using the RAPD or AFLP techniques (Lodhi et al. 1995; Dalbo et al. 2000; Fischer et al. 2004). However, and more recently, two maps are published using mainly SSRs markers and these serve as reference maps (Adam-Blondon et al. 2004; Riaz et al. 2004). SSR markers are known to be highly transferable among grapevine genotypes (Thomas and Scott, 1993; Adam-Blondon et al., 2004). Moreover, grape microsatellites are widely used for many applications, including mapping, genotyping, and breeding (Bowers et al., 1996; Scott et al., 2000). Taking into account that these markers provide a unique DNA fingerprint (Ciprianiet al. 1994), they are considered as the best choice for cultivar identification (Crespan, 2004), for the detection of clonal differences, and for the verification of synonymies or homonymies (Regneret al., 2000; Franks et al., 2002; Ulanovsky et al., 2002).

On the other hand, the RFLP technique proved to be very reproducible, although it is very demanding in terms of labor and time.

It is worth to mention here that different markers methods may give different views of diversity, and genomic compartment in which the markers reside might affect the diversity pattern seen (Jing, 2007). Accordingly, it is highly preferable to use more than one technique for genetic diversity assessment.

1.4 Simple Sequence Repeats (SSRs)

Genetic profiling of individuals is nowadays based on SSR(Simple Sequence Repeat) markers, which have a number of positive features that make them superior to any other type of molecular marker developed so far for DNA fingerprinting (Schlotterer, 2004).

*Vitis m*icrosatellites markers have been developed by various laboratories. As early as 1994, Thomas and Scott (1994) identified 26 grapevine cultivars, in addition to 6 *Vitis* species and *Muscadinia rotundifolia* L. by means of microsatellites. After that they established five microsatellite loci (VVS1, VVS2, VVS3, VVS4 and VVS5) from grapes; VVS2 and VVS5 showed the most polymorphic ones. Moreover, Bowers et al (1996) developed another four new microsatellite loci (VVMD5, VVMD6, VVMD7 and VVMD8) 77 cultivars of *V. vinifera* L.were analyzed. Later, Bowers et al (1999) developed an additional 22 VVMD loci for CT repeat motifs, and ran successfully studies with grape cultivars.

In addition to previous studies, and more recently, two distinctive maps that contain mainly SSRs were released to serve as reference maps (Adam-Blondon et al. 2004; Riaz et al., 2004). Researchers rely heavily on these maps to characterize and identify the existing grape cultivars.

Chapter Two
Materials and Method

2.1 Study site

This study was conducted at Hebron district in Palestine, where four locations were selected for sample collection, these are: Al Arrub, Dura, Halhul, and Hebron city (fig.1). The latitude, longitude, and altitude of these locations are shown in table (1).

Table (1) Geographical position of grape accessions

Location of grape	Latitude	Longitude	Altitude	Average precipitation (mm)*
Hebron	32.3 Nº	35.5 Eº	888 m	646
Dura	31.31 Nº	35.1 Eº	839 m	457
Halhul	34.31 Nº	35.5 Eº	916 m	531
Al Arrub	37.12 Nº	35.7 Eº	857 m	534

*Source: Palestinian Meteorological Department, 2012

Dura located 6 km West of Hebron city, Halhul located 6 km North of Hebron city, and Al Arrub located 11 km North of Hebron city. The Mediterranean climate prevails in Hebron District (ARIJ, 2009), where it is characterized by long hot dry summer and moderately cold winter. The average annual rainfall in the study locations range between 457mm in Dura and 646 mm in Hebron (Table 1).

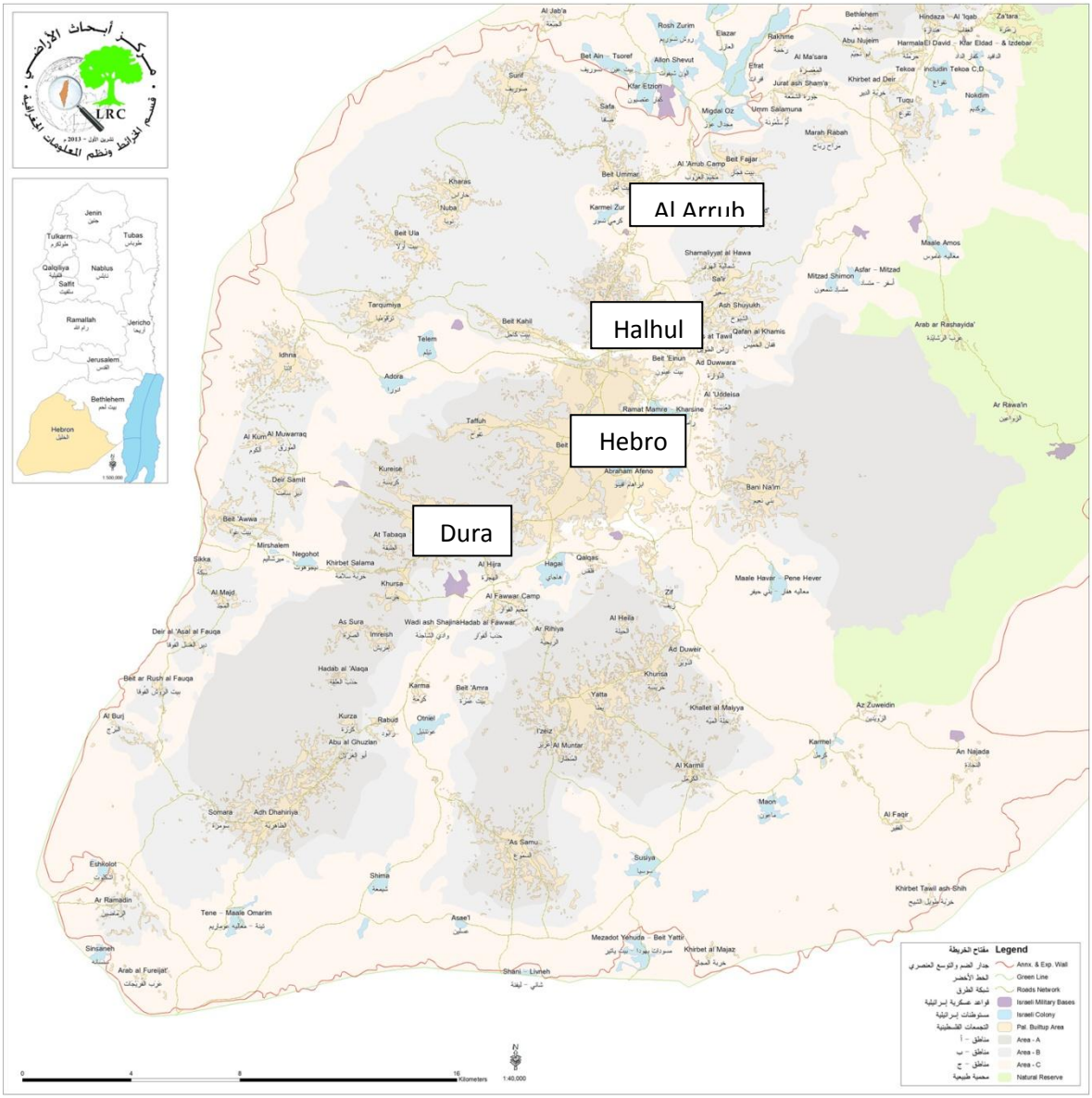


Figure1: The location of the four study sites in Hebron district.

2.2 Plant Sampling

In total, 29 grape accessions (assumed cultivars) were collected from the study sites for both the morphological and genetic characterization. Names of accessions, sources and locations are shown in table (2).

Table (2) Sources of 29 grape accessions

Accession	Location	Farmer
1 Dapougy	Dura	Mahmoud Abu Sharar
2Dapougy	Hebron	Abd El Jawad Sultan
3Dapougy	Halhul	Yousef Abu Rayan
4Halawany	Halhul	Yousef Abu Rayan
5Halawany	Hebron	Abd El Jawad Sultan
6Halawany	Dura	Mahmoud Abu Sharar
7Jandaly	Hebron	Abd El Jawad Sultan
8Jandaly	Al Arrub	Al Arrub agricultural school
9Hamadany	Al Arrub	Al Arrub agricultural school
10Hamadany	Halhul	Yousef Abu Rayan
11Shamee	Halhul	Saied Melhem
12Shamee	Halhul	Yousef Abu Rayan
13Shamee	Hebron	Abd El Jawad Sultan
14 Zane	Halhul	Yousef Abu Rayan
15 Zane	Hebron	Abd El Jawad Sultan
16 Zane	Halhul	Abu Zyad Melhem
17Bairute	Hebron	Abd El Jawad Sultan
18Bairute	Halhul	Yousef Abu Rayan
19Bairute	Halhul	Abu Zyad Melhem
20Mutartash	Halhul	Farid Abu Rayan
21Mutartash	Hebron	Abd El Jawad Sultan
22MalekatLubnan	Halhul	Halhul
23Daraweshy	Al Arrub	Al Arrub agricultural school
24Fhesy	Al Arrub	Al Arrub agricultural school
25Marawy	Halhul	Shehde Melhem
26Balooty	Al Arrub	Al Arrub agricultural school
27Salty	Al Arrub	Al Arrub agricultural school
28Masry	Hebron	Abd El Jawad Sultan
29Betuny	Halhul	Yousef Abu Rayan

2.2.1 Samples for morphological assessment

For morphological assessment, measurements were made on leaves, shoots, and clusters for five grape varieties, namely: Zane, Betuny, Halawany, Dapougy, and Shame. The cultivars were grown in the same

farm at Halhul. Five trees from each cultivar were used, as replicates. From each tree, two samples were collected and averaged.

Grape morphological measurements were taken on shoots at woody stage in February and March, on leaves in April to June, and on clusters in September 2010.

The detailed morphological characters were shown in table (3), and evaluated according to descriptor list for grape varieties and *Vitis* species (OIV, 2001).

Table (3) The collected morphological characteristics

Leaves	Description	Shoot	Description	Berry	Description
OIV51	Color of upper side of blade	OIV101	Cross section	OIV223	Berry shape
OIV67	Shape of blade	OIV102	Structure of surface	OIV225	color of skin
OIV68	Number of lobes	OIV103	Main color		
OIV70	Area of anthocyanin coloration of main vein	OIV1	Aperture of tip		
OIV76	Shape of teeth	OIV2	dist. anth. On prostrate hair of tip		
OIV79	Degree of opening overlapping of petiole sinus	OIV4	Density of prostrate hairs on tip		
OIV80	Shape of base of petiole sinus	OIV6	Attitude		
OIV81-1	Teeth in the petiole sinus	OIV7	color of dorsal side of internodes		
OIV81-2	Petiole sinus base limited by veins	OIV8	color of ventral side of internodes		
OIV601	Length of vein N1	OIV16	Number of concecutive tendrils		
OIV602	Length of vein N2	OIV17	Length of tendrils		
OIV603	Length of Vein N3				
OIV605	Length petiole sinus to upper lateral leaf sinus				
OIV606	Length petiole sinus to lower lateral leaf sinus				
OIV607	Angle between N1 and N2				
OIV612	Length of tooth N2				
OIV613	Width of tooth N2				
OIV614	Length of tooth N4				
OIV615	Width of tooth N4				
OIV618	Opening/ overlapping of petiole sinus				

2.2.1.1 Woody shoot characteristics

Cross section:

Cross section at the internodes was made for 10 internodes during dormancy period in February from the middle and upper third of woody shoots, and the shape of cross section for 10 internodes was examined and recorded according to the index values in (figure 2).

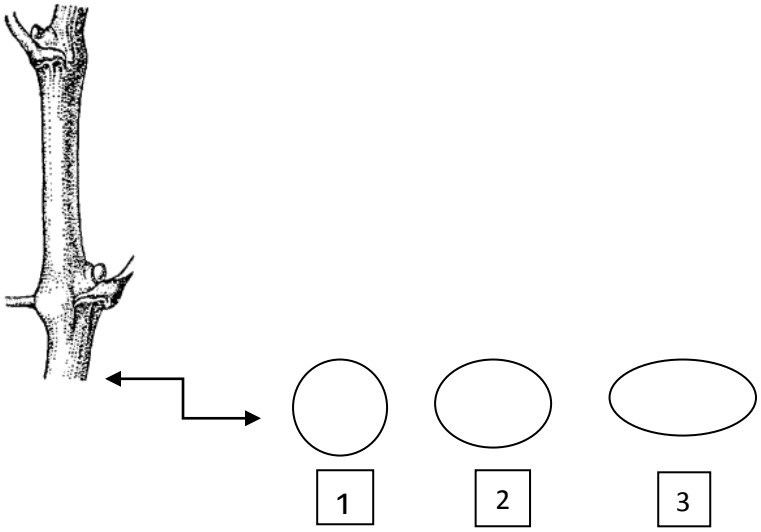


Figure 2: Woody shoot cross section shapes, (1) circular (2) elliptic (3) oblate.

Structure of surface:

Structure of the surface for 10 internodes from the middle and upper third of several woody shoot was examined after leaf fall or during dormancy (figure 3).

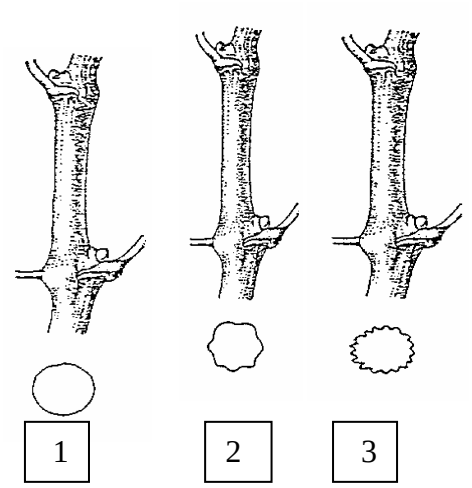


Figure 3: Woody shoot structure of surface shapes, (1) smooth (2) ribbed (3) striate.

Main color:

Main color for 10 internodes from the middle third of several woody shoots was examined after leaf fall until early winter. The data recorded according to the following index: (1) yellow (2) brownish (3) red violet (4) grey.

Aperture of tip (young shoot):

The opening of 10 shoot tips was examined during flowering (figure 4). Shoot tip was scoped above the first unfolded leaf.

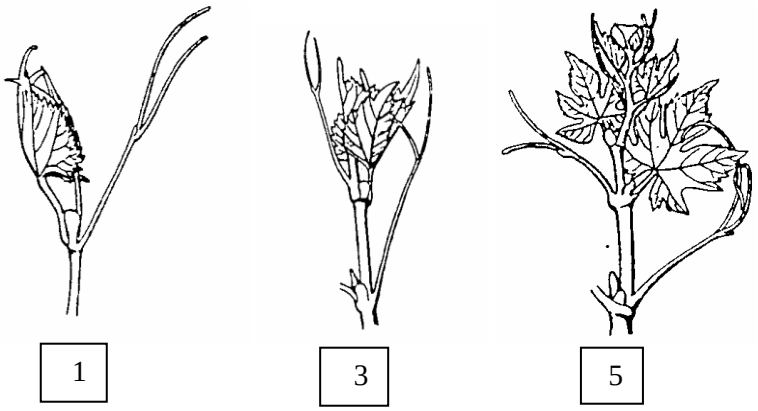


Figure 4: Shapes of young shoot opening of the shoot tip, (1) closed (3) half open (5) fully open.

Distribution of anthocyanin on hairs (young shoot)

Distribution of anthocyanin on hairs for 10 shoot tips was examined during flowering (figure 5); they were scoped above the first unfolded leaf. Leaves of closed and half open shoot tips were considered unfolded to record the corresponding part of the tip. The data was recorded according to the following index: (1) absent (2) piping (3) overall.

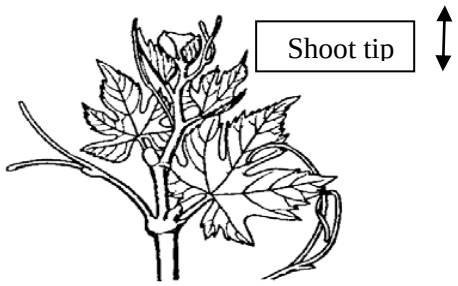


Figure 5: Young shoot tip

Density of prostrate hair on shoot tip (young shoot)

Ten shoot tips were examined during flowering; they were scoped above the first unfolded leaf (figure 6). Leaves of closed and half open shoot tips were considered unfolded to record the corresponding part of the tip. The data was recorded according to the following index: (1) none or very low (3) low (5) medium (7) high (9) very high.

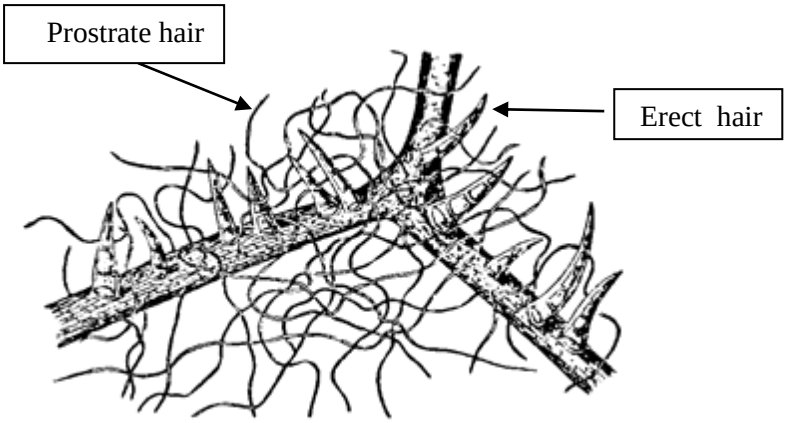


Figure 6: Young shoot, density of prostrate hairs on the shoot tip.

Attitude (shoot) before tying:

Attitude for 10 shoots was examined during flowering Using the following index values: (1) erect (3) semi erect (5) horizontal (7) semi drooping (9) drooping, as shown in figure 7.

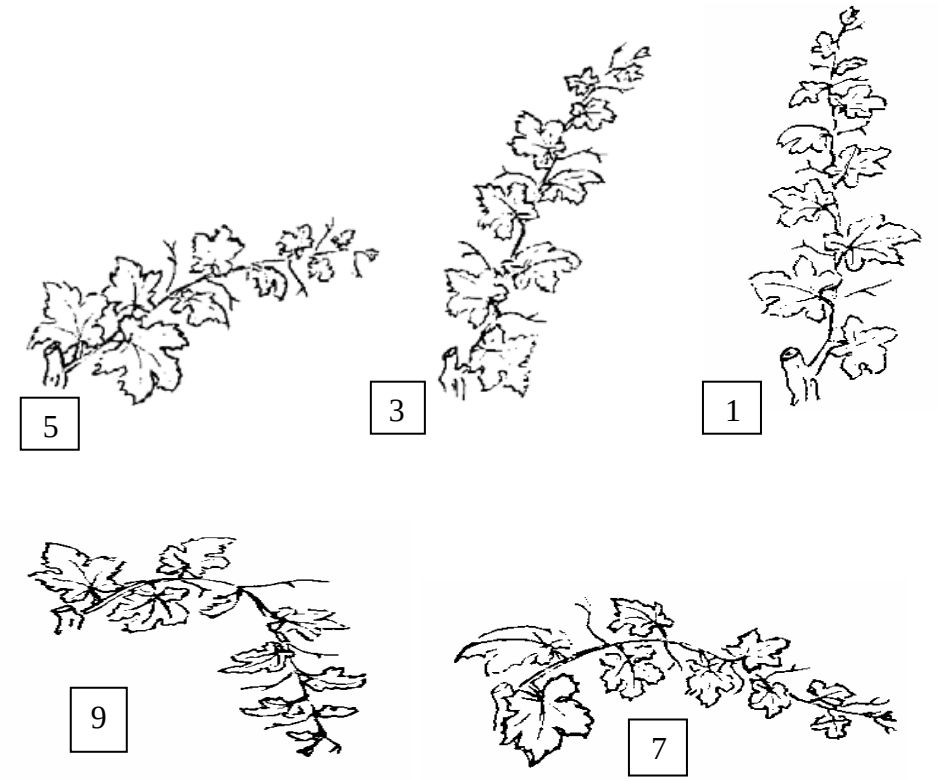


Figure 7: Attitude shapes

Color of the dorsal side of internodes (shoot)

Color of the dorsal side for 10 internodes was examined during flowering at the middle third of shoot (figure 8). Dorsal side is generally exposed to direct sunlight.

The color index was: (1) green (2) green and red (3) red.

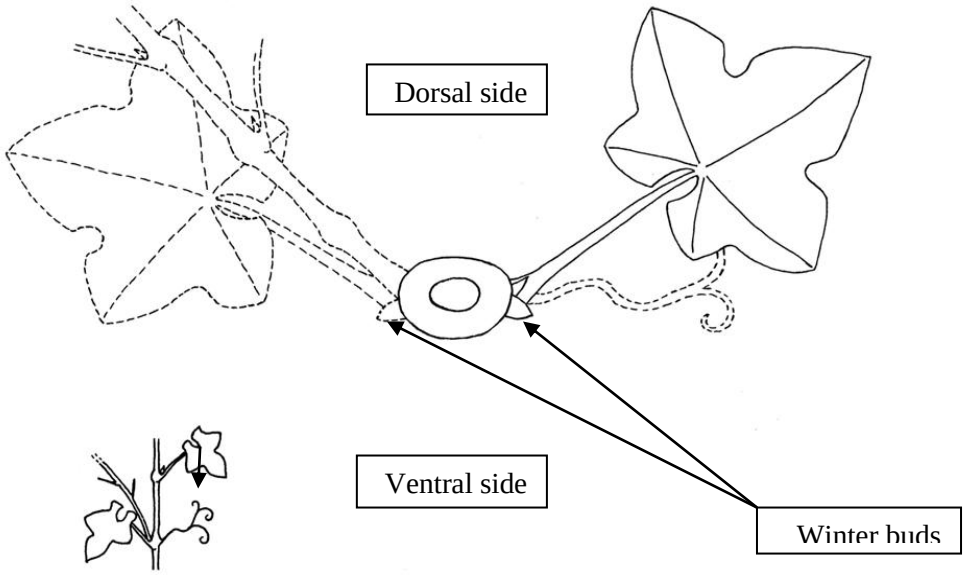


Figure 8: Color of the dorsal and ventral side of internodes

Color of the ventral side of internodes:

Color of the ventral side for 10 internodes was examined during flowering at the middle third of shoot (figure 8). Ventral side is generally not exposed to direct sunlight

The color index was: (1) green (2) green and red (3) red, as shown in figure 8.

Number of consecutive tendrils:

Ten shoots were examined for the number of consecutive tendrils during flowering at the middle third of shoot (figure 9).

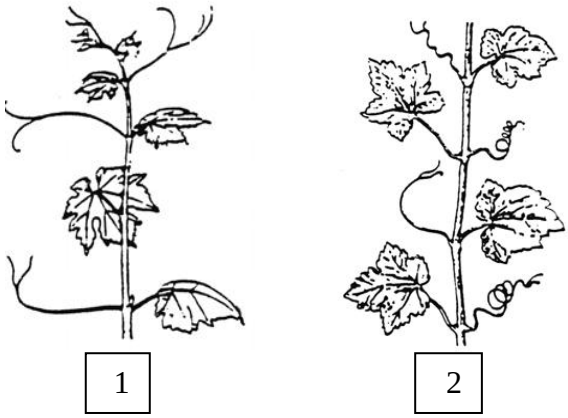


Figure 9: Number of consecutive tendrils at the middle third of a shoot, (1) two or less (2) three or more

Length of tendrils (shoot):

Ten tendrils were examined during flowering at the middle third of a shoot (figure 10).

The index used for recording the data was: (1) very short about 10 cm (3) short about 15 cm (5) medium about 20 cm (7) long about 25 cm (9) very long about 30 cm and more.

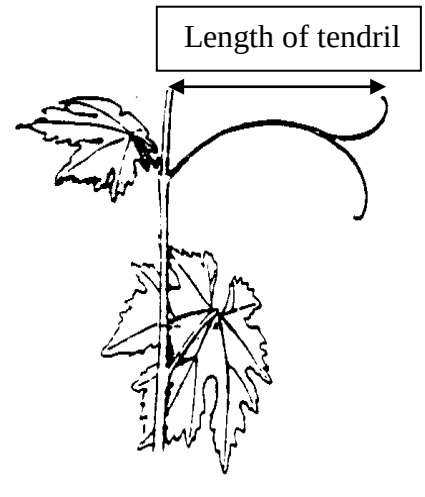


Figure10: Length of tendril on shoot

2.2.1.2 Young leaf characteristics

Color of upper side of blade:

Color of upper side of blade for the 4th distal leaf of 10 shoots was examined during flowering (figure 11).

The color index was: (1) green (2) yellow (3) bronze (4) copper reddish.

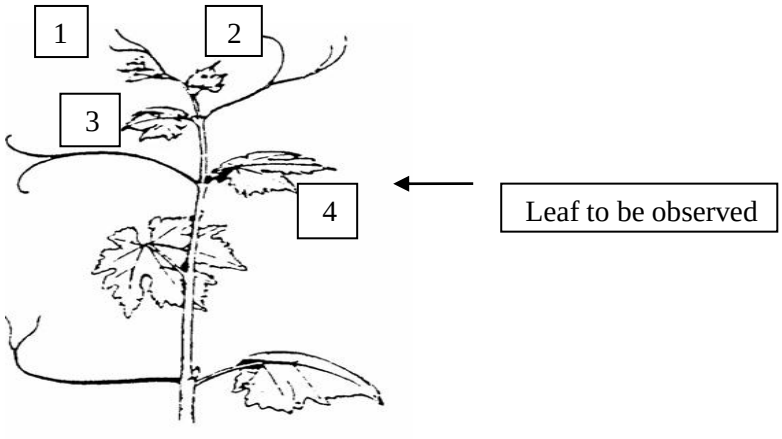


Figure11: Color of the upper side of blade of 4th leaf

Shape of blade (mature leaf):

Shapes of blade for 10 mature leaves from the middle third of several shoots were examined between berry set and veraison (figure12).

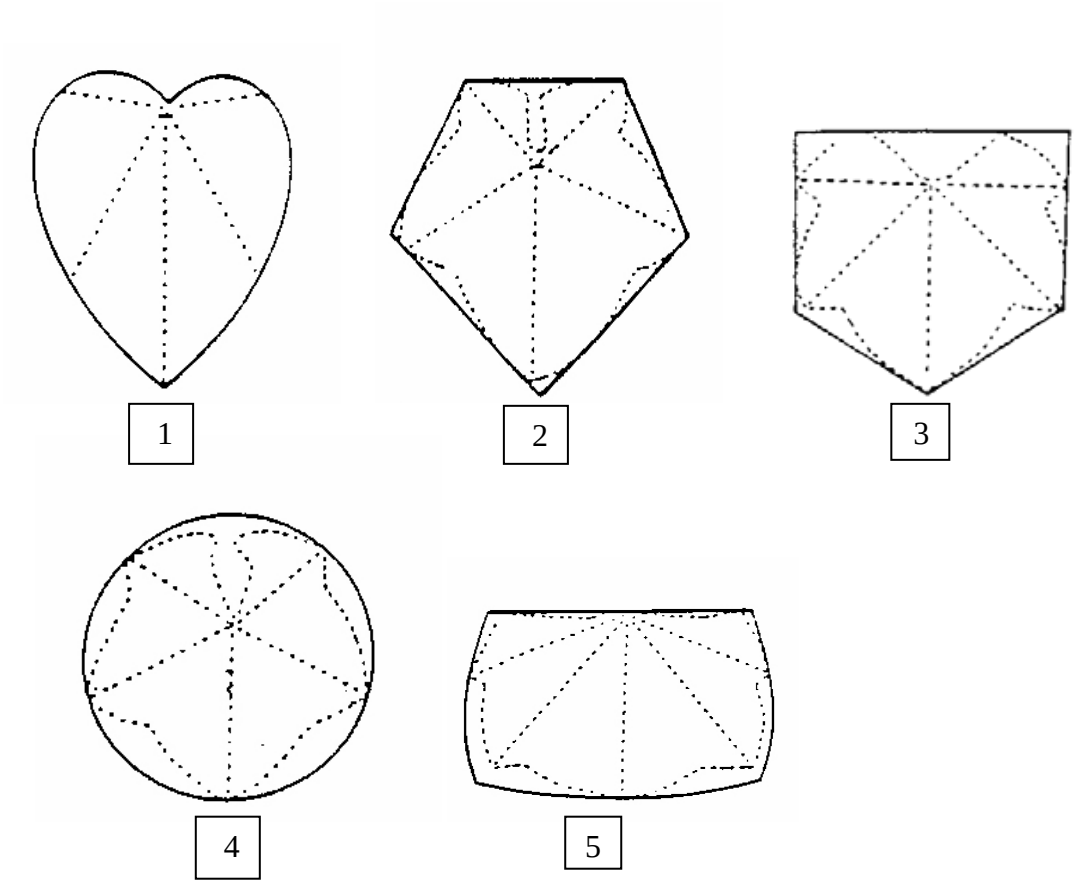


Figure 12: Five different shapes of the mature leaf blade, (1) cordate (2) wedge shaped (3) pentagonal (4) circular (5) kidney shaped

Number of lobes (mature leaf):

Number of lobes for 10 mature leaves from the middle third of several shoots between berry set and veraison was examined, L1: main lobe, L2 to L11: lateral lobes (figure 13).

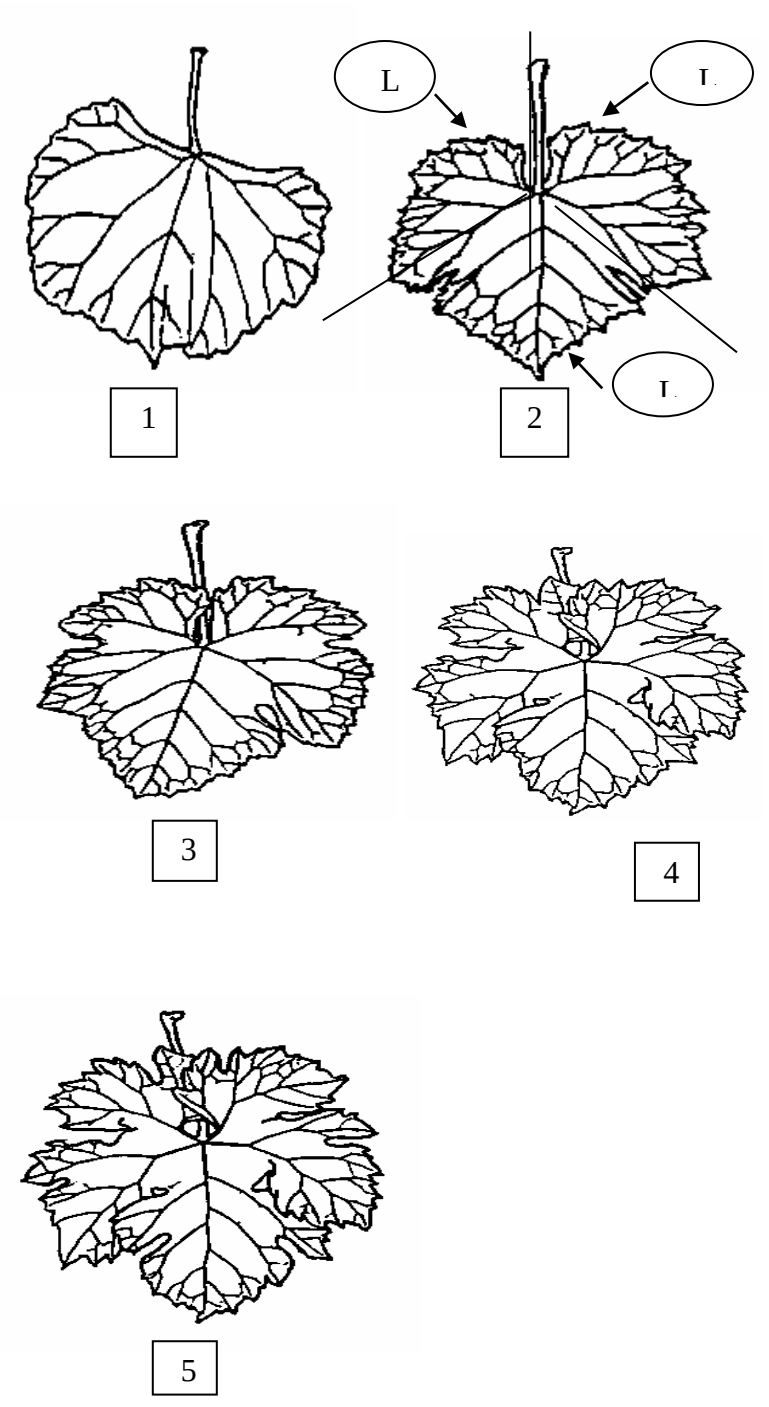


Figure 13: Number of lobes in blade of mature leaf, (1) one (entire leaf) (2) three (3) five (4) seven (5) more than 7

Area of anthocyanin coloration of main veins on upper side of blade

Ten mature leaves from the middle third of several shoots were noted from berry set to veraison. The distribution of anthocyanin coloration on the main veins N1-N3 was assessed and recorded according to the following index:

(1) Absent (2) only at the petiolar point (3) up to the 1st bifurcation (4) up to the 2nd bifurcation (5) beyond the 2nd bifurcation, as shown in figure 14.

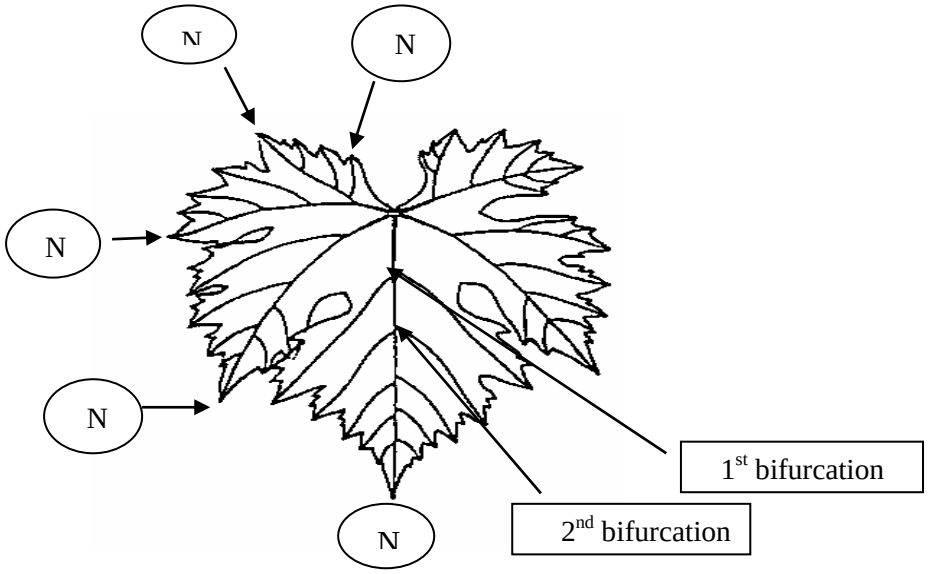
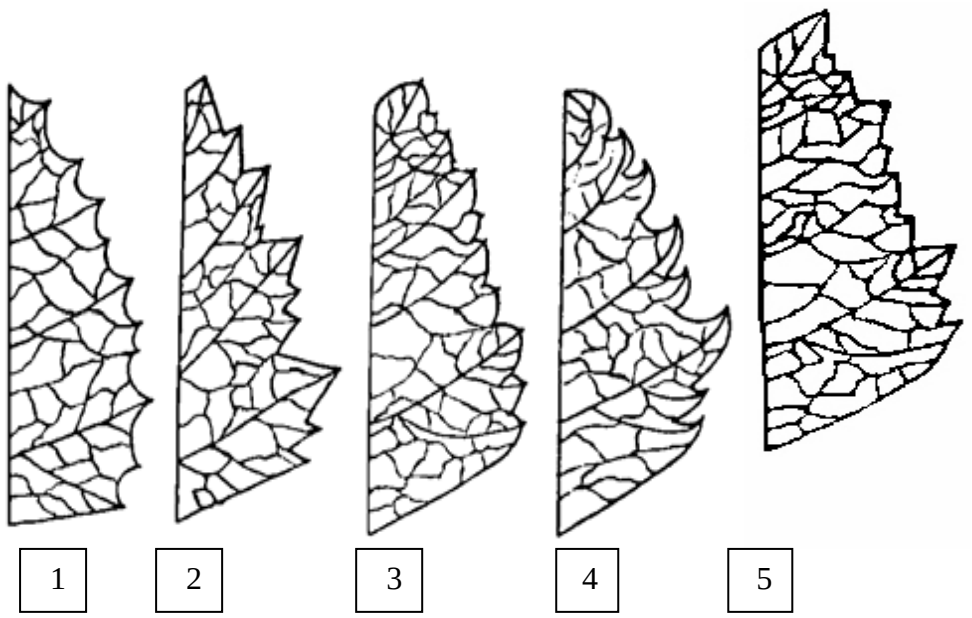


Figure 14: Area of anthocyanin coloration on main veins on upper side of blade.

Shape of teeth (mature leaf):

Shape of teeth for 10 mature leaves from the middle third of several shoots was examined between berry set and veraison (figure15).

Shape of teeth between N2 and N3 excluding teeth of N2 and N3 was examined.



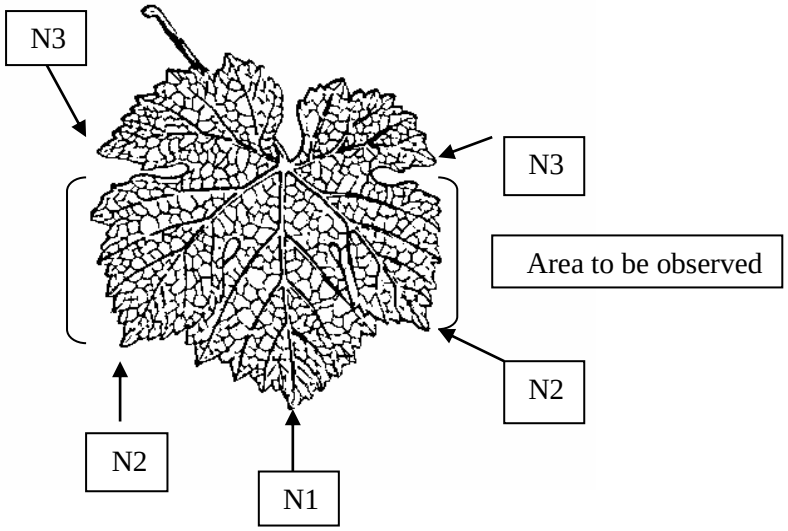


Figure 15: Shapes of teeth and the studied area in the blade of mature leaf, (1) both sides concave (2) both sides straight (3) both side convex (4) one side concave and other convex (5) mixture between both side straight and both side convex

Degree of opening /overlapping of petiole sinus:

Degree of opening/overlapping of petiole sinus for 10 mature leaves from the middle third of several shoots between berry set and veraison was examined. Leaves must be flattened for notation (figure 16).

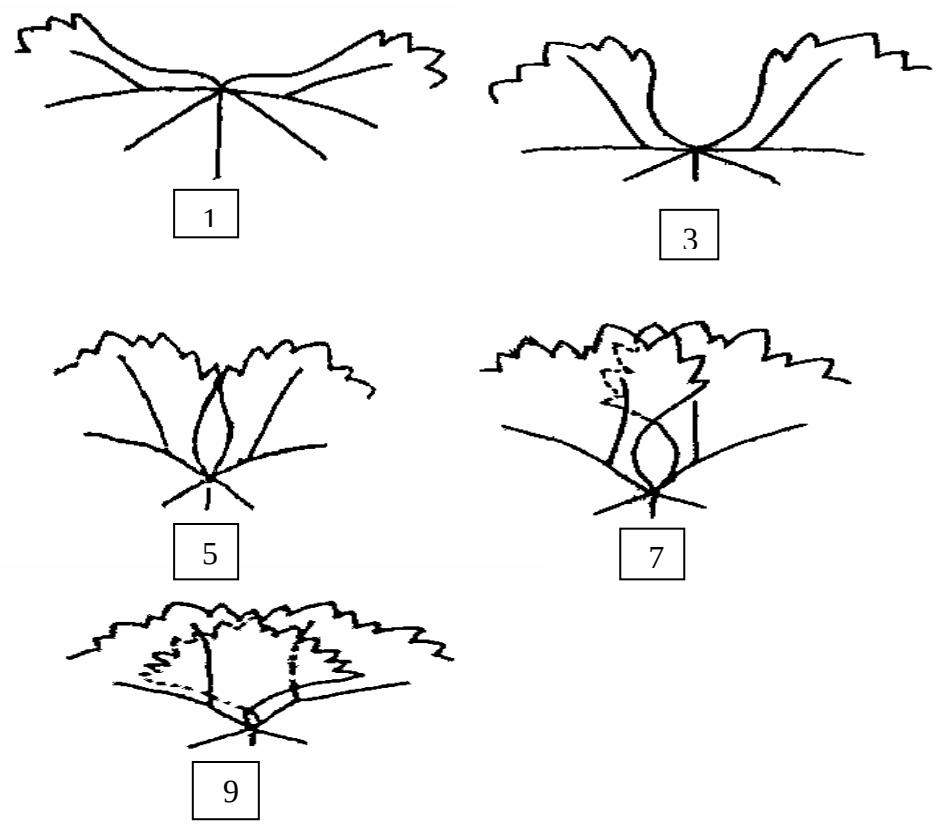


Figure 16: Degree of opening overlapping of petiole sinus in mature leaf, (1) very wide open (3) open (5) closed (7) overlapped (9) strongly overlapped

Shape of base of petiole sinus (mature leaf):

Shape of base of petiole sinus for 10 mature leaves from the middle third of several shoots was examined between berry set and veraison. Lower

third of the petiole sinus was observed and recorded according to (figure 17).

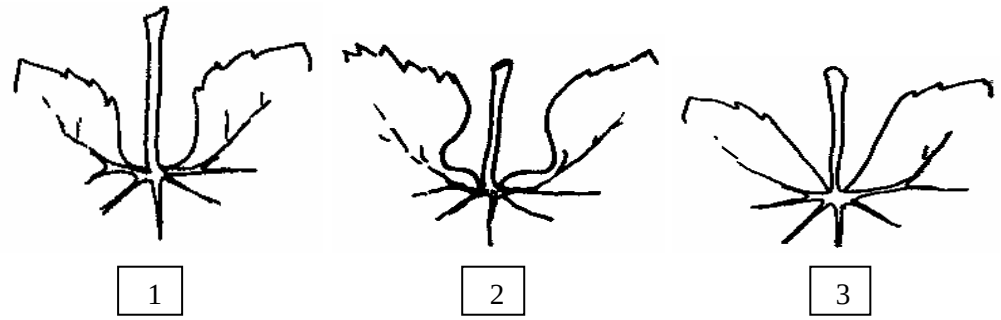


Figure 17: Shapes of base of petiole sinus, (1) U –shaped (2) brace – shaped (3) V-shaped

Teeth in the petiole sinus (mature leaf):

Teeth in the petiole sinus for 10 mature leaves from the middle third of several shoots were examined between berry set and veraison (figure 18). The index used for the recoding of data was: (1) none (9) present. Reading 9: occurrence at least once on ten leaves.

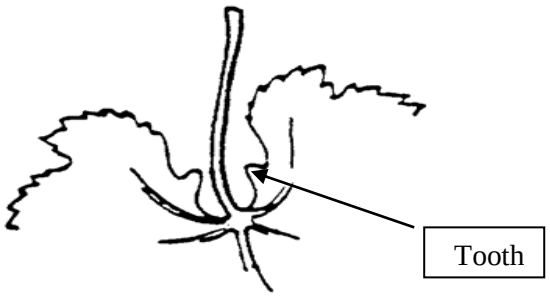
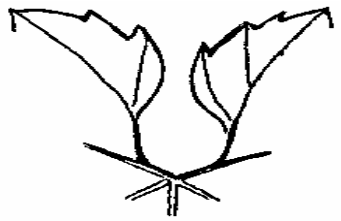


Figure 18: Teeth in the petiole sinus

Petiole sinus base limited by veins (mature leaf)

Ten mature leaves from the middle third of several shoots were examined between berry set and veraison (figure 19).

The index was: (1) not limited (2) on one side (3) on both sides. Readings 2 and 3: occurrence at least once on ten leaves.



3

Figure19: Petiole sinus base limited by vein

Length of vein N1 (mature leaf):

Length of vein N1 for 10 mature leaves from the middle third of several shoots was measured.

The index for length was: (1) very short {75mm} (3) short {105mm} (5) medium {135mm} (7) long {165mm} (9) very long {195mm} and more, as shown in (figure 20).

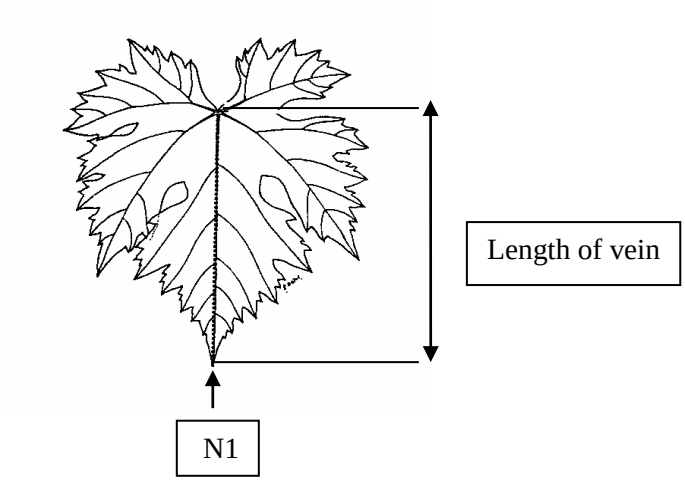


Figure 20: Length of vein N1 for mature leaf

Length of vein N2 (mature leaf):

Length of vein N2 for 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured.

The index for length was: (1) very short {65mm} (3) short {85mm} (5) medium {105mm} (7) long {125mm} (9) very long {145mm} and more, as shown in (figure 21).

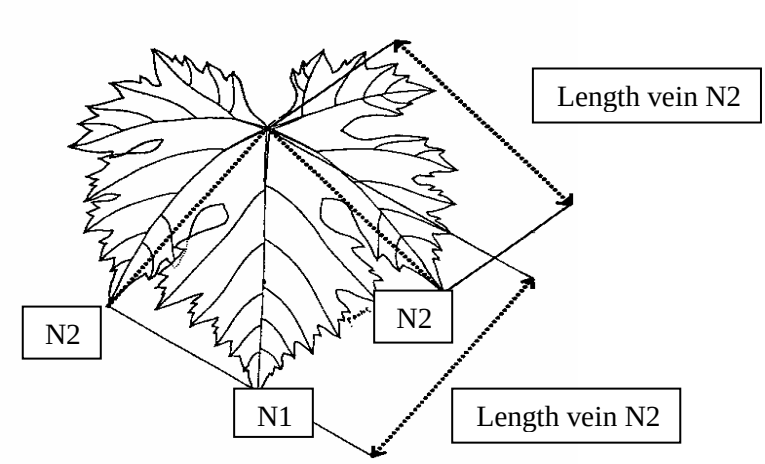


Figure 21: Length of vein N2 on both halves of the leaf

Length of vein N3 (mature leaf):

Lengths of vein N3 for 10 mature leaves from the middle third of several shoots on both halves of the leaf were measured.

The index for length was: (1) very short {35mm} (3) short {55mm} (5) medium {75mm} (7) long {95mm} (9) very long {115mm}, as shown in (figure 22).

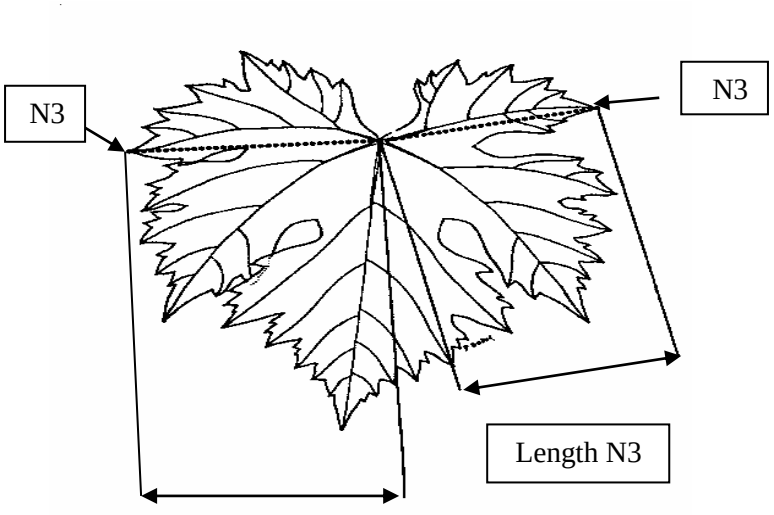


Figure 22: Length of vein N3 on both halves of the leaf

Length petiole sinus to upper lateral leaf sinus (mature leaf):

Distance from petiole sinus to upper lateral leaf sinus 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured.

The index for length was: (1) very short {30mm} (3) short {50mm} (5) medium {70mm} (7) long {90mm} (9) very long {110mm}, as shown in (figure 23).

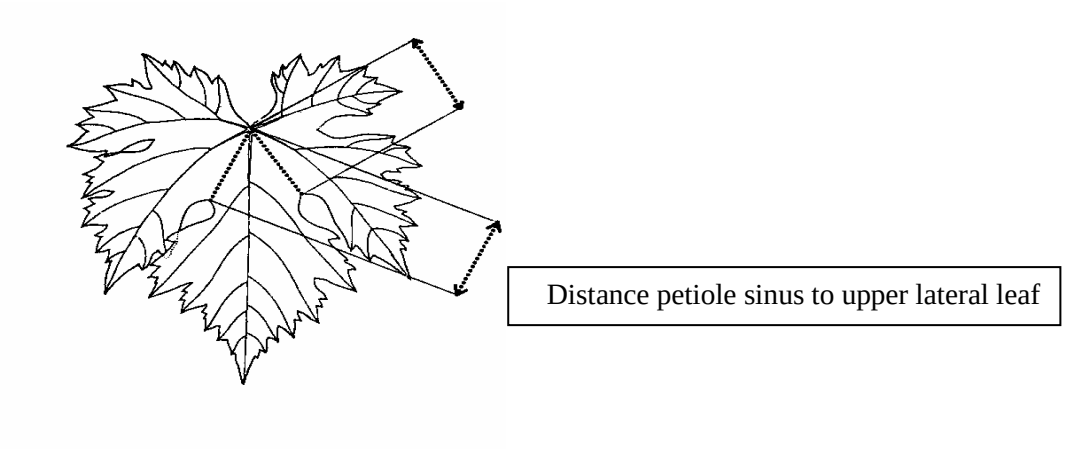


Figure23: Length petiole sinus to upper lateral leaf sinus

Length petiole sinus to lower lateral leaf sinus (mature leaf):

Distance from petiole sinus to lower lateral leaf sinus for 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured and recorded according to the following index: (1) very short {30mm} (3) short {45mm}(5) medium {60mm} (7) long {75mm} (9) very long {90mm}, as shown in (figure 24).

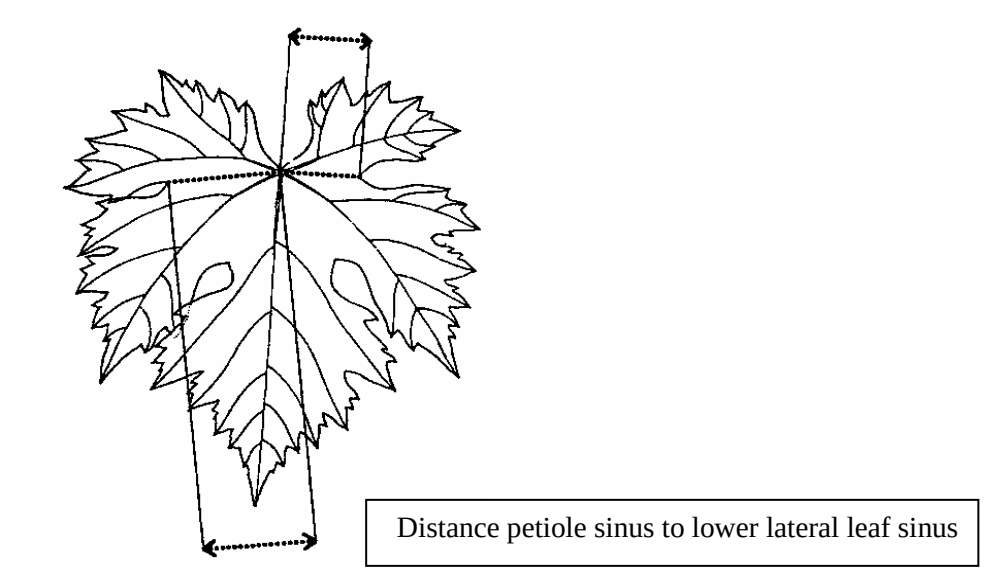


Figure 24: Length petiole sinus to lower lateral leaf sinus of mature leaf

Angle between N1 and N2 measured at the first ramification (mature leaf):

Angle between N1 and N2 for 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured on the tangents formed before these veins first branch.

The index for the angle was: (1) very small 30º (3) small 30º-45º (5) medium 46º-55º (7) large 56º-70º (9) very large 70º and more, as shown in (figure 25).

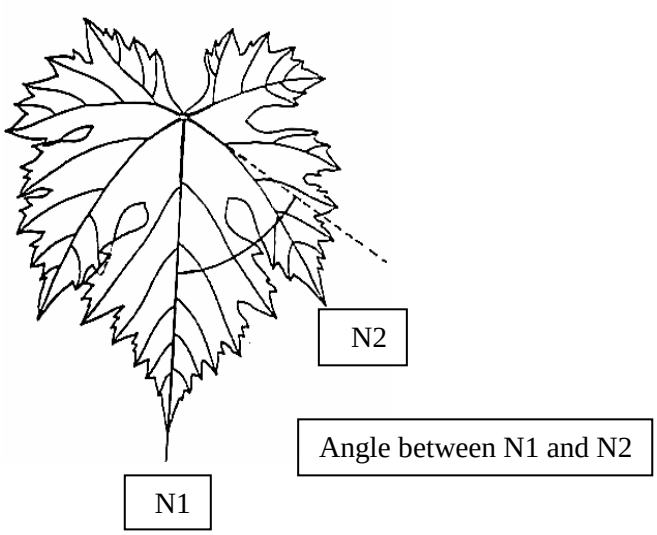


Figure25: The angle between N1 and N2 measured at the first ramification for mature leaf.

Length of tooth of N2:

Length of tooth of N2 for 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured and recorded

according to the following index: (1) very short 6 mm (3) short 10 mm (5) medium 14 mm (7) long 18 mm (9) very long 22 mm, as shown in (figure 26).

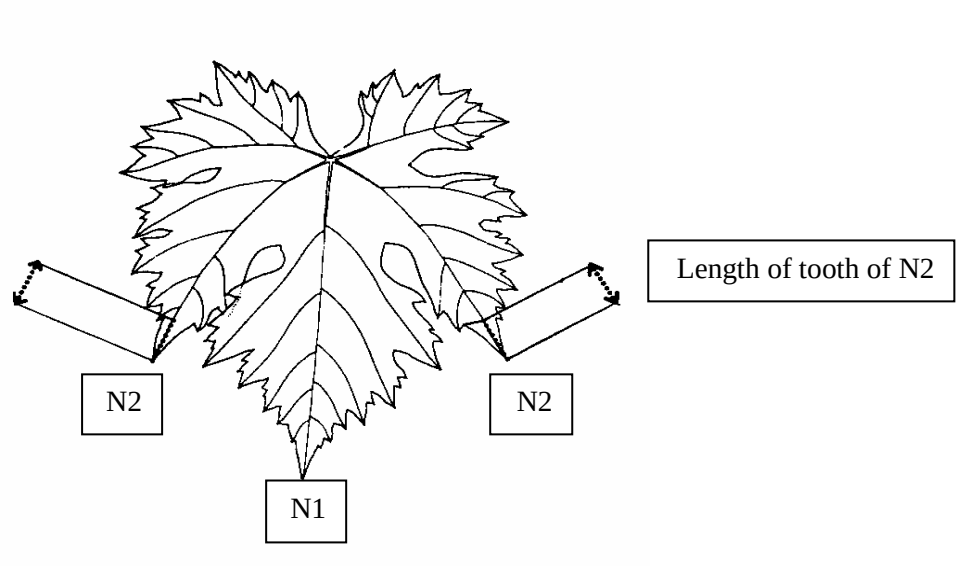


Figure26: Length of tooth of N2 for mature leaf

Width of tooth of N2:

Width of tooth of N2 for 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured.

The index for the width was: (1) very narrow 6 mm (3) narrow 10 mm (5) medium 14 mm (7) wide 18 mm (9) very wide 22 mm, as shown in (figure 27).

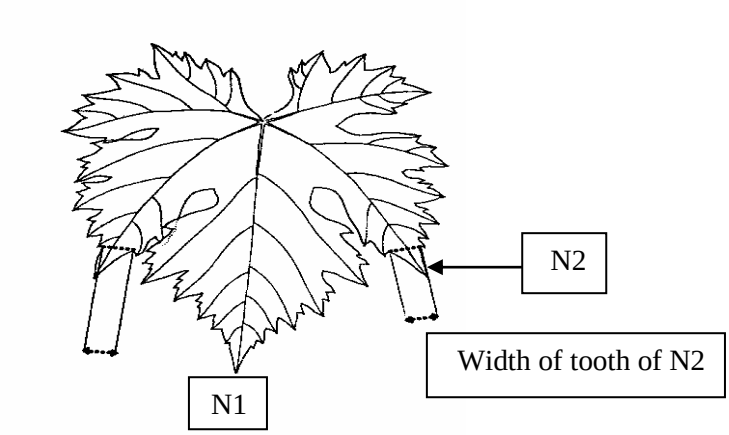


Figure 27: Width of tooth of N2 for mature leaf

Length of tooth of N4:

Length of tooth of N4 for 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured.

The index for the length was: (1) very short 6 mm (3) short 10 mm (5) medium 14 mm (7) long 18mm (9) very long 22 mm, as shown in (figure 28).

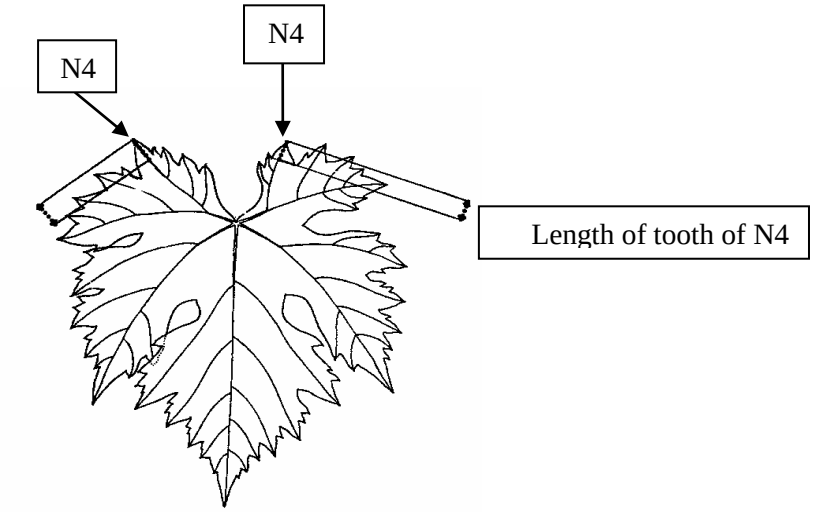


Figure28: Length of tooth of N4 for mature leaf

Width of tooth of N4:

Width of tooth of N4 for 10 mature leaves from the middle third of several shoots on both halves of the leaf was measured.

The index for the width was: (1) very narrow 6 mm (3) narrow 10 mm (5) medium 14 mm (7) wide 18 mm (9) very wide 22 mm, as shown in (figure 29).

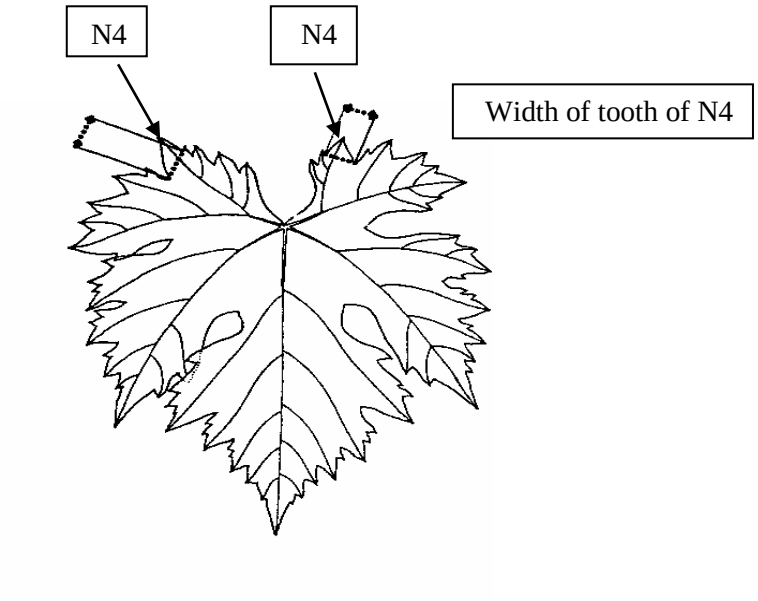


Figure 29: Width of tooth of N4 for mature leaf

Opening overlapping of petiole sinus (mature leaf):

Opening overlapping of petiole sinus for 10 mature leaves from the middle third of several shoots was measured as follow:

1: wide open sinuses: measured the distance between the blades at half the height of the petiole sinus .The value was recorded as negative.

5: closing sinuses: was measured at the shortest distance between the blades. The value was recorded as negative.

7: strongly overlapping lobes: was measured at the widest overlap between the blades. The value was recorded as positive.

The index used for recording the data was: (1) wide open -35 mm (3) open -15 mm (5) closed -5 mm (7) overlapping 25 mm (9) very overlapping 45 mm, as shown in (figure 30).

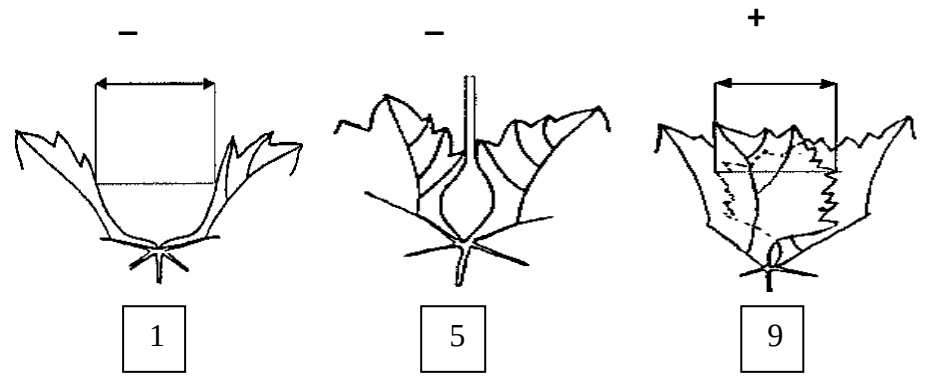


Figure 30: Opening /overlapping of the petiole sinus for mature leaf

2.2.1.3 Cluster characteristics

Berry shape:

Thirty berries not deformed by compression taken from the middle part of 10 bunches was observed at maturity and the data was recorded as shown in figure (31).

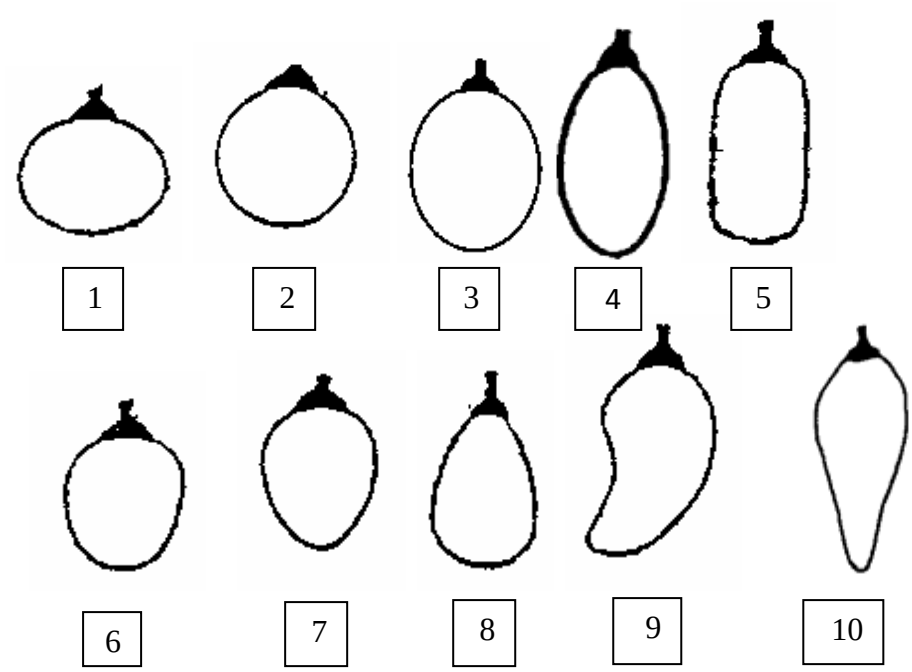


Figure 31: Berry shapes for cluster at maturity: (1) abloid (2) globos (3) broad ellipsoid (4) narrow ellipsoid (5) cylindric (6) obtuse ovoid (7) ovoid (8) obovoid (9) horn shaped (10) finger shaped

Color of skin (berry):

Color of skin of 30 berries taken from the middle part of 10 bunches was examined.

The index used for recording the data was: (1) green yellow (2) rose (3) red (4) grey (5) dark red violet (6) blue black.

2.2.2 Statistical analysis for morphological characterization

For morphological data, the average and standard deviation (by using Microsoft Office Excel) were used to give the last result of the studied character for ten replicates of shoots or leaves or cluster for every studied grape variety. Least significant difference (LSD) at $p \leq 0.05$ was

employed to estimate the significant differences between the treatment means for lengths (ampelometric) characters.

2.2.3 Samples for genetic assessment

For each accession, young grape leaves were collected in April and May of year 2010. Directly after leaves of accessions were collected from the field they were placed in paper bags and taken to the lab. The leaves for each accession were grounded using pestle and mortar in liquid nitrogen to fine powder, and they were filled in monocular eppendorf tubes in laboratory. These accessions became prepared for DNA extraction.

2.2.3.1 Plant material

Young grape leaves were collected for (28 assumed grape accessions) from Hebron district (Dura, Al Arrub, Halhul, and Hebron), as shown in table (2), except Betuny cultivar.

2.2.3.2 DNA extraction, purification, quantification

2.2.3.2.1 DNA extraction and purification

A weight of 100 mg of leaf powder of each accession was transferred into a 1.5 mL eppendrof tube and mixed with, 400 µL of buffer AP1 and 4 µL of RNase A stock solution (100 mg/mL) were added to the tube, and the mixture was vigorously vortexed, incubated at 65 ^{o}C, and mixed 2-3 times during incubation by inverting.

A quantity of 130 µL of buffer AP2 was added to the lysate, which was mixed, incubated on ice for 5 minutes, and then centrifuged at 20,000 x g. The supernatant was applied to the QIAshredder Mini Spin Column and it was carefully centrifuged at 20,000 x g for 2 minutes so as not to disturb the pellet. The flow-through fraction (liquid) was transferred to a new 2

mL eppendrof tube without disturbing the cell-debris pellet and a quantity of 1.5 volumes of buffer AP3/E was added to the cleared lysate and mixed by pipetting. A quantity of 650 µL of the mixture was applied to the DNeasy Mini Spin Column placed in a 2 mL collection tube which was centrifuged at 6,000 x g while its flow-through was discarded. The rest of the mixture was applied as aforementioned. Subsequently, the DNeasy Mini Spin Column was placed in a new 2 mL collection tube, and 500 µL of buffer AW was added to it. The tube was centrifuged at 6,000 x g for 1 min, and the flow-through was discarded, reusing the collection tube in the next step. A total volume of 500 µL of the same buffer was used once more with centrifuging at 20,000 x g for 2 min. The DNeasy Mini Spin Column was transferred to a 1.5 mL eppendrof tube and a quantity of 30 µL of buffer AE was added twice with a separation time of at least 5 min between them. Before storing at 20 ^{o}C, the tube was centrifuged at full speed for 1 min.

2.2.3.2.2 Estimation of DNA quantification

DNA quality and quantity was determined on a 0.8% agarose gel stained with ethidium bromide by visual comparison with known quantities of Lambda DNA as a standard. Final concentration of DNA was adjusted to 50ng/µl.

2.2.3.3 SSR (Simple Sequence Repeats) primer sequence

Eight SSR primers were used according to Bowers et al.(1996), Sefc et al.(1999), Scott et al. (2000), and Riaz et al.(2004), ,

All reactions were conducted using a thermocycler in PCR (PTC-200) to obtain the polymorphic primers. The names and sequences of eight SSR primers were used are shown in table 4.

Table (4) The names and sequences of eight SSR primers used to detect polymorphism in grape accessions

Name	Sequence	
	Forward	**Reverse**
VRZAG62	GGT GAA ATG GGC ACC GAA CAC ACG C	CCA TGT CTC TCC TCA GCT TCT CAG C
VRZAG79	AGA TTG TGG AGG AGG GAA CAA ACC G	TGC CCC CAT TTT CAA ACT CCC TTC C
VVMD27	GTA CCA GAT CTG AAT ACA TCC GTA AGT	ACG GGT ATA GAG CAA ACG GTG T
SCU05VV	CAAGCAGTTATTGAAGCTGCAAGG	TCATCCATCACACAGGAAACAGTG
SCU11VV	AATTGATAGTGCCACGTTCTCGCC	ACGCCGACAAGAATCCCAAGG
SCU08VV	CGAGACCCAGCATCGTTTCAAG	GCAAAATCCTCCCCGTACAAGTC
VMC8A7	GCAGCAACTCTCTTACACACCG	GTGGGAGCACTGGTTGCTTTAG
SCU15VV	GCCTATGTGCCAGACCAAAAAC	TTGGAAGTAGCCAGCCCAACCTTC

2.2.3.4: SSR (Simple Sequence Repeats) PCR reaction mixture and program.

DNA amplification mixture was done by using a PTC -200Peltier thermal cycler. Amplification was carried out in 25µl volumes, containing (5µl) 30 ng template DNA, 2µl 50mM Mgcl2, 2µl 10X (10nM Tris-HCl, PH 8.8 and 50mM KCl), 2µl of a 20 mM dNTPs solution, 4 µl primer at 10 pmol/µl, and one unit tag polymerase. PCR program was as following: initial step of 4 min at 94 Cº, second step of 45 s at 94, 1 min at 56 Cº, and 1 min at 72 Cº, after that go to second step for 34 cycles, and final elongation step at 72 Cº for 7 min. Samples were kept at 4 Cº until analysis (Arnold et al., 2002).

2.2.3.5: SSR gel processing, evaluation and generating the binary data matrix.

Amplified products (15 µl) were mixed with 7 µl of orange gel loading buffer and analyzed by electrophoresis in 2.5% agarose gels (Hy Labs) in 1X TAE buffer at 4 volt/cm for 4h as well as detected by staining with ethidium bromide (Sigma). A 100 bp DNA ladder was used as standard marker (Fermentas). Consequently, amplicons were visualized and photographed black and white on Polaroid type film with UV trans-illuminator (ImageMaster®VDS).

DNA bands were scored (1) for presence and (0) for absence for each primer-genotype combination. Only reliable and clear bands were scored for the estimation of genetic similarity.

2.2.3.6: SSR data analysis.

Data matrix was utilized to generate genetic similarity data among genotypes using. Jacquard's similarity coefficient formula as the following:

$$S_{ij,Jaccard} = \frac{n_{11}}{n_{11} + n_{01} + n_{10}}$$

Where *nxy* is the number of characters that have state *x* in individual i and state *y* in individual *j*. Un-weighted pair group method using arithmetic averages (UPGMA) (Schluter and Harris, 2006) phenogram was then calculated from the Jaccard's similarity using Fingerprint Analysis with missing data (FAMD) software version 1.108 beta. Tree view software (Win32) version 1.6.6 was used to visualize the resulted trees.

Chapter Three

Results

3.1 Morphology

3.1.1 Woody shoot characteristics

The morphological characteristics of the woody shoots of five grape varieties are shown in table (5) and figure (32). Data show that Zane and Shame have oblate cross section for the woody shoot, while Dapougy, Halawany, and Betuny have elliptic shape.

Table (5) Results of some morphological characters for woody shoots of five grape cultivars, (LSD, P≤ 0.05)

OIV N#	morphological character	Grape cultivars				
		Zane	Betuny	Halawany	Dapougy	Shame
OIV101	Cross section	Oblate	Elliptic	Elliptic	Elliptic	Oblate
OIV102	Structure of surface	Smooth	Smooth	Smooth	Smooth	Smooth
OIV103	Main color	Brown	Brown	Brown	Brown	Brown
OIV1	Aperture of tip	Fully Open	Fully Open	Half Open	Half Open	Half Open
OIV2	distribution. anth. On prostrate hair of tip	Piping	Absent to Piping	Piping	Piping	Overall
OIV4	Density of prostrate hairs on shoot tip	High	Medium	Low	Medium	Medium
OIV6	Attitude	Semi Erect	Semi Erect	Erect	Semi Erect	Semi Erect
OIV7	color of dorsal side of internodes	Green Red	Green Red	Green Red	Green Red	Green
OIV8	color of ventral side of internodes	Green	Green	Green	Green	Green
OIV16	Number of concecutive tendrils	Tow or less	Tow or less	Tow or less	Tow or less	Tow or less
OIV17	Length of tendrils	1 ±0 **b**	2 ±1.4 **ab**	1 ±0 **b**	1 ±0 **b**	1 ±0 **b**

OIV17: 1: very short about 10cm, 3: short about 15cm, 5: medium about 20cm, 7: long about 25cm, 9: very long about 30cm and more.

Figure 32: Cross sections for woody shoots of the studied grape cultivars.

Also a very high similarity was observed in the structure of the surface (Fig. 33 and 34) and the color of the woody shoots for the five studied grapevines (Zane, Betuny, Halawany, Dapougy, and Shame). These cultivars showed the same index value which indicates a smooth surface structure with brownish woody shoot color.

Figure 33: Shoot surface structure for five studied grapevines

Figure 34: Photo show the main color of some woody shoots of the five studied grapevines.

As for shoot tips, those of Zane and Betuny were fully open, while for Halawany, Dapougy, and Shame it was half open. The distribution of anthocyanins differed also between cultivars. Data in table (5) show that Zane, Halawany, and Dapougy have piping distribution of anthocyanin on prostrate hairs of shoot tip. On the other hand, Shame has nearly overall distribution of anthocyanin, whereas in Betuny some were absent, and some were piping. Another criterion is the density of prostrate hairs on shoot tip, which was high in the Zane grapes, medium for Shame, Dapougy, and Betuny, and low for Halawany grape. Concerning the growth of shoots, it was observed that Zane, Betuny, Dapougy, and Shame have semi erect shoot, whereas Halawany has erect shoot. In addition, the internodal color differs between the investigated cultivars. The dorsal sides of internodes were green for Shame, but for Dapougy, Zane, Halawany, and Betuny they were mixed between green and red color. On the other hand, all cultivars have a green color on the ventral side of the shoots. As for the number of consecutive tendrils, all cultivars have two or less. However, results reveal that four of the grapevine cultivars under investigation, namely Zane, Halawany, Dapougy, and Shame, have the same length of tendrils (around 10 cm), which is considered as very short tendrils. The exception with small significant difference was Betuny, which had short to very short tendrils (10 - 15cm).

3.1.2 Mature leaf characteristics

The results of various characteristics of mature leaves of the five grape cultivars are shown in Table (6).

Table (6) The average index values and stander errors of some morphological character for mature leaves of five grape cultivars (LSD, $P \leq 0.05$)

OIV N#	Morphological character	Grape cultivars				
		Zane	Betuny	Halawany	Dapougy	Shame
OIV51	Color of upper side of blade	Copper reddish	Bronze	Yellow	Copper reddish	Bronze
OIV67	Shape of blade	Pentagonal	Circular	Wedge	Circular	Circular
OIV68	Number of lobes	Five	Five	Five	Five	Five
OIV70	Area of anthocyanin coloration of main vein	At petiolar point	Absent	Absent	At petiolar point	At petiolar point
OIV76	Shape of teeth	Straight	Convex	Convex	Straight	Convex
OIV79	Degree of opening overlapping of petiole sinus	Open to Close	Closed to Ovelapped	Open	Closed	Open
OIV80	Shape of base of petiole sinus	U-Shaped	U-Shaped	V to U Shaped	U-Shaped	U-Shaped
OIV81-1	Teeth in the petiole sinus	None	None	None	None	None
OIV81-2	Petiole sinus base limited by veins	Not limited	Not limited	Not limited	Not limited	Not limited
OIV601	Length of vein N1	1.8 ±1**a**	1 ±0 **a**	1 ±0 **a**	1.6 ±0.97 **a**	1 ±0 **a**
OIV602	Length of vein N2	2.4 ±1.3 **a**	2 ±1.1 **ab**	2.2 ±1.0 **ab**	2 ±1.1 **ab**	1.4 ±0.84 **b**
OIV603	Length of Vein N3	3.2 ±1.1 **a**	3.2 ±0.63 **a**	3.2 ±1.5 **a**	3 ±0 **a**	2 ±1.1 **b**
OIV605	Length petiole sinus to upper lateral leaf sinus	1 ±0 **a**	1.3 ±0.48 **a**	1.7 ±0.48 **a**	1 ±0 **a**	1 ±0 **a**
OIV606	Length petiole sinus to lower lateral leaf sinus	1.4 ±0.84 **b**	1 ±0 **b**	2.2 ±1 **ab**	1.2 ±0.63 **b**	1.4 ±0.84 **b**
OIV607	Angle between N1 and N2	6.4 ±0.97 **a**	6.4 ±0.97 **a**	6.2 ±1 **a**	5.8 ±1 **ab**	5.8 ±1 **ab**
OIV612	Length of tooth N2	3 ±0.94 **a**	1.8 ±1 **b**	2.2 ±1 **ab**	1.8 ±1 **b**	1.8 ±1 **b**
OIV613	Width of tooth N2	4.8 ±1.1 **a**	2.4 ±1.3 **b**	3.2 ±1.1 **b**	3.9 ±0.97 **ab**	2.4 ±1.3 **b**
OIV614	Length of tooth N4	2.4 ±1.3 **a**	1.8 ±1 **ab**	2 ±1.1 **a**	1.8 ±1 **ab**	1.8 ±1 **ab**
OIV615	Width of tooth N4	3.4 ±1.3 **a**	2.2 ±1 **b**	2.2 ±1 **b**	2.8 ±1.5 **a**	2 ±1.1 **b**
OIV618	Opening/ overlapping of petiole sinus	3.2 ±0.63 **b**	4.2 ±1 **a**	3.6 ±0.97 **b**	3 ±0 **b**	3.6 ±0.97**b**

OIV601: 1:very short(75mm), 3:short(105mm), 5:medium(135mm), 7:long(165mm), 9:very long(195mm) and more. **OIV602:** 1:very short(65mm), 3:short(85mm), 5:medium(105mm), 7:long(125mm), 9:very long(145mm). **OIV603:** 1: very short (35mm), 3: short (55mm), 5: medium (75mm), 7: long (95mm), 9: very long (115mm). **OIV605:** 1: very short (30mm), 3: short (50mm), 5: medium (70mm), 7: long (90mm) 9: very long (110mm). **OIV606:** 1:very short(30mm), 3:short(45mm), 5:medium(60mm), 7:long(75mm), 9:very long(90mm). **OIV607:** 1: very small (30^o), 3: small (30^o-45^o), 5: medium (46^o-55^o), 7: large (56^o-70^o), 9: very large 71^o and more. **OIV612:** 1: very short (6mm), 3: short (10mm), 5: medium (14mm), 7: long (18mm), 9: very long (22mm). **OIV613:** 1:very narrow(6mm), 3:narrow(10mm), 5:medium(14mm), 7:wide(18mm), 9:very wide(22mm). **OIV614:** 1:very short(6mm), 3:short(10mm), 5:medium(14mm), 7:long(18mm), 9:very long(22mm). **OIV615:** 1:very narrow(6mm), 3:narrow(10mm), 5:medium(14mm), 7:wide(18mm), 9:very wide(22mm). **OIV618:**1: wide open (-35mm), 3: open (-15mm), 5: closed (-5mm), 7: overlapping 9: very overlapping.

Results in table (6) show that the color of upper side of the blade is bronze in Betuny, and Shame, yellow in Halawany, and copper reddish in Zane and Dapougy. As for the shape of blade, it is nearly circular for Shame, Dapougy, and Betuny, pentagonal for Zane, and wedge shaped for Halawany it was. Further, all grape cultivars under investigation have leaves with five lobes (Fig. 35).

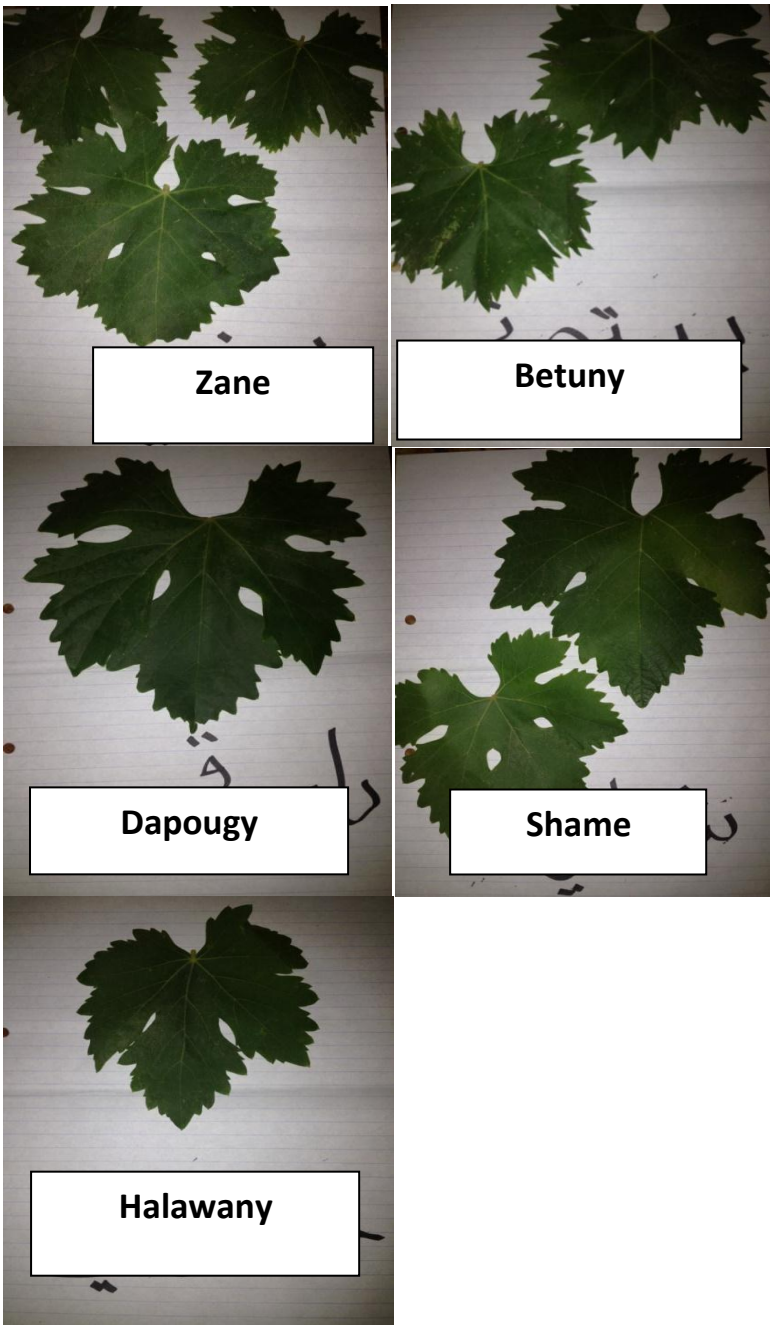


Figure 35: photos show number of lobes in leaves of five studied grapevines.

In addition, data show that the area of anthocyanin coloration of the upper side of blade is at petiolar point for Zane, Dapougy, and Shame varieties, whereas Betuny and Halawany varieties show no anthocyanin coloration.

The teeth shape for Zane and Dapougy varieties were on both sides straight, while in Betuny, Halawany and Shame cultivars the shapes of teeth were on both side convex. The different cultivars have different degree of opening. In Halawany and Shame cultivars they are open petiole sinus, and in Zane it was open for some leaves and closed for others. For Dapougy, it is closed petiole sinus, and with Betuny some leaves are closed, and some have overlapped petiole sinus. Another criterion is the shape of base of petiole sinus, which was similar with Zane, Betuny, Dapougy, and Shame cultivars that have U-shape. However, for Halawany, the shape of base of Petiole sinus is inconsistent; sometimes it was V-shaped and in others it was U–shaped. For all cultivars there are no teeth in the petiole sinus, the petiole sinus base is not limited by veins, and the length of vein N1 is considered as very short. However, there is significant differences appear between five grape cultivars in the following ampelometric characters such as length of vein N2 of mature leaves for Dapougy, Halawany, and Betuny cultivars is very short to short, whereas for Zane it is short. For Shame it is very short. As for the length of N3 veins, it is short for Zane, Betuny, Halawany, and Dapougy varieties, but for Shame it is short to very short. In addition, the length of petiole sinus to upper lateral leaf sinus, it is very short for all investigated grape cultivars (Zane, Dapougy, Shame, Halawany, Betuny). On other hand, the length of petiole sinus to lower lateral leaf sinus is very short for Zane, Betuny, Dapougy, Shame cultivars, and short to very short for Halawany cultivar.

In addition, the angle between N1 and N2 is large for Zane, Betuny, and Halawany cultivars and medium to large for Dapougy and Shame cultivars. Moreover, the length of tooth of N2 is short for Zane cultivar, short to very short for Halawany, and very short for Betuny, Dapougy, and Shame. Further, the width of tooth of N2 is medium for Zane, narrow for Betuny, Halawany, and Shame, and for Dapougy it is from narrow to medium. In addition, the length of tooth of N4 is short to very short for Zane and Halawany, and very short for Betuny, Dapougy, and Shame cultivars. Moreover, the width of tooth of N4 is narrow for Zane and Dapougy, and narrow to very narrow for Betuny, Halawany, Shame cultivars. In addition to that, results show a closed petiole sinus to small opening for Betuny, and open petiole sinus for Halawany, Zane, Dapougy, and Shame.

3.1.3 Cluster characteristics

The berry shape characteristics for the five grape cultivars are shown in table (7).

Table (7) Results of some morphological character of cluster for five grape cultivars

Morphological character	Grape cultivars				
	Zane	Betuny	Halawany	Dapougy	Shame
Berry shape	Narrow Ellipsoid	Broad Ellipsoid	Globose	Obtuse Ovoid to Broad Ellipsoid	Globose
color of skin	Green Yellow	Blue Black to Dark RedViolet	Red to Rose	Green Yellow	Dark Red Violet to Rose Red

Results in table (7) show that berry shape is different between cultivars. For Zane it is narrow ellipsoid, broad ellipsoid berry for Betuny,globose berry shape for Halawany and Shame, and obtuse ovoid berry shape to some broad ellipsoid for Dapougy (Fig. 36).

As for the skin color, it is green yellow with Zane and Dapougy, and Betuny has some berries with dark red violet skin color and others with blue black color. Halawany has red berry skin color with few berries that have rose color. Shame berries are dark red violet to rose red color.

Betuny cluster

Zane cluster

Halawany cluster

Dapougy cluster

Shame cluster

Figure 36: photos show skin color for berries of five studied grape cultivars (Betuny, Zane, Halawany, Dapougy, and Shame).

3.1.4 Similarity between five grape cultivars among all morphological characters.

Results in Table (8) show that the highest overall morphological similarity among the investigated grape varieties is between Zane and Dapougy (69.7%). Moreover, high morphological similarity is observed between Dapougy and Shame (63.6%). The lowest morphological similarity is between Zane and Betuny, Zane and Halawany, and Zane and Shame.

Table (8) Similarity percentage of morphological characterization between five studied grapevines.

	Zane	Betuny	Halawany	Dapougy	Shame
Zane	100%				
Betuny	48.5	100%			
Halawany	48.5	54.5	100%		
Dapougy	69.7	60.6	51.5	100%	
Shame	48.5	60.6	51.5	63.6	100%

3.2 Genetic Results

3.2.1 SSR markers analysis

Some genetic parameters were measured such as approximate band size (bp), total number of SSR bands, monomorphic bands, polymorphic bands, polymorphic percentage for every SSR primer, and genetic distance (similarity) and tree view of 28 grape accessions.

3.2.1.1 Approximate band size, monomorphic bands, polymorphic bands, and polymorphic percentage.

The SSR analyses were carried out to examine genetic diversity, and polymorphism level among and within 28 grape accessions. As shown in table (9), the 28 grape accessions were analyzed using 8 polymorphic SSR primers; analyses gave 50 polymorphic bands ranging in size from 169 to 260 bp. The number of allele per locus amounts 4 to 9, and the observed polymorphic percentage was 100% with all eight primers, which indicates that SSR markers are highly informative and sensitive.

Table (9) Band size, total SSR band, monomorphic, polymorphic, and polymorphic percentage for the 8 SSR primers.

Primer	Approximate band size(bp)		Total # of SSR band	Monomorphic band	Polymorphic band	Polymorphic%
	Min	Max				
VRZAG62	190	204	5	0	5	100%
VRZAG79	197	260	9	0	9	100%
VVMD27	190	197	4	0	4	100%
SCU05VV	185	194	4	0	4	100%
SCU11VV	240	260	7	0	7	100%
SCU08VV	220	230	7	0	7	100%
VMC8A7	169	180	6	0	6	100%
SCU15VV	199	216	8	0	8	100%

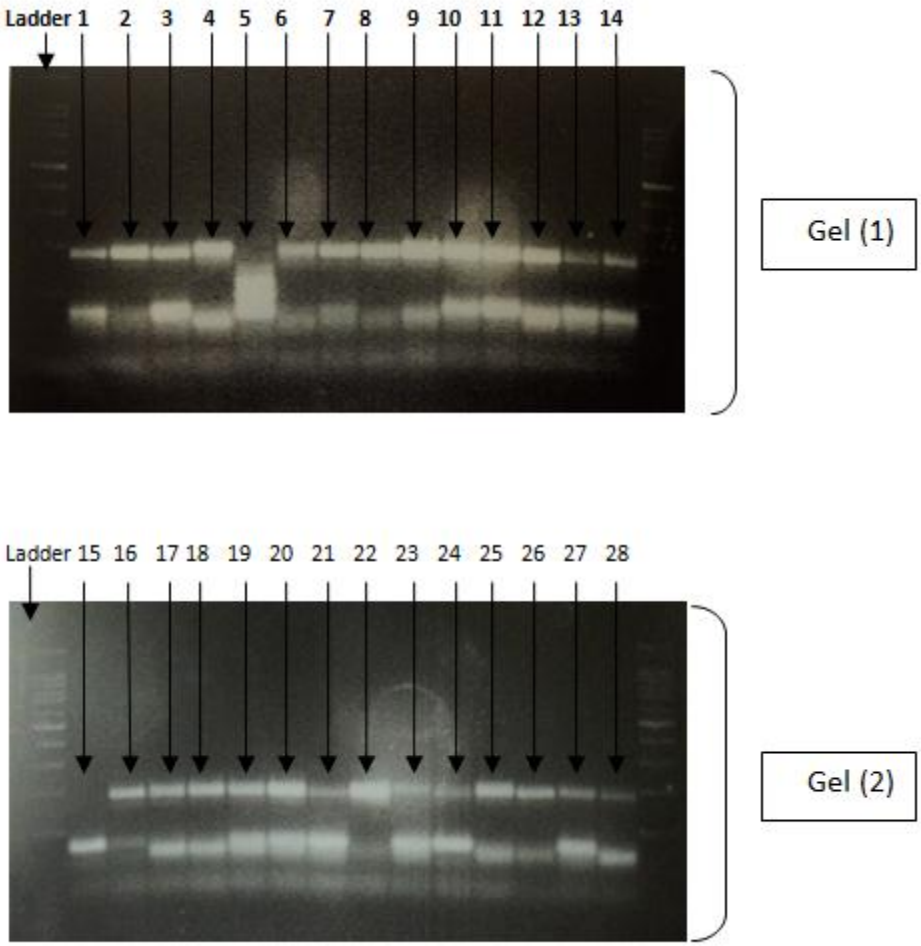


Figure 37: Results of VVMD27 primer show polymorphic bands for the 28 studied grape accessions and the order of accessions in two gels (gel 1 contains the first 14 grape accessions in table 2, and gel 2 contains the second 14 accessions) as the same order of these accessions in table (2).

3.2.1.2 Genetic distance (similarity) between different grape accessions.

From distance diagram (table 10), it is obvious that the greatest shared allele distance (d) detected is 1.00, and the smallest distance is 0.2, which indicate great dissimilarities between the accessions 14 (Zane) and 1 (Dapougy), 25 (Marawy) and 6 (Halawany), 28 (Masry) and 7 (Jandaly). On the other hand, the greatest similarity is between 1 (Dapougy) and 2

(Dapougy). Accordingly, it is obvious that the high distance values are the reflection of a large diversity among grape accessions that are collected from Hebron district.

Table (10) Genetic distance between 28 grape accessions.

	1Dap.	2Dapo.	3Dap.	4Hal.	5Hal.	6Hal.	7Jan.	8jan.	9Ham.	10Ham.	11Sham.	12Sham.	13Sham.	14Zan.	15Zan.	16Zan.	17Bair.	18Bair.	19Bair.	20Mtart.	21Mtart.	22M.L.	23Dar.	24Fhes.	25Mar.	26Balo	27Sal.	28Mas.
1Dapougy	0.00																											
2Dapo.	0.20	0.00																										
3Dap.	0.54	0.42	0.00																									
4Halawany	0.88	0.88	0.75	0.00																								
5Hal.	0.88	0.88	0.95	0.67	0.00																							
6Hal.	0.87	0.87	0.88	0.80	0.62	0.00																						
7Jandaly	0.93	0.93	0.94	0.87	0.94	0.85	0.00																					
8jandaly	0.82	0.82	0.89	0.76	0.76	0.73	0.62	0.00																				
9Hamadany	1.00	1.00	0.95	0.83	0.89	0.81	0.80	0.53	0.00																			
10Hamadany	0.95	0.95	0.95	0.83	0.89	0.88	0.88	0.71	0.43	0.00																		
11Shame	0.94	0.94	0.88	0.81	0.88	0.79	0.67	0.82	0.67	0.67	0.00																	
12Shame	0.94	0.94	0.95	0.82	0.89	0.80	0.87	0.76	0.69	0.69	0.73	0.00																
13Shame	0.94	0.94	0.95	0.82	0.89	0.71	0.87	0.76	0.69	0.69	0.73	0.33	0.00															
14Zane	1.00	1.00	1.00	0.90	0.95	0.89	0.89	0.86	0.67	0.67	0.84	0.56	0.56	0.00														
15Zane	0.88	0.88	0.82	0.82	0.75	0.80	0.94	0.76	0.76	0.83	0.88	0.82	0.82	0.79	0.00													
16Zane	0.89	0.82	0.83	0.89	0.89	1.00	1.00	0.95	0.84	0.84	0.95	0.95	0.95	0.80	0.60	0.00												
17Bairute	0.84	0.84	0.85	0.85	0.79	0.83	1.00	0.80	0.74	0.74	0.95	0.72	0.79	0.70	0.85	0.74	0.00											
18Bairute	0.76	0.76	0.78	0.84	0.78	0.75	0.88	0.65	0.85	0.85	0.95	0.71	0.78	0.81	0.78	0.85	0.33	0.00										
19Bairute	0.67	0.75	0.83	0.95	0.76	0.73	0.88	0.71	0.90	0.90	0.95	0.83	0.89	0.91	0.83	0.84	0.59	0.36	0.00									
20Mtartash	0.71	0.78	0.85	0.90	0.79	0.83	0.89	0.80	0.96	0.91	0.95	0.85	0.90	0.87	0.85	0.80	0.70	0.53	0.29	0.00								
21Mtartash	0.95	0.95	0.95	0.89	0.89	0.94	0.80	0.78	0.84	0.90	1.00	0.76	0.83	0.80	0.83	0.78	0.74	0.65	0.71	0.59	0.00							
22M.Lubnan	0.90	0.84	0.79	0.85	0.95	0.95	0.82	0.86	0.91	0.91	0.90	0.85	0.85	0.70	0.79	0.67	0.76	0.68	0.74	0.63	0.67	0.00						
23Daraweeshy	0.87	0.88	0.88	0.88	0.82	0.65	0.79	0.64	0.68	0.81	0.90	0.73	0.73	0.76	0.76	0.77	0.57	0.50	0.56	0.60	0.51	0.77	0.00					
24Fhesy	0.90	0.84	0.85	0.95	0.85	0.83	0.82	0.74	0.80	0.91	0.90	0.72	0.72	0.87	0.79	0.74	0.70	0.61	0.59	0.63	0.50	0.63	0.39	0.00				
25Marawy	0.95	0.89	0.90	0.90	0.95	1.00	0.88	0.90	0.79	0.85	0.95	0.84	0.84	0.81	0.95	0.65	0.68	0.86	0.85	0.81	0.56	0.61	0.75	0.61	0.00			
26Balooty	0.95	0.95	0.95	0.89	0.76	0.88	0.94	0.78	0.78	0.90	1.00	0.76	0.83	0.86	0.76	0.71	0.59	0.65	0.71	0.74	0.43	0.86	0.51	0.50	0.56	0.00		
27Salty	0.83	0.76	0.78	0.78	0.90	0.95	0.94	0.85	0.95	0.90	0.95	0.90	0.90	0.81	0.78	0.56	0.81	0.80	0.79	0.68	0.79	0.44	0.84	0.75	0.67	0.79	0.00	
28Masry	0.94	0.94	0.94	0.88	0.88	0.94	1.00	0.89	0.89	0.82	1.00	0.81	0.88	0.78	0.81	0.67	0.63	0.69	0.75	0.78	0.67	0.78	0.71	0.78	0.69	0.46	0.60	0.00

3.2.1.3 SSR markers result tree

From UPGMA tree (fig. 38), there is rapprochement between accessions of grape, such as 4 (Halawany from Halhul) and 5 (Halawany from Hebron) and 6 (Halawany from Dura). Moreover, there is rapprochement between 8 (Jandaly from Al Arrub) and 7 (Jandaly from Hebron).

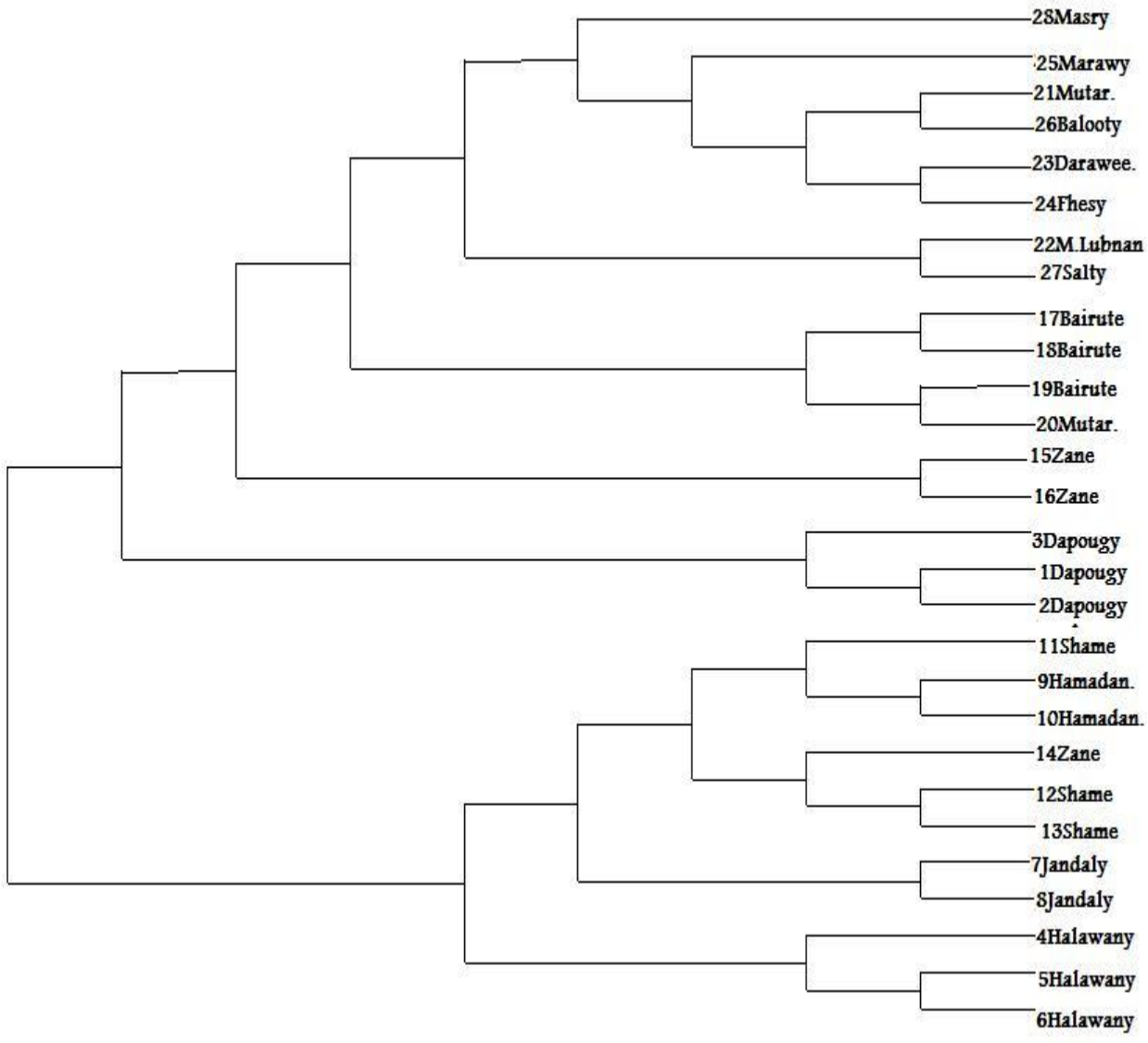


Figure 38:UPGMA tree based on 50polymorphic bandsobtained from eight SSR primers.

In addition, there are many examples for such rapprochement in tree. On the other hand, it is obvious that there are clear differences between some

accessions, that are considered to be the same variety species e.g: (21Mtartash from Hebron) and (20Mtartash from Halhul)). That indicates the two accessions are genetically different; they homonym but not for the same genotype. Furthermore, there are raproachment between (14Zane from Halhul) with (12Shame from Halhul) and (13Shame from Hebron), which indicates that there are genetic similarity between 22 (Zanee) and the two Shame accessions. This tree appears separation (differences) between assumed accessions, because it identified every three or two expected similar accessions in one variety.

Chapter Four

Discussion

4.1 Morphological characterization of five grapevines

Morphological characterization of woody shoot, young and mature leaves, and clusters were performed for five grape vine cultivars grown in Halhul at the same field.The results revealed similarities between the different grape cultivars for many morphological characteristics.

4.1.1 Woody shoots characters:

The results showed similarities in some morphological characters between the different grapevine cultivars. All five cultivars have the following characters: smooth structure of wood shoot surface, brownish color of woody shoot, green color of ventral side of internodes, and two or less consecutive tendrils. Similar results was obtained by Ekhvaia and Akhalkatsi (2010), where they found that the number of consecutive tendrils was two for all grape varieties they studied, and green color for ventral side of internodes.

There was variability between the five varieties in the aperture of the tip, the opening of the shoot tip (fully open in some and half open in others),the density of prostrate hairs on shoot tip between high, medium, and low; and the distribution of anthocyanin on prostrate hairs on shoot tip; some were piping, some overall, others absent. Ekhvaia and Akhalkatsi (2010) and Hassan et al. (2011) found an open shoot tip for their studied grape varieties, and variability between grape varieties in density of prostrate hairs on shoot tip, and distribution of anthocyanin coloration on prostrate hairs of the shoot tip.

On the other hand, the grapevine leaf hairs feature is one of the morphological characters with ampelographic value. Number and length of leaf hairs may differ between *Vitis* species and varieties and may be influenced by various environmental conditions. In addition, the hairs of abaxial leaf surface may play a role, due to its hydrophobic nature, in reducing the contact area for water droplets on leaf lamina; this reduces usually the wettability of the epidermis. However, the presence of very dense leaf may lead to a reduction in water retention capacity on leaf surface (Brewer et al., 1991; Kortekamp and zyprian, 1999), which is crucial for infection process (Zaiter et al., 1990). In this sense, density of abaxial leaf hairs is related, although indirectly, to the degree of tolerance of *Vitis* species to various pathogens (Stand and Kassemeyer, 1995). In this respect, high number of hairs does not automatically lead to better resistance, although hairs might be part of the resistance mechanism (Boso et al., 2010).In the current study, there were differences and similarities between the five varieties in woody shoot cross section character; Zane and Shame have oblate cross section, and Dapougy, Halawany, Betuny have elliptic cross section.

Concerning the woody shoot characters, most of the investigated cultivars were similar, such as attitude character, which is semi-erect for Zane, Betuny, Dapougy, Shame, and erect for Halawany. Further, the color of dorsal side of internodes is green and red color for Betuny, Halawany, Zane, Dapougy, and green for Shame. In addition to that, the length of tendrils is very short for Shame, Dapougy, Halawany, Zane, and short to very short for Betuny. These results are similar to those reported by Hassan et al (2011). Also Pour and Shakouri (2012) found that the color of dorsal side of internodes was green for some studied grape varieties and green and red for others.

4.1.2Young and mature leaf characteristic

Great similarities, five common characters for all studied grape varieties were recorded, namely number of lobes, which was five lobes for all; teeth in petiole sinus, as no teeth was recorded for all, petiole sinus base, which was not limited by veins with very short (N1), and the length of petiole, which was very short petiole sinus to upper lateral leaf sinus. Muganu et al (2009) and Pour and Shakouri (2010) found that number of leaf lobes for some studied grape varieties were five lobes , no teeth in petiole sinus for some studied grape varieties, and that petiole sinus base was not limited by veins for some varieties.

The color of the upper side of the 4th leaf blade was variable between the five grape varieties from bronze, yellow to copper reddish color. Also there were differences between cultivars in the shape of the blade, as some cultivars have circular, whereas others have pentagonal and wedge shaped. It was observed that the area of anthocyanin pigmentation of main vein on upper side of blade for Zane, Dapougy, and Shame, just at the petiolar point, whereas it was absent with Betuny and Halawany. These results are similar to those reported by Hassan et al. (2011) and Pour and Shakouri (2012), they found variability between grape varieties in color of upper side of blade. In their study, some varieties had coppery color, whereas other had bronzy and green bronzy, also they found variability in the shape of blade and variability of anthocyanin coloration on main veins.

In many plants, the bright red anthocyanin coloration is abundant in young and senescing leaves (Lee, 2007). However, anthocyanin biosynthesis is often initiated under drought, extreme temperature, and excessive light. Such changes reflect the role of these pigments as

indicator of plant stress (Neil and Gould, 1999). Moreover, it is generally accepted that the main physiological function of anthocyanin is to protect from light (photo protection). In this sense, pigments found in vacuoles of epidermal cells function as filters and an internal light trap for excessive solar radiation (Chalker and scott, 1999; Close and Beadle, 2003; Styne et al., 2002).

4.1.3 Cluster

The ampelographic character berry shape was distinct for the five investigated grape cultivars. The grape clusters vary also widely in shape and size, depending on the cultivar and their position on the shoot.

Further, the berry skin color vary also among grape cultivars, this is in agreement with Muganu et al (2009), Ekhvaia and Akhalkatsi (2010), they found that black berry skin color for some of their studied grape cultivars, red color for some others, and green yellow color for others, also they found variability in berry shape of studied cultivars.

The color of grape berry color is a very relevant trait and a wide variation, either genetic or somatic, exists among grape cultivars ranging from yellow-green (white) to dark blue berries. It is well known that berry color results from the synthesis, and consequently the accumulation of anthocyanins in the skin; the biosynthesis of these compounds is mainly regulated by MYB transcription factors (Lijavetzky et al) and VvmybA1 gene (This et al., 2006).

Concerning the morphological characteristics of bunch and berry anatomy, which may affect grape resistance to pathogens; loose bunches with movable berries may mean less decay. In this sense, tight bunches

have micro-environmental conditions in the fruit zone, that lead to increases in air temperature, much low aeration and high relative humidity. It is well known that such conditions promote pathogen growth, as reported for *B. cinerea* (Commenil et al., 1997). In addition the frequent berry skin cracks may lead to the release of free water, and consequently the germination of *B. cinerea* conidia. Furthermore, the increase of physical contact between berries during throughout the growing season leads to the formation of flattened areas, which in turn affects the structure of epicuticular wax. Under such condition, higher number of gray mold infections occur compared no flattened berries (Percival et al., 1993; Marios et al., 1985). In this sense, recent studies recorded a negative correlation between berry degree of resistance to *E. necator* and bunch density, (Cadle-Davidson et al., 2010). On the other hand, a positive correlation was recorded among berry resistance to gray mold and berry skin thickness, which reflects the number of epidermal cell layers (Vannuccini, 1982). In another study, the intravarietal assessment of Spanish Albariño variety clearly shows that genotypes with small berries and short pedicels are less susceptible to gray mold (Alonso-Villaverde et al., 2011).

4.1.4 Morphological similarity between five grape cultivars

Through the use of OIV descriptors for grape varieties, it is clear that Dapougy and Zane have the highest similarity among the studied varieties. Also there are some degree of similarity between Dapougy and Betuny, and between Betuny and Shame. These results show that Dapougy cultivar may be considered as a diverse cultivar that has many common traits with other cultivars (Zane, Shame, and Betuny). Because of this diversity, it could be used to improve the hereditary qualities for

some weak or worse grape cultivars. On the other hand, Shame and Betuny have many common morphological traits with other studied grape varieties. Halawany grape nearly considered the lowest cultivar that has common morphological traits with other studied grape cultivars. But generally there is high similarity between the five studied grapes in morphological characteristics that carried out on them.

4.2 Genetic characterization

4.2.1 SSR analysis

The employment of SSR markers in this study was due to the fact that these are highly transferable among grapevine genotypes (Scotte et al., 2000). Moreover, SSR markers are widely used in molecular research, since they are numerous, co dominant, and highly polymorphic, informative (Marquez-Lema et al., 2010; Nybom, 2004).

In this study, the 28 grapevine varieties showed high genetic diversity with polymorphic percent equal 100% (from 8 SSR markers). Results clearly show that Halawany accessions 6, 5, and 4, which were collected from Dura, Hebron, and Halhul, respectively, have the same genetic profiling and should be regarded as one variety, namely Halawany. Furthermore, Jandaly accessions 8 (from Al Arrub) and 7 (from Hebron) are identical. Similarly Dapougy accessions 2, 1, and 3 from Hebron, Dura, and Halhul, respectively, are identical. Similar results were recorded for Bairute accessions 17, 18, and 19Bairute, which were collected from Hebron, Halhul, Halhul respectively. Similar results were also recorded for Hamadany (accessions 9, and 10). For Zane, the accessions 15, and 16 were identical, whereas accession 14 was similar to Shame accessions 12 and 13. In this sense, accordingly, it is obvious that

allelic diversity reflects high heterozygosity among grape varieties that were collected from Hebron area. Such trend was reported by Ekhvaia and Akhalkatsi (2010). The molecular characterization using SSR markers is reported in various studies such as that of Martinez et al (2006), and Karatas et al (2007), Muganu et al (2009).

The final aspect is the relatedness of grape cultivars based on both morphological and genetic characterization. It is important to mention here that the identification of genes that govern morphological and physiological traits was not possible in this study. However, coupling genetic relatedness and morphological characters may be beneficial in determining the possible genetic background of the most important agronomic traits, in particular resistance to fungal pathogens. In this sense, Ekanayake et al., (1985) recorded the polygenic inheritance of root characters and reported that long root and high root numbers are controlled by dominant alleles, whereas thick root tips are controlled by recessive alleles (Armento-Soto et al., 1983).

In our results, connections between genetic relatedness and morphological similarities can be seen, although partially. As an example, there are morphological similarity between Halawany and Shame (51.5%), which reflects itself in genetic relatedness. Another example is the Dapougy, and Zane (69.7%), which reflects itself also in the dendrogram.

In conclusion, morphologically, the present study reveals major differences and similarities (common traits) between five investigated grape cultivars (Zane, Betuny, Halawany, Dapougy, and Shame). Berry

shape and berry skin color are the most morphological characters that appear differences between grape varieties.

Genetically, this study reveals differences between assumed grape accessions. Fingerprinting techniques proved to be important in evaluation of grape genetic diversity and as a tool for cultivar identification.

Generally, this study considered as a small step in the process of characterization and definition of grape varieties in Palestine, and it is crucial to continue working on identifying all grape genotypes in Palestine.

References

Adam-Blondon, AF., Roux, C., Claux, D., Butterlin, G., Merdinoglu D., and This, P. (2004). Mapping 245 SSR markers on the Vitisvinifera genome: a tool for grape genetics. TheorAppl Genet. 109: 1017–1027.

Alleweld, G., Spiegel-Roy, P., and Reisch, B. (1990). Grapes (Vitis). In: Moore, I.N., Ballington, J.L. (Eds.), Genetic resources of temperate fruit and nut crops. Acta Hortic. 290: 291–337.

Afghan, S., Haider, M.S., Shah, A.H., Rashid, N., Iqbal, J., Tahir, M., and Akhtar, M. (2005). Detection of genetic diversity among sugarcane genotypes using RAPD Markers. Sugarcane International. 23(6): 15-19.

Alonso-Villaverde, V., Voinesco, F., Viret, O., Spring, J.L., and Gindro, K. (2011). The effectiveness of stilbenes in resistant Vitaceae: Ultra structural and biochemical events during Plasmopara viticola infection process. Plant Physiology and Biochemistry. 49: 265-274.

Applied Research Institute-Jerusalem (ARIJ). (2009). Palestinian Localities Study in Hebron district; Al Arrub. Jerusalem, Palestine.

Applied Research Institute-Jerusalem (ARIJ). (2009). Palestinian Localities Study in Hebron district; Dura. Jerusalem, Palestine.

Applied Research Institute-Jerusalem (ARIJ). (2009). Palestinian Localities Study in Hebron district; Halhul. Jerusalem, Palestine.

Applied Research Institute-Jerusalem (ARIJ). (2009). Palestinian Localities Study in Hebron district; Hebron. Jerusalem, Palestine.

Armento-Soto, J. L., Chang, T. T., Loresto, G. C., and O'Toole, J. C. (1983). Genetics and genetic improvement of drought resistance in crop plants. J. Soc. Adv. Breed. Asia Oceania. 15: 103–116.

Arnold, C., Rossetto, M., Mcnally, J., and J. Henry, R. (2002). The application of SSRs characterized for grape (*Vitis vinifera*) to conservation studies in Vitaceae. American Jornal of Botany. 81(1): 22-28.

Bassermann, J. (1923). Geschichte des Weinbaus. Frankfurt. Am. Main, Frankfurter verlags. An. Statt. A. G. Vol: 1.

Bazzaz, FA. (1996). Plants in changing environments: linking physiological, population and community ecology. Cambridge: Cambridge University Press.

Bonser, S I., and Aarssen, L W. (1994). Plastic allometry in young sugar maple (Acer saccharum): adaptive responses to light availability. Am. J. Bot. 81: 400-406.

Boso, S., Villaverde, V.A., Santiago, J.L., Gago, P., Durrenberger, M., Duggelin, M., Kassemeyer, H.H., and Martinez, M.C. (2010) Macro- and microscopic leaf characteristics of six grapevine genotypes (Vitis spp.) with different susceptibilities to grapevine downy mildew. Vitis. 49(1):43-50.

Bowers, J. E., Dangl, G. S., Vignani, R., and Meredith, C. P. (1996). Isolation and characterisation of new polymorphic simple sequence repeat loci in grape (Vitisvinifera). Genome 39: 628–633.

Bowers, J. E., Boursiquot, J. M.,This, P., Chu, K., Johanssen, H., and Meredith, C. (1999). Historical Genetics: The parentage of Chardonnay,

Gamay and other wine grapes of Northeastern France. Science. 285: 1562-1565.

Brewer, C.A., Smith, W.K., and Vogelmann, T.C. (1991). Functional interaction between leaf trichomes, leaf wettability and optical properties of water droplets. Plant Cell and Environment. 14: 995-962.

Cadle-Davidson, L., Chicoine, D.R., and Consolie, N.H. (2010). Variation within and between Vitis species for foliar resistance to the powdery mildew pathogen Erysiphenecator. Plant Disease. 95: 202-211.

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. Photochem. Photo biol. 70:1-9.

Cipriani, G., Frazza, G., Peterlunger, E., and Testolin, R. (1994). Grapevine fingerprinting using microsatellite repeats. Vitis. 33: 211-215.

Close, D.C., and Beadle, C.L. (2003). The ecophysiology of foliar anthocyanin. Bot. Rev. 69: 149-161.

Cody, M.L., and Mooney, H.A. (1978). Convergence versus non-convergence in Mediterranean-climate ecosystems. Annu Rev Ecol Syst. 9: 265–321.

Commenil, P., Brunet, L., and Audran, J.C. (1997).The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. Journal of Experimental Botany. 48: 1599-1607.

Crespan, M. (2004). Evidence on the evolution of polymorphism of microsatellite markers in varieties of Vitisvinifera L. Theor. Appl. Genet. 108: 231-237.

Crossley, M.N., Dennison, W.C., Williams, R.R., and Wearing, A.H. (2002). The interaction of water flow and nutrients on aquatic plant growth. Hydrobiologia. 489: 63–70.

Cunningham, S.A., Summerhayes,B.A., and Westoby, M. (1999). Evolutionary divergences in leaf structure and chemistry comparing rainfall and soil nutrient gradients. EcolMonogr. 69:569–588.

Dalbo, M.A., Ye, G.N., Weeden, N.F., Steinkellner, H., Sefc, K.M., and Reisch, B.I. (2000). A gene controlling sex in grapevines placed on a molecular marker-based genetic map. Genome. 43: 333–340.

Dettweiler, E., Jung, A., Zyprian, E., and To¨ pfer, R. (2000). Grapevine cultivar Mu¨ ller-Thurgau and its true to type descent. Vitis. 39: 63–65.

Dion, R. (1977). Histoire de la vigne et du vin en France des origines au XIX\gravee me sie` cle. Flammarion, Paris. P: 768.

Dolph, G.E., and Dilcher, D.L. (1980). Variation in leaf size with respect to climate in the tropics of the Western Hemisphere. Bull Torrey Bot Club. 107: 154–162.

Ekanayake, I. J., O'Toole, J. C., Garrity, D. P. and Masajo, T. M. (1985). nheritance of root characters and their relations to drought resistance in rice. Crop Sci. 25: 927–933.

Ekhvaia, J., Akhalkatsi, M. (2010). Morphological variation and relationships of Georgian populations of Vitis vinifera L. subsp. sylvestris (C.C. Gmel.) Hegi. Flora.50396: No. of pages 10.

Faria, M., Magalh˜aes, A. R., Ferreira, M.A., Meredith, C.P., Monteiro, F.F., and Agric, J. (2000). Vitisvinifera must varietal authentication using microsatellite DNA analysis SSR. J Agric Food Chem. 48(4): 1096-1100.

Ferguson, I.B. (2004). The plant response: stress in the daily environment. Journal of Zhejiang University SCIENCE. 5(2): 129-132.

Fischer, B.M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K.J., Topfer, R., and Zyprian, E.M. (2004). Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. TheorAppl Genet. 108:501–515.

Fonseca, C.R., Overton, J.M., Collins, B., and Westoby, M. (2000). Shifts in trait combinations along rainfall and phosphorus gradients. J Ecol. 88:964–977.

Franks, T., Botta, R., and Thomas, M. R. (2002). Chimerism in grapevines: Implications for cultivar identity, ancestry and genetic improvement. Theor. Appl. Genet. 104: 192-199.

Fregoni, M. (1991). Origines de la vigne et de la viticulture. Musumeci. Quart Italie. P: 160.

Galet, P. (1956). Cépages et vignobles de France. Précis d´Ampélographie Practique. Tome I: Les Vignes Américaines. Imp. Déhan, Montpellier, France.

Galet, P. (2000). Cépages et Vignobles de France. L`Ampélographie Française (Vol. II): 2éme ed. Imp. Déhan, Montpellier, France.

Gedroc, J.J., McConnaughay, K.D.M., and Coleman, J.S. (1996). Plasticity in root/ shoot partitioning: optimal, ontogenetic, or both? Functional Ecology.10: 44–50.

Grando, M.S., Demicheli, L., and Scienza, A. (1996). Characterization of Vitis germplasm using random amplified polymorphic DNA markers. Genetic Res. Crop Evol. 43: 187-192.

Grime, J. P. (1979). Plant strategies and vegetation processes. John Wiley & Sons, Chichester, UK.

Hamann, O. (1979). On climatic conditions, vegetation types, and leaf size in the Galápagos Islands. Biotropica. 11:101–122.

Hancock, J.F. (1992). Plant Evolution and the Origin of Crop Species Prentice-Hall, Inc., Englewood Cliffs, NJ. pp. 297.

Hassan, NA., El-Homosany, A., Gomma, AH., and Shaheen, MA. (2011). Morphological and Issr Polymorphisms in Some Egyptian Grapes (*Vitis vinefera L*.) Collection. World Applied Sciences Journal. 15 (10):1369-1375.

Heslop-Harrison, J. (1964). Forty years of genecology. Advances of Ecological Research. 2: 159–247.

Horn, Henry, S. (1971). The adaptive geometry of trees. Princeton, NJ: Princeton University Press. P: 144.

Huglin, P. (1986). Biologie et Écologie de la Vigne. Ed. PayotLausane, París.

Hussain, K., Nisar, M.F., Nawaz, K.,Abdul Majeed, and Bhati, K.H. (2010). Morphological traits vs. genetic diversity: reliable basis for sugarcane varieties identification. E-Journal of Life Sciences. 1(2): 41-43.

Hutchings, M. J., and DE Kroon, H. (1994). Foraging in plants: the role of morphological plasticity in resource acquisition. Advances in Ecological Research. 25: 159–238.

Jing, R., Johnson, R., Seres, A., Kiss, G., Mike, J., Maggie, A., Knox, R., Ellis, N., and Flavell, A. (2007). Gene-Based Sequence Diversity Analysis of Field Pea (Pisum). Genetics. 177: 2263-2275.

Karatas, H., Degirmenci, D., Velasco, R., Vezzulli, S., Bodur, C., and Agaoglu, Y.S. (2007). Microsatellite fingerprinting of homonymous grapevine (Vitisvinifera L.) varieties in neighboring regions of South-East Turkey. Scientia Horticulturae. 114: 164–169.

Kirchheimer, F. (1938). Aus der Geschichte der rebengewachse. Wein u. Rebe., 20:188-192.

Kleif, M., Ibrahim, A., and Othman, A. (1991). Grapes Planting, Care and Production. Al-Ma'ref Inc., Alexandria.

Knight, H., and Knight, M.R. (2001). Abiotic stress signaling pathways: specificity and cross- talk. Trends Plant Sci. 6: 262-267.

Kortekamp, A., and Zyprian, E. (1999). Leaf hairs as a basic protective barrier against downy mildew of grape. J. Phytopathology. 147: 453-459.

Kramer, Paul J., Kozlowski,Theodore T. (1979). Physiology of woody plants. New York, NY. Academic Press. P: 811.

Lamikanra, O. (1993). Identification of grape cultivars from their seed polypeptide composition. Phytochem. 32: 1199-1202.

Langlet, O. (1971). Two hundred years genecology. Taxon. 20: 653–722.

Lee, D. (2007). Nature’s Palette: The Science of Plant Color. University of Chicago Press, Chicago. P: 409.

Li, B., Suzuki, J-I., and Hara, T. (1999). Competitive ability of two Brassica varieties in relation to biomass allocation and morphological

plasticity under varying nutrient availability. Ecological Research. 14: 255–266.

Lijavetzky, D., Ruiz-García, L., Cabezas, J. A., de Andrés, M. T., Bravo, G., Ibáñez, A., Martínez-García, J., Vicente-Renedo, T., Carreño, J., Cabello, F., Ibáñez, J., and Martínez-Zapater, J. M. Genetic Control of Berry Skin Color.

Lodhi, M.A., and Reisch, B.I. (1995) In situ hybridization in Vitisvinifera L. Theor. Appl. Genet. 90: 11–16.

LUO, S., and HE, P. (2001). Discrimination of wild grapes native to China by RAPD markers. Vitis.40: 163–168.

Marois, J.J., Bledsoe, A.M., and Gubler, W.D. (1985). Effect of surfactants on epicutilcular wax and infection of grape berries by Botrytis cinerea. Phytopathology. 75: 1329.

Marquez-Lema, A., Velasco, L., and Prez-Vich, B. (2010). Transferability, amplification quality, and genome specificity of microsatellites in Brassica Carinata and related species. J Appl Genet. 51(2): 123-131.

Martinez, M. C., Boso, S., and Santiago, J. L. (2005). Los Clones de Albariño (Vitisvinifera L.) Seleccionados en el Consejo Superior de

Investigaciones Científicas. Departamento de Publicaciones del CSIC, Biblioteca de Ciencias, Madrid.

Martinez, L.E., Cavagnaro , P.F., Masuelli , R.W., and Zuniga, M. (2006). SSR-based assessment of genetic diversity in South American Vitisvinifera varieties. Plant Science. 170: 1036–1044.

Mondini, L., Noorani, A., and A.Pagnotta, M. (2009). Assessing Plant Genetic Diversity by Molecular Tools. Diversity. 1:19-35.

Mooney, H.A. (1977). Convergent evolution in California and Chile: Mediterranean climate ecosystems. Dowden, Hutchinson and Ross, Stroudsberg, Pa. pp: 85-143.

Mooney, H.A., and Dunn, E.L. (1970). Convergent evolution of Mediterranean-climate evergreen sclerophyllous shrubs. Evolution. 24:292–303.

Muganu, M., Dangl, G., Aradhya, M., Frediani, M., Scossa, A., and Stover, E. (2009). Ampelographic and DNA characterization of local grapevine accessions of the Tuscia area (Latium, Italy). American Journal of Enology and Viticulture. 60:110-115.

Neill, S., and Gould, K. (1999). Optical properties of leaves in relation to anthocyanin concentration and distribution. Can. J. Bot. 77:1777-1782.

Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecology. 13:1143-1155.

OIV (Organisation Internationale de la Vigne et du Vin). (1984). Code des Caractères Descriptifs des Variétés et Espèces de*Vitis*. Off. Int. Vigne Vin. Ed. Dedon. Paris, France.

OIV (Organisation Internationale de la Vigne et du Vin). (2001). Code descriptor list for grape varieties and vitis species. Paris, France.

Ortiz, J. M. , Martín, J. P. , Borrego, J.,Chávez, J. , Rodríguez, I. , Muñoz, G., and Cabello, F. (2004). Molecular and morphological

characterization of a Vitis gene bank for the establishment of a base collection.Genetic Resources and crop Evolution. 51: 403-409.

Parkhurst, D., and Loucks, O. (1972). Optimal leaf size in relation to environment. J Ecol. 60:505–537.

Pelsy, F. (2010). Molecular and cellular mechanisms of diversity within grapevine varieties. Heredity. 104(4): 331-340.

Percival, D.C., Sullivan, J.A., and Fisner, K.H. (1993). Effect of cluster exposure, berry contact and cultivar on cuticular membrane formation and occurrence of bunch rot (Botrvtiscinerea) with three Vitisvinifera L. cultivars. Vitis. 32: 87-99.

Pearson, R.C., and Goheen, A.C. (1990). Compendium of Grape Diseases. APS Press, St. Paul, MN. p: 93.

Pour, M. A., and Shakouri, M. J. (2012). Recognition of Morphological Traits of Grapevine in Piranshahr and Sardasht. Annals of Biological Research. 3(2):1070-1075.

Reich, P.B., Walters, M.B., and Ellsworth, D.S. (1997). From tropics to tundra: global convergence in plant functioning. Proc Ntl Acad Sci USA. 94:13730–13734.

Regner, F., Wiedeck, E., and Stadlbauer, A. (2000). Differentiation and identification of White Riesling clones by genetic markers. Vitis. 39: 103-107.

Riaz, S., Dangl, G.S., Edwards, K.J., and Meredith, C.P. (2004). A microsatellite marker based framework linkage map of *Vitis vinifera* L. Theor Appl Genet. 108:864–872.

Saatchi, S., Houghton, R., Avala, R., Yu, Y., and Soares, J.V. (2007). Spatial distribution of live aboveground biomass in Amazon Basin. Global Change Biology. 13:816-837.

Santiago, J. L., Boso, S., Martinez, M. C., Pinto-Carnide, O., and Ortiz, J. M. (2005). Ampelographic comparison of grape cultivars (Vitisvinifera L.) grown in northwestern Spain and northern Portugal. Am. J. Enol. Vitic. 56: 287-290.

Sattler, R. (1978). What is theoretical plant morphology? In: R. Sattler (ed). Theoretical plant morphology. Acta. Biotheor. 27: 5-20.

Schlotterer, C. (2004). The evolution of molecular markers – just a matter of fashion. Nat Rev Genet. 5:63-69.

Schluter, B.M., and Harris, F.A. (2006). Analysis of multi locus fingerprinting data sets containing missing data. Molecular Ecology. 6: 569-572.

Scott, K. D., Eggler,P., Seaton,G., Rossetto, M., Ablett, E. M., Lee, L. S, and Henry, R. J. (2000). Analysis of SSRs derived from grape ESTs. Theor Appl Genet. 100: 723–726.

Sefc, K.M., Regner, F., Turetschek, E., Glo¨ ssl, J., and Steinkellner, H. (1999). Identification of microsatellite sequences in Vitisriparia and their applicability for genotyping of different Vitis species. Genome. 42:367–373.

Sefc, K. M., Lefort, F., Grando, MS, Scott, K., Steinkellner, H., and Thomas, M. (2001). Microsatellite markers for grapevine: A state of the art.Amsterdam: Kluwer Publishers.

Staudt, G., and Kassemeyer, H. H. (1995). Evaluation of downy mildew resistance in various accessions of wild Vitis species. Vitis. 34: 225-228.

Steyn, W.J., Wand, S.J.E., Holcroft, D.M., and Jacobs, G. (2002). Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. New Phytol. 155:349-361.

Subden, R.E., Krizus, A., Lougheed, S.C., and Carey, K. (1987). Isozyme characterization of Vitis species and some cultivars. Am. J. Enol. Vitic. 38: 176-181.

Sultan, S.E. (1987). Evolutionary implications of phenotypic plasticity in plants. Evol Biol. 21:127–178.

Sultan, S. E. (1992). What has survived of Darwin's theory? Evolutionary Trends in Plants. 6: 61–71.

Sultan, S. (2005). Grapevines Establishing Planting Training Pruning Servicing. pp: 24-31.

Sultan, S., Bazzaz, F. (1993). Phenotypic plasticity in *Polygonum persicaria* II. Norms of reaction to soil moisture and the maintenance of genetic diversity. Evolution. 47:1032–1049.

Sultan, S.E., Wilczek, A.M., Bell, D.L., and Hand, G. (1998). Physiological response to complex environments in annual Polygonum species of contrasting ecological breadth. Oecologia. 115: 564–578.

This, P., Lacombe, T., and Thomas, M.R. (2006). Historical origins and genetic diversity of wine grapes. Trends Genet. 22: 511–519.

Thomas, M.R., Scott, N.S., (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. 86: 985-990.

Thomas, M. R., Cain, P., and Scott, N. S. (1994). DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. Plant Molecular Biology. 25(6): 939-949.

Thomas, M.R. et al. (1993) Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theor. Appl. Genet. 86: 173–180.

Vannuccini, L. (1892). I vitignitoscani. In "Annuariogenerale di Viticolturaed Enologia, anno I.

Weiher, E., and Keddy, P.A. (1995). The assembly of experimental wetland plant communities. Oikos. 73: 323–335.

Weising, K., Nybom, H., Wolff, K., &Kahl, G. (2005). DNA fingerprinting in plants: principles, methods and applications. CRC Press Taylor & Francis Group. pp: 472.

Williams, D. G., and Black, R. A. (1993). Phenotypic variation in contrasting temperature environments: growth and photosynthesis in *Pennisetum setaceum* from different altitudes on Hawaii. Functional Ecology. 7: 623–633.

Wilson, Brayton F. (1995). Shrub stems: form and function. In: Gartner, Barbara L., ed. Plant stems: physiology and functional morphology. New York, NY: Academic Press. P: 440.

Winkler, A.J., Cook, J.A., Kliewer, W.M., and Lider, L.A. (1974). General Viticulture. University of California Press, Berkeley, CA. p: 710.

Zaiter, H.Z., Coyne, D.P., Staedman, J.R., and Beaver J.S. (1990). Inheritance of abaxial leaf pubescence in beans. J. Amer. Soc. Horticult. Sci. 115: 1158-1160.

Zhang, J. (1996). Interactive effects of soil nutrients, moisture and sand burial on the development, physiology, biomass and fitness of Cakike dentula. Annals of Botany. 78: 591–598.

Zohary, D. (1995). Domestication of the Grapevine Vitisvinifera L. in the Near East. In The origins and Ancient History of Wine. Gordon and Breach. pp: 23–30.

المراجع العربية

ابراهيم حسن محمد السعيدي . زراعة وانتاج الكروم (1984) . وزارة التعليم العالي والبحث العلمي، جامعة الموصل، الجمهورية العراقية .

جبار عباس حسن ومحمد عباس سلمان . انتاج الأعناب (1989) . وزارة التعليم العالي والبحث العلمي، جامعة بغداد.

د. سفيان عبدالرحمن شكري سلطان . كروم العنب انشاء زراعة تربية تقليم خدمة (2005) . وزارة الاعلام الفلسطينية ، الخليل ، فلسطين.

الملخص باللغة العربية

التشخيص الجيني والشكلي لأصناف العنب (*Vitis*) في منطقة الخليل

تمت دراسة عدة أصناف من نبات العنب في أربع مناطق من محافظة الخليل وهي دورا، الخليل، حلحول، العروب .

تشمل الدراسة قسمين: (1) دراسة الصفات الشكلية لخمسة أصناف عنب في منطقة حلحول باستخدام قائمة صفات شكلية للعنب (OIV, 2001) وهذه الأصناف هي الزيني، البيتوني، الحلواني، الدابوقي، و الشامي.

(2) التشخيص الوراثي لثمانية وعشرون من أصناف مختلفة للعنب من الأربع مناطق المذكورة سابقا.

تم التشخيص الشكلي على ثلاثة مراحل وهي: مرحلة الأغصان الخشبية في فصل الشتاء، ومرحلة الأوراق الصغيرة والناضجة في فترة الربيع وبداية الصيف، ومرحلة القطوف الناضجة أيضا في الصيف.

وقد أظهرت الدراسة اختلاف في بعض الصفات الشكلية بين الخمسة أصناف مثل شكل الورقة، لون سطح الورقة، شكل الثمرة، لون الثمرة. وأيضا ظهرت صفات كثيرة مشتركة بين الخمسة أصناف. وكانت أعلى نسبة تشابه حوالي 69.7% بين صنفي الزيني والدابوقي.

التشخيص الوراثي تم قياسه باستخدام بادئات (SSRs) وكانت نتائج هذه الاختبارات على شكل شجرة قرابة لثمانية وعشرون عينة عنب . وقد أظهرت نتائج جدول التشابه مسافة قليلة جدا بين عينات العنب التي تشكل نفس الصنف وكانت أقل مسافة مسجلة (d=0.2)، وأيضا كانت أعلى مسافة (d=1) بين الأصناف المختلفة، وهذا يدل على التنوع الجيني في أصناف العنب في هذه المنطقة.

استنادا الى النتائج التي تم الحصول عليها، هناك حاجة ملحة لأعادة النظر في تسمية بعض أصناف العنب، علاوة على ذلك ينبغي استخدام تقنيات البصمة الوراثية لتوصيف جميع أصناف الكرمة في محافظة الخليل من قبل مؤسسة علمية مختصة لأن العنب هو محصول اقتصادي مهم جدا في منطقة الخليل.

استخدام الصفات الشكلية بالإضافة الى البصمة الوراثية أعطت نتائج واضحة ومتكاملة أكثر من استخدام كل طريقة على حدة.