MOLECULAR ANALYSIS OF HISTORICAL WINE GRAPE VARIETY CANDIDATES FOUND IN URLA

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MOLECULAR ANALYSIS OF HISTORICAL WINE GRAPE VARIETY CANDIDATES FOUND IN URLA

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ABSTRACT

Urla had been an important center of viticulture and wine making due to its suitable ecology for vineyards. It is also known that there are some grape varieties endemic to this area. However, for various reasons, the viticulture in Urla started to diminish in the beginning of the twentieth century, almost getting to the point of extinction in the mid-century. As the interest for wines and wine grapes rose in the 1990s, vineyards have started to be rebuilt in this area. However, mostly foreign varieties are grown in these vineyards; the local cultivars are non-existent now, except for some small vineyards. Rebuilding of vineyards and wineries would be very valuable for both economic and touristic development of Urla. Detecting and registering local grape cultivars and producing high-quality chateau wines from these grapes would create a greater added value.

Five grapevines that might represent different red wine grape varieties have been found in Urla. Although these vines might represent historical local grape cultivars, they might also be some examples of grape varieties that are already known. In this study the vines that were found in Urla were compared to the black grape cultivars collected from the Aegean Region, and to the major Turkish and the world red wine grape varieties, using molecular methods. For the molecular analyses, SSR (Simple Sequence Repeat) markers were utilized first. 14 grapevine varieties that show close relationship to the vines found in Urla were further analyzed with AFLP (Amplified Fragment Length Polymorphism) markers. UPGMA graph and genetic similarity coefficient values of the AFLP analysis indicated that Urla karası 4 and Urla karası 5 belong to grapevine accessions certainly different from the analyzed samples. However, in order to determine whether or not the vines found in Urla represent economically valuable novel red wine grape varieties, these should be further propagated and their wine qualities analysed

Keywords: *Vitis vinifera*, grapevine, SSR, AFLP

ÖZET

İzmir, Urla'nın ekolojisinin bağ yetiştiriciliği için çok elverişli olması bu yörenin antik çağlardan beri önemli bir bağcılık ve şarapçılık merkezi olmasını sağlamıştır. Ayrıca yöreye özgün üzüm çeşitlerinin de bulunduğu bilinmektedir. Fakat, çeşitli nedenlerden dolayı Urla yöresindeki bağcılık 20. yüzyılın başlarında azalmış, yüzyılın ortalarında ise kaybolma noktasına gelmiştir. 1990'lardan itibaren şarapçılığa ve şaraplık üzüme artan ilgi nedeniyle Urla yöresinde yeniden bağlar kurulmaya başlanmıştır. Fakat bu bağlarda çoğunlukla yabancı üzümlerin yetiştirildiği, birkaç küçük bağ dışında yerli çeşitlere fazla yer verilmediği görülmektedir. Urla yöresinin tarihini yansıtan bağcılık ve şarapçılığın tekrar canlanması, bu yörenin hem ekonomik hem de turistik açıdan gelişebilmesi için büyük önem taşımaktadır. Öyle ki, yerel tarihi üzüm çeşitlerinin saptanması ve tescil edilmesi ile bu çeşitlerle tesis edilecek bağlardan kaliteli şaraplık üzüm üretilerek özel şato şaraplarına işlenmesi büyük bir katma değer yaratacaktır.

Urla'da farklı kırmızı şaraplık üzüm çeşitlerini temsil edebileceği düşünülen beş asmaya rastlanmıştır. Bu asmalar, tarihi çeşitleri temsil edebilecekleri gibi, hali hazırda bilinen üzüm çeşitlerinin örnekleri de olabilir. Çalışma kapsamında, Urla'da bulunan asmalar Ege Bölgesi siyah üzüm çeşitleri, belli başlı kırmızı şaraplık Türk ve Avrupa üzümleriyle karşılaştırılmıştır. Moleküler karşılaştırmada önce SSR (Simple Sequence Repeat) markörlerinden yararlanılmıştır. SSR analizlerinde Urla'da bulunan asmalara yakın olduğu görülen 14 üzüm çeşiti AFLP (Amplified Fragment Length Polymorphism) markörleri ile daha detaylı incelenmiştir. AFLP analizi sonucunda elde edilen UPGMA grafiği ve genetic benzerlik katsayıları Urla karası 4 ve Urla karası 5'in incelenen örneklerden farklı olduğunu göstermektedir. Bununla beraber, Urla'da bulunan asmaların ekonomik değer taşıyan yeni kırmızı şaraplık üzüm çeşitlerinin örnekleri olup olmadıklarının anlaşılması için bu çeşitlerin çoğaltılarak elde edilecek üzümlerin şaraba işlenerek kalitelerinin araştırılması gerekmektedir.

Anahtar kelimeler: *Vitis vinifera*, asma, SSR, AFLP

To my family with all my heart

&

In memory of my grandmother

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ABBREVIATIONS

AFLP	Amplified Fragment Lenght Polymorphism
GS	Genetic Similarity
GD	Genetic Distance
IBA	Indole Butyric Acid
PAGE	Polyacrylamide Gel Electrophoresis
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Lenght Polymorphism
SSR	Simple Sequence Repeat
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

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1 INTRODUCTION

Grape is one of the Old World (Europe, Asia, Africa and the surrounding islands) fruits domesticated in the land of Turkey according to archaeological findings. It is the second most cultivated temperate crop in the world after olive. The importance of this fruit does not only arise due to wine production, but also production of raisin (a dried grape), juice, table fruit, and jam jelly. Despite being one of the grape cultivation (viticulture) starting centers, Turkey has insufficiently evaluated this vulnerable fruit in the past times as a result of wars and ignorance.

Grape cultivation followed the trade routes and migration of ancient tribes over the 2000 years, so its distribution extends over a larger area. Currently, over 6000 varieties are documented, including wine, table and raisin types. However, problem arises while the cultivar names are taken into consideration. Poor documentation of new grape cultivar results in the presence of variants within cultivars (clones), the substitution of local or regional names for the original cultivars names, and transliteration. In addition to these, synonymies (the existence of multiple systematic names to label the same organism) of the numerous cultivars arose as it possesses wide distribution and long cultivation history.

Ampelography is the traditional methods of distinguishing the identity and relationships among *V. vinifera* (the most widely cultivated grape) cultivars based on the plant's vegetative and reproductive traits. However, this method does not suitable for closely related cultivars, because genotype-environment interactions affect the results.

Apart from the traditional ampelographic method, biochemical and molecular markers are also being used to characterize and classify grape germplasm collections. Among the molecular markers, the microsatellites are the markers of choice for population genetic analysis due to their multiallelic, abundant, highly polymorphic, and co-dominant nature.

Tandemly repeated simple sequence motifs that contain a high variation in repeat number between individuals are the characteristic of the microsatellites (Simple Sequence Repeats - SSRs). High level of polymorphism of SSRs makes them indispensable markers for organisms where little information could be extracted from other marker types. Applications of microsatellite markers comprises of parentage testing, individual or cultivar identification, pedigree reconstruction and studies of population structure.

Up to now *Vitis* SSR primers have been developed by three groups; Thomas and Scott [29], Bowers *et al*.[43], Sefc *et al*.[45], which are mainly exhibit role in identification and discrimination of cultivars in order to simplify the management of cultivar collections and to control the trade of plant material. Although the usefulness of these markers has been assessed in the vine-growing regions, due to presence of the predominant and null alleles for certain alleles in some populations, the data of a given marker differ among the cultivars from different regions.

Amplified fragment length polymorphism (AFLP) is another marker system for genetic studies, because this molecular marker system is not affected by environmental factors such as SSR. By the help of AFLP analysis, the whole genome can be scanned *via* large number of markers. In plants, production of more than 150 loci – specific bands *via* PCR technology provides genetic distance data between samples that can be very informative for genetic diversity, phylogeny, and the geographic origins of genotypes and gene pools of plants.

Data, gained by molecular markers such as SSR and AFLP, are combined with a computer program to obtain genetic diversity relationship among plant genotypes. Cluster analysis is a common exploratory classification method employed in most diversity analyses. It is particularly useful in discovering natural groupings among entries or items without assumption on the number of groups or group structure. The groupings are visualized as sub-clusters and clusters connected by branches and are called dendrograms, phenograms or simply trees. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) the most straightforward method for tree construction was used to visualize the cluster pattern.

Klazomenai (seaport of Urla) was the transition line between Izmir and Chios, and it was an ancient Greek city of Ionia. The ruins of city indicate that olive oil was firstly exported

across the sea. In addition to that, wine production of this region was proudly explained in the ancient historical books. Although exports of olive oil and the wine were observed by the way of sea in this region, increasing number of migrants from the (ex-Ottoman lands) Greek Island, Chios at the mid nineteenth century, wars, and ignorance resulted in the diminishing of vineyards.

The objective of this study was to regain the local grapevine varieties of Urla for red wine production, which have been vanishing for decades. In order to achieve this, molecular studies was conducted on the candidate varieties which were thought to have historical value. Comparative genetic analysis between candidates varieties and the Aegean zone black and red wine grape varieties, also most known red wine grape varieties of Turkey and of Europe resulted with the enough knowledge about whether they are the original historical local red wine varieties or not. AFLP and SSR markers were used in molecular analysis with such a high number of grapevine varieties for the first time in Turkey. One or more cultivars may be registered in the case of determination of uniqueness of them *via* AFLP and SSR markers. Consequently, modern molecular techniques were used for the first time in registration of new cultivars in Turkey.

2 OVERVIEW

2.1 Historical origins of grapevine

Formal agriculture was started ~ 10,000 years ago, as a result of deliberate breeding and working of human begins with the local environment. After changing lifestyle from migratory to sedentary, the first signs of horticulture were also started in the Old World. This change in the lifestyle, so starting of horticulture, observed several millennia after establishment of grain agriculture. As a consequence, the first evidences of the fruit – tree cultivation appear at Near East in Chalcolithic context (4^{th} millennium BC) [1].

Olive, grape vine, fig and date palm seem to have been the first principal fruit crops domesticated in the Old World, especially in the countries bordering the Mediterranean Sea. They emerged as important additives to the cereals and pulses in the Bronze Age after the establishment of horticulture. The Latin words hortus (garden plant) and cultura (culture) form the horticulture that requires a sedentary lifestyle. This can be considered as the main difference between agriculture and horticulture. Since, although cereals and pulses are annual, fruit trees are perennial. Thus, after several months from sowing, grains of those cereals and pulses can be harvested; whereas, orchards bear fruits 3 – 8 years after planting. Therefore, short harvest time of grains permits the move from place to place while it is not possible with horticulture [1].

Among these four fruit crops (olive, grapevine, fig, and date palm) of the Old world, grapes have contributed significantly to food production in the Mediterranean basin, providing fresh fruits rich in sugar, easily storable dried raisins, and juice for fermentation of wine. The latter became an important trade element in the countries around this area, and also around the world. By the end of the Tertiary era, the genus *Vitis* were to be found

widespeared throughout Japan, eastern Asia, north America, and Europe; mainly, within the latitudinal band between 30º and 50º north (Figure 1)[2]. During its spread throughout this latitudinal band, discrimination between cultivated (*sativa*) and wild type (*silvestris*) grapevine, based on just morphology, became a difficult task. Therefore, within their extensive natural distribution, identification of the place or places that people first began to cultivate vines for the production of wine is extremely difficult [2].

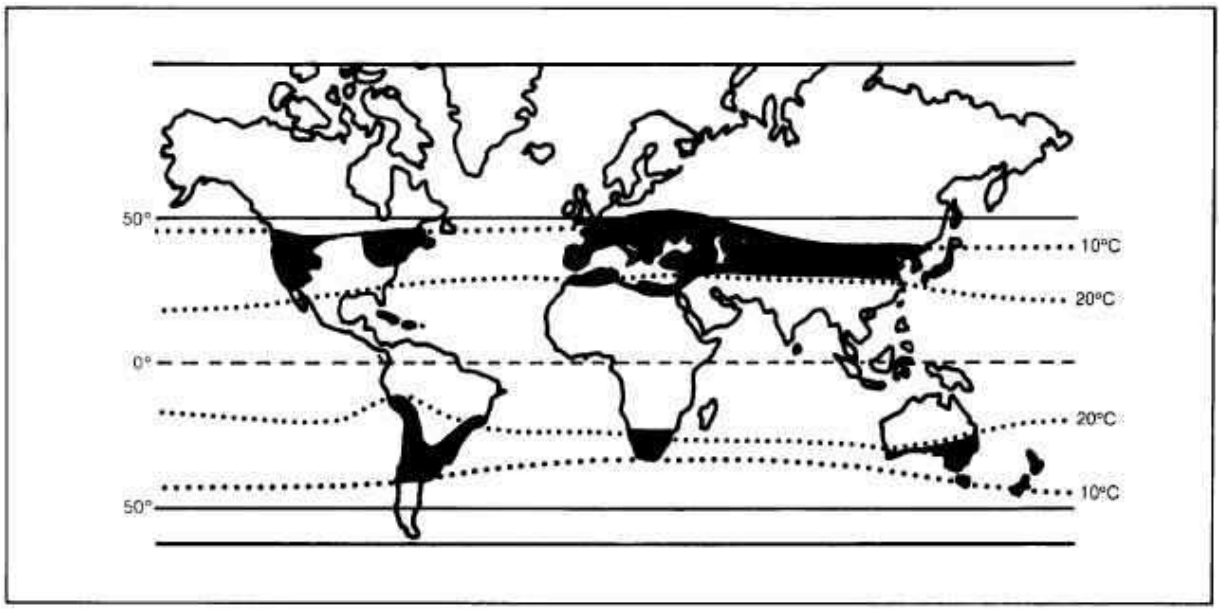


Figure 1. Best areas for viticulture lie between the 10°C and 20°C annual isotherms, equating approximately to the warm temperate zones between latitudes 30° and 50° north and south [2]

Archaeo – botanical, cultural and historical data are informative to comprehend the origin of the grapevine. However, these data can not be regarded as conclusive evidence [3]. It is thought that the *Vitis vinifera* ssp. *sativa* grapevine has been domesticated in the Near East region or in the Transcaucasian region (the southern portion of the Caucasus region between Europe and Asia, extending from the Greater Caucasus to Turkish and Iranian borders, between the Black and Caspian Seas) [4]. Although wild grapes grew widely in Europe and Asia, the original domestication of wine grapes has taken place between the Caucasus, eastern Turkey, and the Zagros region. There is some evidence supporting this mostly accepted view, such as the remains of cultivated grape seeds and evidence for wine making found in Iran as early as the fourth millennium BC [5, 6]

Although wild grape was one of the food sources of Palaeolithic hunter-gatherer populations in many prehistoric sites across Europe [7], domestication did not occur until the Neolithic period (c. 8500 – 4000 B.C.) [8]. After the first domestication of the grape, trades and conquerors (e.g., the Romans) caused gradual spread of the grape cultivation all over the Mediterranean region and Europe [9, 10]. Domesticated grapevine first appeared in the South-eastern Mediterranean regions, Palestine, Southern Lebanon and Jordan [11]. Afterwards, during the first half of the 3rd millennium B.C., domesticated grapevines appeared in Minor Asia (Anatolia), Southern Greece, Crete and Cyprus. At the beginning of the 2nd millennium B.C., the Southern Balkans were the next countries that showed up with the domestication of grapevines [4], whereas their first appearance in Southern Italy dates back to the second half of the 2nd millennium B.C. The second part of the 1st millennium was the date for domestication of grapevine in the Northern Italy, Southern France, Spain and Portugal [4].

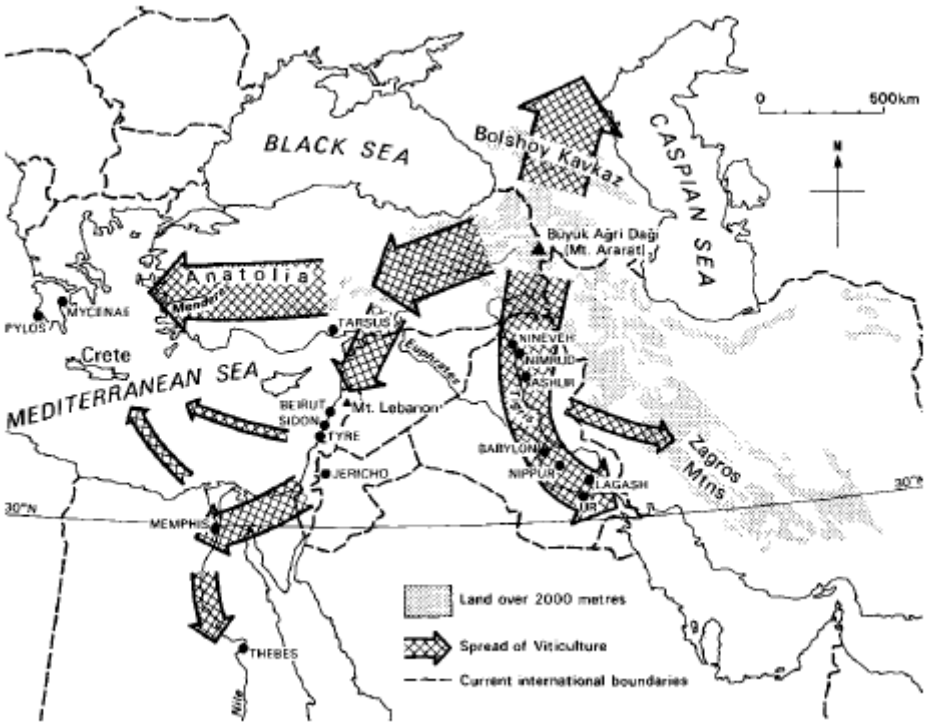


Figure 2. Movement of the grapevines at the ancient time [2]

During its journey from Caucasian region to the Mediterranean and Europe, Turkey especially Anatolia represents the unique location. Moreover, its surrounding areas might have served as a secondary center of diversification for current grape varieties. Natural hybridizations, mutations, and artificial selections result in rich grape germplasm in this region [6, 11, 12].

2.2 Domestication of the wild grapevine

One of the most widely cultivated and economically important fruit crops is the Eurasian grape (*Vitis vinifera* L.) [7]. It is thought that domestication of the wild populations of *Vitis vinifera* spp. *sylvestris* resulted in the cultivated grapevines (*Vitis vinifera* spp. *sativa*) [13]. These wild grapevines can be still found in small isolated populations along riverbank forests from the Atlantic coast of Europe to Tajikistan and the western Himalayas [7].

The obvious difference between wild type and domesticated grapevine is the mating system. The wild type grapevine (*V.vinifera* ssp. *sylvestris*) is characterized with having dioecious mating system resulting in anemophilous pollination (whereby pollen is distributed by wind); whereas domesticated grapevine (*V. Vinifera* ssp. *sativa*) possesses a self – pollination mating system (hermaphrodite; an organism that has both male and female sex organs during its life). This trait, hermaphrodism, was the crucial trait for the ancient farmers in order to guarantee the fruit production from the whole planted grapevine individual. Nowadays, whole cultivated grapevines are hermaphrodite. The domestication process consists of the selection of hermaphrodite genotypes producing both larger and sweeter berries of attractive colors and the development of techniques for their vegetative propagation [1].

The bizarre circumstance is the Lambrusco accessions, since they exhibit the characteristics that observed in both domesticated and wild grapevine. These accessions are considered as ancient hypothetical domesticated ancestors derived from wild grapevine [13, 15]. Their extreme position in the grapevine classification is under discussion (Table 1).

Table 1. Comparative morphology of wild and domesticated grapevine based on Olmo [16].

Type	Wild grapevine	Domesticated grapevine	Lambrusco accessions
Mating system	Dioecious	Hermaphrodite	Hermaphrodite
Habitat	Humis soils	Dry habitats	Dry habitats
Berry shape	Small, round or oblated	Large and elongated	Small and round, in several case irregular; dimension is very variable
Trunk	Often branches, slender, bark separated in very long thin strips	Thick bark separates in wider and more-coherent strips	Similar to domesticated grapevine
Seeds	Small, rounded body, high width/length ratio (>0.70)	Large, pyriform body, lower width/length ratio (<0.60)	Similar to domesticated grapevine
Fruit clusters	Small, globular to conical, irregular set, berry maturity variable in cluster	Large, elongated, compact to well-fitted, berry, uniform in maturity	Small, conical and irregular set
Leaves	Small, usually deeply three-lobed. Petioles short and slender, dull aspects	Large, many entire, or with shallow sinuses, petiole thick, glabrous to drowny	Small, usually deeply and three-lobed

Dioeciousness (functionally equivalent structures occurring on different individuals) and outbreeding are the two main characteristics of the wild type grapevine plants that enhance the heterozygosity. This high number of heterozygosity, in fact, has a vital role for the plants, since it prevents the presence of the deleterious recessive traits in the plant genome [16]. Therefore, during the domestication process, selection of the highly heterozygous plants is inevitable for agronomically important genotypes. However, selfing of these genotypes results in decreasing the heterozygosity that leads to substantial inbreeding depression within the offspring.

2.3 Genetic variation in the Anatolian grapevine

Anatolia, or Latin name of Asia Minor, has a diverse topography and climate that encourage a huge diversity of plant and animal communities. It is believed that together with Transcaucasia, they are likely homelands of viticulture and the earliest 'wine culture' [1, 6, 12, 13]. Today, wild grapevine is not only grape planted in this region, but also hundreds of grape cultivars are grown for wine and table grapes. Based on the recent archaeological and chemical evidence, the upland region of the Taurus Mountains in Eastern Anatolia, the Caucasus Mountains (including Transcaucasia) and the northern Zagros Mountains of Iran can be described as the starting region of wine culture [6]. Recent chemical analyses of Neolithic pottery from Georgia (Shulaveris-Gora) and Eastern Anatolia (Çayönü) confirm that the same beverage was being produced over a broad area of the mountainous Near East. Moreover, it was dating back to the early 6^{th} millennium BC.

As Anatolia is considered as the cradles of viticulture, it is not so surprising that, more than 1000 grape accessions exist in the National Germplasm Repository Vineyard at Tekirdağ Viticulture Research Institute in Thrace, Turkey [17, 18]. Most of them can be considered as indigenous to Anatolia, The white 'Sultani Çekirdeksiz' ('Sultanina' or'Thompson Seedless', especially for table grape production), 'Emir', 'Narince' and 'Misket' and the red 'Öküzgözü' and 'Boğazkere' are the most striking and important indigenous varieties. The genetic relationships among and between these gene pools of grape cultivars were investigated in this research by DNA profiling.

2.4 Cultivar Identification

Old practice of growing seedlings, which was noticed just by chance not by the breeding activity, resulted in a great genetic diversity in the grape cultivars. According to Alleweldt, more than 14.000 putative cultivars are the evidence of this breeding habit [19]. Moreover, migration and trades are the main reasons of the dispersion of the grape cultivars or populations from place to place. As a consequence of these, high number of synonyms (the existence of multiple systematic names to label the same organism), and ambiguities in cultivar identification are observed and they require a reliable method to be solved.

When the crop improvement is taken into consideration, characterization and identification of varieties, cultivars, sports, and clones become an important issue [20, 21]. Moreover, the importance of the identification and conformity analysis of the different vegetatively propagated lines is coming from the economical value of this fruit, in the viticulture industry. One of the more interesting applications of reliable identification tools is for the characterization and discrimination of clones, since it is an important aspect in the production of high quality wines. In particular, problems are observed around the identification of young plants during the process of multiplication and international exchanges. The protection of varietal names also causes a debate between wine growers and nurseries as well as the concerns of breeders [22].

The grapevine is a vegetatively propagated plant that possesses more than 6000 varieties according to ampelographic studies [14]. In grapevine, ampelography and ampelometry have been the main and traditional biotype identification methods used for these purposes. They are based on the possession of particular chemical profiles, e.g. phenolics and terpenics, and on protein electrophoretic profiles of the samples [23]. Therefore, they are not genetics markers, and hence, several false attributions have been made. In addition, morphological characters are instable, and also they can not be used in the juvenile stages or in the isolated parts as a result of clonal and environmental variability [22]. Thus, the requirements of the more rapid and reliable approach to these problems underlines the necessity of tools by which the genetic differences at the clonal level can be observed.

Polymerase chain reaction (PCR) – based on DNA marker technologies such as simple sequence repeats (SSRs) or microsatellites, and amplified fragment length polymorphism (AFLP) is now available for many crop species, including barley [24], citrus [25], carrot [26], and grapevine [27, 28]. A range of molecular markers has been suitable for cultivar identification of grapevine [29, 30]. In 1992, Thomas and Scott firstly revealed the applicability of microsatellite DNA in the grape cultivars for genomic studies [29]. Moreover, SSRs provide considerable resolving power for accurate variety labeling [31, 32, 33], pedigree reconstruction [33, 34] and genetic resources analysis [35]. SSRs have also been used to differentiate closely related cultivars and also it is suitable for fingerprinting [9, 36]. However, since the analysis of AFLPs offers the possibility to screen a larger number of anonymous loci than any other tool available at this time, it permits the identification of closely related individuals.

Apart from these markers, isozymes [37] and restriction fragment length polymorphisms (RFLPs) [30], as well as random amplified polymorphic DNAs (RAPDs) have been widely used for identifying grapevine varieties until the understanding of usefulness of SSR and AFLP markers become dominant.

2.5 Ampelography

Ampelography can be defined as the field of botany concerned with the identification and classification of grapevines, *Vitis* spp. Morphological characters of leaves, and shoot tips, fruit clusters, and berries are mainly analyzed and compared according to this identification method [38]. It was firstly emerged in order to discriminate the grapevines suitable for wine-making. Moreover, especially in the 19^{th} century, disease and pest resistant grapevines discriminated *via* ampelography were the choice of planting, because viticulture had a phylloxera (pest) problem that affects the planted grapevines severely in these days. However, this method has some drawbacks. First of all, number of experts on ampelography is very restricted. Secondly, environmental factors, individual plant biology, and life history have an influence on the expression of morphological characters. Moreover, morphological traits of plants can be analyzed after 4 or 5 years. It is not possible to look for these traits in juvenile plants [38]. Also, visual comparison of the morphological traits of some genetically related cultivars is almost impossible as they have very similar traits [39]. On the other hand, DNA based classification methods eliminate these drawbacks.

2.6 Simple Sequence Repeats (SSRs) or Microsatellites

SSR, also called microsatellite and minisatellite, is a DNA region containing a relatively short base pair motif that is repeated in tandem, and motif of SSR can be described as a particular sequence of DNA basepairs (e.g. CACACA…). Thus, SSR contains two distinct mononucleotide motifs (A/T and C/G), six distinct dinucleotide motifs, and ten distinct trinucleotide motifs. Delseny *et al.* (1983) [40] showed the presence of SSR motifs in plant nuclear genome. Then, it is shown gradually that SSRs are abundant across genomes and reveal high level of polymorphism. It is estimated that 10^4 to 10^5 microsatellite loci are widespread randomly throughout the genome of eukaryotes. This ratio provides a valuable polymorphic source in eukaryotes as genetic markers. Their distribution in genome is not random across coding and noncoding regions. To be more specific, mono-, di-, and tetranucleotide motifs were located in noncoding region across 54 plant species. However, triplet motifs are more common within coding region. Moreover, frequency of motifs is variable between species; for example, although $(AC)_n$ is the most common dinucleotide repeat in human beings, $(AT)_n$ is the most common dinucleotide repeat in *Arabidopsis thaliana* [41].

After development of the PCR, the usefulness and availability of these markers on targeting specific loci became more convenient than using molecular probes *via* classical hybridization methods. PCR amplified microsatellite markers comprise advantages by being locus specific and highly polymorphic. High-resolution electrophoresis enables the determination of allele size. The markers posses a co-dominant feature, so it is possible to discriminate homozygotes and heterozygotes. However, the identification and establishment of microsatellite markers in an organism have some disadvantages. It requires the construction and screening of genomic libraries, and also the design and optimization of the PCR primers. On the other hand, once the microsatellite primers are optimized, they can be used across closely – related species of the same genus. This is the case for the *Vitis* species [41].

2.6.1 Identification of cultivars of *Vitis via* SSR markers

In 1993, applicability of the repetitive DNA for identifying grapevine cultivars was firstly shown by Thomas *et al* [31]. This study also revealed that microsatellite sequences are abundant in grapevine and very informative for identifying *V. vinifera* cultivars. In the same year, Thomas and Scott [29] demonstrated by pedigree analysis that the microsatellite alleles had a co-dominant Mendelian inheritance. Moreover, their suitability for genetic analysis and investigation of genetic relatedness were confirmed [42]. Afterwards, SSR is used for wide range of applications ranging from cultivar identification to pedigree construction and genome mapping. Other groups around the world also developed additional markers, and all published markers are available in the Greek *Vitis* database.

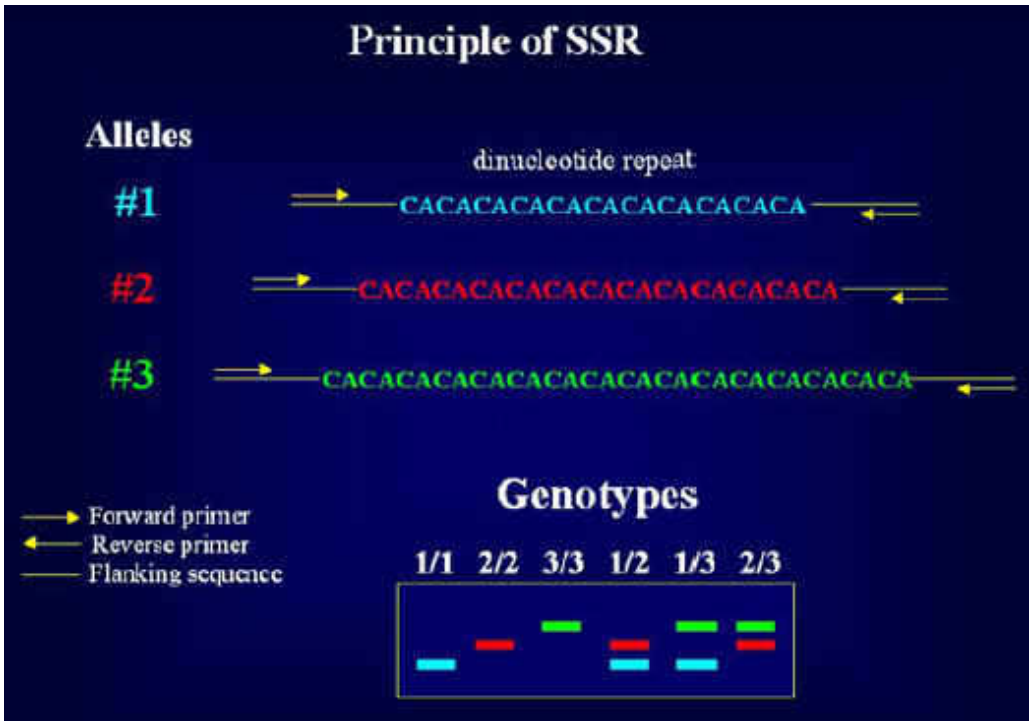


Figure 3. Representative diagram of a principle of SSR: Presence and absence of an allele affects the genotyping of a dinucleotide SSR locus. If the individual only possesses the allele 1 or allele 2 or allele 3, it results in an expected band pattern. Whereas, if the individual possess the combination of these two alleles, such as allele 1 and allele 2, expected band pattern of these two alleles is observed at the same time.

GENRES#081 was a European Union research project that aimed to develop reference microsatellite profiles for true-to-type identification of grapevine accessions. In this project, ten European laboratories worked with six microsatellite primer pairs to comprehend the reproducibility of different methods and to standardize the allele scoring by defining reference alleles [38]. VVS2 [31], VVMD5, VVMD7 [43], VVMD27 [44], VrZAG62, and VrZAG79 [45] were set as markers of choice as a consequence of this project. It is demonstrated that these six microsatellite markers are convenient for grapevine cultivar characterization as a result of their high degree of allelic polymorphism and high discrimination power. Therefore, six microsatellite markers are known as a standard set of microsatellite markers.

As a consequence of GENRES#081 and other researches parallel to this project, VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79 were used in this study.

2.7 Amplified Fragment Length Polymorphism (AFLP)

AFLP was firstly described as a new technique for DNA fingerprinting in 1995 by Keygene NV, Wageningen, and The Netherlands [46]. AFLP analysis is used frequently for characterization of cultivars, parentage analysis, identification of clones, establishing the genetic relationship and molecular mapping [47, 48]. It based on the selective restriction fragment amplification techniques, which results in a highly informative pattern of 40 to 200 bands. AFLP analysis has a polymorphic banding pattern due to

I. Mutations in the restriction sites,
II. Mutations in the sequences adjacent to the restriction sites and complementary to the selective primer extensions,
III. Insertions or deletions within the amplified fragments

Although SSR and AFLP are the two useful classes of molecular markers, they are different in their nature. SSR shows a co – dominant inheritance and high reproducibility, whereas AFLP is preferred for its multiplex nature and is found useful for the identification of clonal variation in grape [48].

AFLP analysis has a various applications in plant molecular genetics;

- **Phylogeny and diversity:** As many as 150 loci – specific bands production *via* multilocus PCR technology provides different AFLP patterns. It is informative about genetic diversity, phylogeny, and the geographic origins of genotypes and gene pools of plants.
- **Breeding:** Since AFLP markers are widespread throughout the whole chromosomes and inherited in a Mendelian way, this technology has four major applications in marker – assisted breeding.
- **Variety identification:** Production of F1 hybrids is valuable because they possess superior agronomic performance than their parental homozygous lines. Nevertheless, self – pollination in the female line and pollen from other male lines result in a contaminating variety. AFLP analysis allows the discrimination of these varieties.
- Germplasm management
- Indirect selection of agronomically important properties (traits)
- Backcross breeding [49]

For AFLP analysis, small amount of DNA is digested with two restriction enzymes; one rare cutting (like *Eco*RI) and one frequent cutting (like *Mse*I). Then, ligation of adapters prevents the rejoining of restricted fragments, and also allows both pre-selective and selective amplification of the restricted fragments. Pre-selective PCR primers are designed according to the *Eco*RI and *Mse*I adapters and it contains extra one nucleotide of A (selective nucleotide) in order to provide the first selection. Afterwards, in the selective PCR part, the fragments are 256-fold reduced *via* the increasing number of selective nucleotide to three. Finally, visualization of the fragments can be achieved by Silver Staining, autoradiography, or automated laser fluorescence sequencers.

2.8 Grape in Turkey

Horticultural Paradise, Turkey is one of the Mediterranean countries that has suitable ecological conditions for most of the fruit species. It is considered to be an important germplasm source for lots of fruit. Cultivation of the more than thirty different fruit tree species from Temperates to Citrus and the other subtropicals observed for centuries. Moreover, these high number of varieties of fruits and nuts are indigenous to this area, such as apple, pear, quince, cherry – sour cherry, plum, grape, hazelnut, pistachio, almond, walnut, olive and chestnut fig. Turkey devotes an approximately 1.8 million hectares to the planting of fruits/olives and 0.7 million ha to the planting of nuts. The total annual fruit production of Turkey is around 15 million tons (including grapes) [50].

The most important fruit crops in tonnage terms are; grapes, apples, oranges, peaches, peaches – nectarines, apricots, mandarins, lemons, figs, plums and cherries (in decreasing order of importance). From these fruits, grapevine was harvested 3,667,000 tons in 1998 in the world, and Turkey supplied 6.3 percentage of the total world production [50].

2.8.1 Viticulture

Viticulture has been an important horticultural practice for centuries in Turkey, which has a quite rich *Vitis* germplasm. Currently, it has about 400 local varieties, and 50 of these varieties are economically important. Total vineyard area of Turkey is 549,000 ha. The three main regions are Aegean, Mediterranean and Central Anatolia [50].

Only 2 – 3 percent of 3.67 million tons of fresh grape production is being processed for wine making. Among the native wine grapes, Emir, Narince, Kalecikkarası, Öküzgözü, Boğazkere, Çalkarası, Papazkarası and Adakarası; and foreign cultivars, Semillon, Riesling, Cabernet Sauvignon and Gamay are being used for high quality wine production [50].

Table 2. A list showing percentages of fresh grape consuming ways [50].

Grape Molasses Production (Including Vinegar)	36.9%
Table Grape	26.7%
Seedy Raisin	17.5%
Seedless Raisin	16.3%
Wine Production	2.6%
Total	100%

2.9 Urla (Clazomenae, Greek: Klazomenai)

Urla is a district of İzmir Province, the third largest city of Turkey that is located at the western coast of Anatolia. It is situated on the road to Çeşme from İzmir and holds typically Aegean characteristics. It used to be an important cultural centre in antiquity. It was originally the site of the Ionian city of Klazomenai, with probably the most ancient regularly used port in the world [51].

According to the oldest ruins documented in Urla went back to the 8000 BC, the Neolithic period, and the importance of this city was coming from its harbor, where both exporting and importing of goods were possible. The antique Clazomenai was famous with its olive oil and wine exported to various Mediterranean and Black sea cities.

Figure 4. Map of Urla region

As Urla peninsula is located on the transition line that connects İzmir and Chios, it is obvious to expect Urla region to become a part of commercial network. However, at the beginning of the sixteenth century, Çeşme harbor became the most important commercial link among Europe. Therefore, the commercial routes coming from Western Anatolia passed by Urla and ended up in Çeşme. This route resulted in an increase in population, agriculture, and commerce of these two cities. In 1566, non – Muslims in Urla was 1500, whereas Muslims was 3500. Nevertheless, after this date, Urla was preferred by the migrants form Chios Island, who were looking for better living conditions. Therefore, at the mid-nineteenth century, Greeks made up the majority of the population. In the late nineteenth and first quarter of the twentieth centuries, Urla was exporting raisins to Europe *via* its harbor. However, afterwards, grape production diminished with the migration of Greek population from Urla in early Republican years. As a consequence, Urla harbor lost its economic importance, and became a holiday village [52].

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

Grapevine accessions from both Turkey and abroad were used in the experiments. All plant materials were kindly provided by the National Germplasm Repository Vineyard at Tekirdağ Viticulture Research Institute in Thrace, Turkey. In addition, five grapevines that might represent different red wine grape varieties were collected from Urla. ~100 mg leaf sample of each grapevine accessions was weighted and stored at -80ºC until DNA isolation was done. Grapevine accessions are listed in Appendix A.

3.1.2 Chemicals

3.1.2.1 Enzymes and Buffers

- Proteinase K (20 mg/ml) – Applichem
- RNase A (100 mg/ml) – Qiagen
- GeneJET Fast PCR Master Mix (2X) – Fermentas
- Recombinant *Taq* DNA Polymerase (1 ul/µl) – Fermentas
- PCR Buffer (10X) – Fermentas
- $MgCl_2$ (25 mM)- Fermentas
- dNTP Mix – Fermentas

3.1.2.2 CHEMICALS AND SOLUTIONS

- CTAB Lysis Solution
- CTAB Precipitation Solution
- NaCl Solution

3.1.2.3 Commercial Kits

- DNeasy Plant Mini Kit – Qiagen
- IRDye® Fluorescent AFLP Kit for Large Plant Genome Analysis – Li- Cor

3.1.3 Equipment

Equipment used in this research are listed in Appendix B.

3.2 Methods

3.2.1 Simple Sequence Repeats

3.2.1.1 Total DNA isolation

Plant materials stored at -80ºC were first mechanically disrupted *via* TissueLyser (Retsch). Then, Qiagen DNeasy Plant Mini Kit protocol was followed exactly to isolate the total DNA from fine powdered leaves of different accessions of grapevine cultivars frozen in liquid nitrogen.

CTAB DNA isolation procedure developed by Doyle and Doyle (1987) was also used with the samples that possess a problem during the SSR – PCR analysis. Especially, this protocol was applied to grapevine accessions collected from Urla.

3.2.1.2 Spectrophotometric Measurement and Dilution of DNA Samples

Nanodrop Spectrophotometer and/or agarose gel electrophoresis were used to determine the concentration and purity of the DNA samples. The purity of samples were evaluated *via* Nanodrop calculation of 230/260 ratio for polysaccharide and salt contamination, and of 260/280 ratio for protein contamination. Pure DNA must be approximately 1.8-2.2 for the former ratio, and it must be around 1.8 for the latter one. All DNA samples were diluted to a final concentration of 50 ng/µl optimum DNA concentration for the SSR – PCR analysis.

3.2.1.3 SSR PCR

3.2.1.3.1 Primers

The cultivars were genotyped at the following microsatellite loci: VVS 2 [42], VVMD 5, VVMD 27 and VVMD 27 [43], ssrVrZAG 62, ssrVrZAG 79 (Table 3). The markers investigated here have recently been chosen as a core set for the screening of grapevine collections in Europe covered by the GENRES#081 research project.

Table 3. Standard set of microsatellite markers used in this study

Name of the primers	Sequence of primers	Base length	Tm	Allele Length Range (bp)
VVMD5-F	ctagagctacgccaatccaa	20	56	226-246
VVMD5-R	tataccaaaaatcatattcctaaa	24		
VVMD7-F	agagttgcggagaacaggat	20	52	233- 263
VVMD7-R	cgaaccttcacacgcttgat	20		
VVMD27- F	gtaccagatctgaatacatccgtaagt	27	56	173-194
VVMD27- R	acgggtatagagcaaacggtgt	22		
VVS2-F	cagcccgtaaatgtatccatc	21	53.4	129-155
VVS2-R	aaattcaaaattctaattcaactgg	25	52.1	
ssrVrZAG62-F	ggtgaaatgggcaccgaacacacgc	25	50	185-203
ssrVrZAG62-R	ccatgtctctcctcagcttctcagc	25		
ssrVrZAG79-F	agattgtggaggagggaacaaaccg	25	50	236-260
ssrVrZAG79-R	tgcccccattttcaaactcccttcc	25		

3.2.1.3.2 PCR Amplification

Extracted grapevine genomic DNAs were PCR-amplified using six pair of primers specific to flanking SSR sequences. PCR reactions were performed in a 25 µl volume and the content of the reaction mixture is summarized in the Table 4.

Cycling parameters were: one cycle of 95 ºC for 9 min; 30 cycles of 95 ºC for 50 sec, 50 ºC for 45 sec, and 72 ºC for 90 sec; final extension for 15 min at72 ºC.

Table 4. Stock and final concentration of each component of the SSR – PCR reaction mixture

PCR	stock	Unit	Each	MM	final conc	unit
MQ			14,9	640,7	n.v.t.	
PCR buffer 10x	10,0	X	2,5	107,5	1,00	x
MgCl2	25,0	mM	1,6	68,8	1,60	mM
dNTP	2,5	mM	2,0	86,0	0,20	mM
PrimerFW	10,0	uM	1,3	53,8	0,50	uM
PrimerRV	10,0	uM	1,3	53,8	0,50	uM
Taq	1,0	U/ul	0,5	21,5	0,02	U/ul
DNA	50,0	ng/ul	1,0	43,0	50	ng
Total			25,0	1075,0		
# Sample	43					

To the samples that did not give any polymorphic SSR-band pattern, GeneJET Fast PCR Master Mix (2X) (Fermentas) was applied according to Table 5.

Cycling parameters were: cycle of 95 ºC for 1 min; 35 cycles of 95 ºC for 1 sec, 50 ºC for 25 sec; followed by 10 sec at72 ºC.

Table 5. Stock and final concentration of each component of the GeneFast SSR – PCR reaction mixture

PCR	stock	Unit	each	MM	final conc	unit
MQ			6,0	258,0	n.v.t.	
PCR buffer 10x	20,0	X	10,0	430,0	10,00	x
PrimerFW	10,0	uM	1,5	64,5	0,75	uM
PrimerRV	10,0	uM	1,5	64,5	0,75	uM
DNA	50,0	ng/ul	1,0	43,0	50	ng
Total			20,0	860,0		
# Sample	43					

3.2.1.4 Agarose gel electrophoresis

PCR products of the reactions were firstly size-fractionated by agarose gel electrophoresis. Gels were prepared at 2% concentration using 0.5X TBE buffer. Prepared gels were run in 0.5X TBE buffer at 100 mV of constant voltage for 40 minutes. In order to determine the size, intensity of each band was compared with a marker (Fermentas).

3.2.1.5 Polyacrylamide gel electrophoresis (PAGE)

The positive PCR products, obtained form agarose gel electrophoresis, were separated on denaturing 7 % polyacrylamide / 8 M urea gels. Gels were prepared using 10X TBE buffer and Acrylamide:Bisacrylamide mix (29:1). Gels were pre-run in 0.5X TBE buffer at 100 V for ~ 1 hour. Before loading, samples were denatured for 5 min at 95 ºC and immediately immersed in ice. After cleaning the wells by pipetting in order to get rid of excess urea, PCR samples mixed with 6X loading dye (including sucrose) were loaded into the wells. Gels were run at ~160 V, approximately for 6 hours. Results were visualized using a solution consisting of Ethidium Bromide and 0,5X TBE buffer.

3.2.2 AFLP

After the SSR – PCR analysis, 14 grapevine accessions that exhibit higher similarity score than 0.5 were compared with the 5 grapevine accessions belong to Urla region and also with the wild type *via* AFLP analysis.

AFLP analysis was performed according to IRDye® Fluorescent AFLP Kit for Large Plant Genome Analysis kit (Li-Cor). As mentioned in the AFLP procedure, high quality and enough quantity of DNA are critical points. According to spectrophotometric results of the templates, although 1 µl of each sample was enough to obtain 100 ng of genomic DNA, this amount did not give any polymorphic band as a result of AFLP analysis. To optimize the quantity of DNA, 3 µl of each sample and 1:10 dilution factor after pre – selective PCR analysis were used during the AFLP procedure.

IRDye® Fluorescent AFLP Kit provides 8 *Mse*I and 8 *Eco*RI primers which result in the 64 primer combinations (8x8). Among these 64 primer combinations, M-CTC was the primer of choice for the *Mse*I forward primer. All 8 *Eco*RI primers were selected as reverse primer. As a consequence, 8 primer combinations were used to discriminate the 20 grapevine accessions.

3.2.3 Data Analysis

Polymorphic SSR and AFLP bands were scored manually as present (1) or absent (0) across all the genotypes. In using molecular marker data, amplified fragments are considered as alleles. Thus, the degree of dis/similarity between two genotypes, is a direct description of allelic variation [53]. Genetic similarity (GS) can be loosely defined as the proportion of molecular markers common between the two individuals being compared. Computation of GS is directly translated to comparing the number of rows of marker fragments of (apparently) similar size separated by electrophoresis. Genetic distance (GD) is the complement of GS (i.e. GD = 1 – GS). The choice of the method to translate the marker data to a data matrix for analysis is critical. In genetic diversity studies, the two most common GS coefficient are Jaccard's [54] and Nei and Li's [53]. For this study, Jaccard's coefficient was used as it is deemed most appropriate when using a dominant marker like AFLP.

The MVSP software package version 3.1 [55] was used to calculate Jaccard's [54] similarity coefficients among the genotypes as follows;

$$S_{ij} = N_{ij} / (N_{ii} + N_{ij} + N_{jj})$$

where S_{ij} is the similarity index between *i*th and *j*th genotype; N_{ij} is the number of bands present in both genotypes, N_{ii} is the number of bands present in *i*th genotype, but absent in *j*th genotype; and N_{jj} is the number of bands present in *j*th genotype, but absent in *i*th genotype. Unweighted pair – group method with arithmetic averaging (UPGMA) was used to construct a dendogram. Also, principal components analysis (PCA) was also carried out to show multiple dimensions of the distribution of the genotypes in a scatter plot.

3.3 Rooting of grapevine cuttings

Hardwood cuttings from each of the 5 Urla grapevine accessions brought to Sabanci University from Urla region on the 6th of March. The length of the cuttings was approximately 25-30 cm. Each cutting was gently scratched at the basal parts, and kept 5 sec in 1 g/1 L IBA (indole butyric acid). Then, they were planted on 1:1 perlit:torf mixture. Until the first shoot formation observed, the grape scions were kept in the growth room. Finally, they were taken to greenhouse to adapt to the natural enviroment before transferred to the National Germplasm Repository in Tekirdağ.

4 RESULTS

In this study, 5 Urla grapevine accessions that might be represent the local historical grape varieties were analyzed by SSR and AFLP markers to investigate the possible genetic relationship with the already known grapevine accessions. Thus, it was aimed to find possible distinct grapevine accession or accessions from the ones provided by the National Germplasm Repository Vineyard at Tekirdag Viticulture Research Institute.

4.1 SSR

A total of 98 grapevine accessions were analyzed with standard set of SSR markers. Of those, 5 are Urla accessions (*V. vinifera* ssp. *sativa*), 79 are Aegean accessions (*V. vinifera* ssp. *sativa*), 13 are Europe accessions (*V. vinifera* ssp. *sativa*), and one is a wild-type accession (*V. Vinifera* ssp. *silvestris*). Sample number 76 was out of the study. Gel pictures of the investigated six microsatellite loci are listed in the Appendix C.

4.1.1 SSR marker VVMD7

Polymorphic SSR – bands were genotyped manually as present (+) or absent (-). Figure 5a is a representative gel picture of the SSR marker VVMD7. Polymorphic bands of VVMD7 were scored according to the presence of 3 bands demonstrated with red rectangle in Figure 5b. It is clear that sample 41 consists of only one band present in the middle; whereas sample 42 possesses two bands, one present in the middle and the other in the bottom. Sample 44 is different from sample 41 and 42 that it has the top and the middle bands. If these three samples' band pattern is used to form the Microsoft Excel™ sheet to analyze these samples' similarities and differences *via* UPGMA, it would be as shown in the Table 6. All grape accessions were analyzed according to this scheme, and Table 7 exhibits the banding pattern of all samples analyzed with SSR marker VVMD7.

Figure 5. a.*top* 7% polyacrylamide / 8 M urea gel was used to separate the PCR product of the SSR – marker VVMD7. Red rectangular highlights the genotyped bands. Marker (M) used is GeneRuler DNA Ladder Mix (Ferments) b.bottom Genotyped bands.

Table 6. Example of a Microsoft Excel™ sheet formed to construct dendogram the dendogram *via* the MVSP software package version 3.1

	bottom	middle	top
Sample 41	-	+	-
Sample 42	+	+	-
Sample 44	-	+	+

Table 7. Genotyped data of all the samples analyzed during the study with SSR – marker VVMD7.

			VVMD7		
No	**ID**	**Name of the cultivars**	**top**	**middle**	**bottom**
1	646-48	Kızıl Üzüm	-	+	+
2		Boğazkere	+	+	-
3	492-45	Duman	+	-	+
4	454-45	İri Kara	-	+	-
5	734-03	Algöynek	-	-	-
6	791-64	Aydın Üzümü	-	-	-
7	639-48	Kadın Parmağı	+	-	+
8		Öküzgözü	+	-	-
9	593-48	Alyanak	+	-	-
10	588-20	Çalkarası	-	+	+
11	491-45	Siyah Gemre	-	-	-
12	549-20	Muhammediye (Mor Üzüm)	+	-	+
13	786-64	Terzi Nasuf	-	+	-
14	824-35	Efe Püskülü	+	+	-
15	849-35	Ekşi Üzüm	+	+	+
16	850-35	Kara Üzüm	-	-	-
17	592-48	Pembe Çekirdeksiz	-	+	+
18	552-20	Fesliken	-	+	-
19	446-45	Antep Şamı	-	+	+
20	693-03	Siyah Parmak	-	+	-
21	784-64	Mor Üzüm	+	-	-
22	502-20	Siyah Dimrit	-	-	-
23	536-20	İri Kızıl	+	+	-
24	500-20	Yediveren	-	+	+
25	626-06	Kalecik Karası	-	+	+
26	547-20	Yanal Üzüm	+	+	-
27	651-48	Yerli Kara	-	+	+
28	848-35	Zeytin Üzümü	-	+	+
29	539-20	Ekşi Kara	-	-	-
30	821-64	Hacı Eyüp	-	+	+
31	484-45	Cami	-	+	+
32	440-45	Kırmızı Şam	+	+	-
33	779-03	Isparta	-	-	+
34	638-48	İstanbul Dimliti	-	+	+
35	538-20	Eşek Memesi	+	+	-
36	825-35	Yediveren	-	+	+
37		Papazkarası	-	-	+
38	589-20	Kınalı	+	+	-
39	438-45	Şaraplık Üzüm	-	-	-
40	696-03	Manda Gözü	-	+	-
41	488-45	Siyah Misket	-	+	-
42	648-48	Katı Kara	-	+	+
43	782-64	Mor Razdağı	-	+	+
44		Adakarası	+	+	-
45	641-48	Pembe Çavuş	-	-	-
46	489-45	Siyah Misket	-	+	-
47	543-20 (534-20)	Aşıkara	-	+	-
48	594-48	Siyah Dimrit	-	+	-

			VVMD7		
No	**ID**	**Name of the cultivars**	**top**	**middle**	**bottom**
49	852-35	Sık Kara	-	+	+
50	647-48	Kara İbrahim	-	+	+
51	597-48	Sofra Karası	-	-	-
52	495-45	Hasan Üzümü	-	-	-
53	452-45	Nar Üzümü	-	-	-
54	496-45	Pembe Şam	+	+	-
55	546-20	Tavşan Böbreği	-	+	+
56	780-03	Söbü Dimrit	-	-	-
57	785-64	Şahin Tırnağı	-	-	-
58	599-48	Kayasar (Kayzer) Dimliti	-	-	-
59	643-48	Kara Büzgülü	-	+	-
60	857-09	Foça Karası	-	-	-
61	823-35	Hacı Azman	-	-	-
62	134-11	Kokulu Kara	-	+	-
63	142-17	Foça Karası	-	-	-
64	498-20	Eski Kara	-	-	-
65	499-20	Hırsız Çalmaz	-	-	-
66	605-48	Aydın Karası	-	-	-
67	822-35	Gelin Dudağı	-	+	+
68	792-64	Siyah Razakı (Razdağı)	-	+	-
69	556-20	Kınalı Tırnak	+	+	-
70	487-45	Hacı Hıdır	-	+	+
71	485-45 (486-45)	Pembe Üzüm	+	+	-
72	688-03	Hevenk (Gelin Parmağı)	-	+	-
73	554-20	Pembe Gemre	-	-	-
74	650-48	Tavşan Böbreği	-	+	+
75	542-20	Sultaniye Tatlı	-	-	-
76	434-45	Siyah Gemre	-	-	-
77	555-20	Devegözü	+	-	+
78	702-03	Veyisoğlu	-	-	-
79	553-20	Büzgülü	-	+	-
80	236-17	Karalahna	+	+	-
81	138-17	Karasakız	+	+	-
82	179-31	Sevgi Karası	+	-	+
83	Özel Koleksiyon Bağı	Gamay	+	-	+
84	Özel Koleksiyon Bağı	Carignane	-	-	-
85	Özel Koleksiyon Bağı	Hamburg Misketi	+	-	-
86	Özel Koleksiyon Bağı	Pinot Noir	-	+	+
87	Özel Koleksiyon Bağı	Alicante Bouschet	-	+	+
88	Özel Koleksiyon Bağı	Syrah	-	-	-
89	Özel Koleksiyon Bağı	Merlot	+	+	-
90	Özel Koleksiyon Bağı	Sangiovese	-	+	-
91	Özel Koleksiyon Bağı	Cabernet Sauvignon	+	-	+
92	Özel Koleksiyon Bağı	Cinsaut	-	+	-
93	UK1	Urla karası 1	+	+	-
94	UK2	Urla karası 2	+	+	-
95	SE	Urla karası 3	+	-	+
96	MK	Urla karası 4	+	+	-
97	MB	Urla karası 5	+	+	-
98	S	Urla karası 6	+	-	-

4.1.2 SSR marker Zag79 and SSR marker Zag62

Gel pictures shown in Figure 6a and Figure 6b were analyzed as the gel picture of the SSR marker VVMD7. Sample 41 and 44 have two bands present at the bottom and at the top; whereas it is clear that sample 42 and 43 possess the top and middle bands (Figure 6a). The presence of middle band in the sample 45 discriminates this sample from the others. Table 8 demonstrates the banding pattern of all the samples with SSR marker Zag79 and SSR marker Zag62.

Example of the gel picture of SSR marker Zag62 was shown in the figure 6b that was analyzed in a same manner with the SSR marker VVMD7 and Zag79.

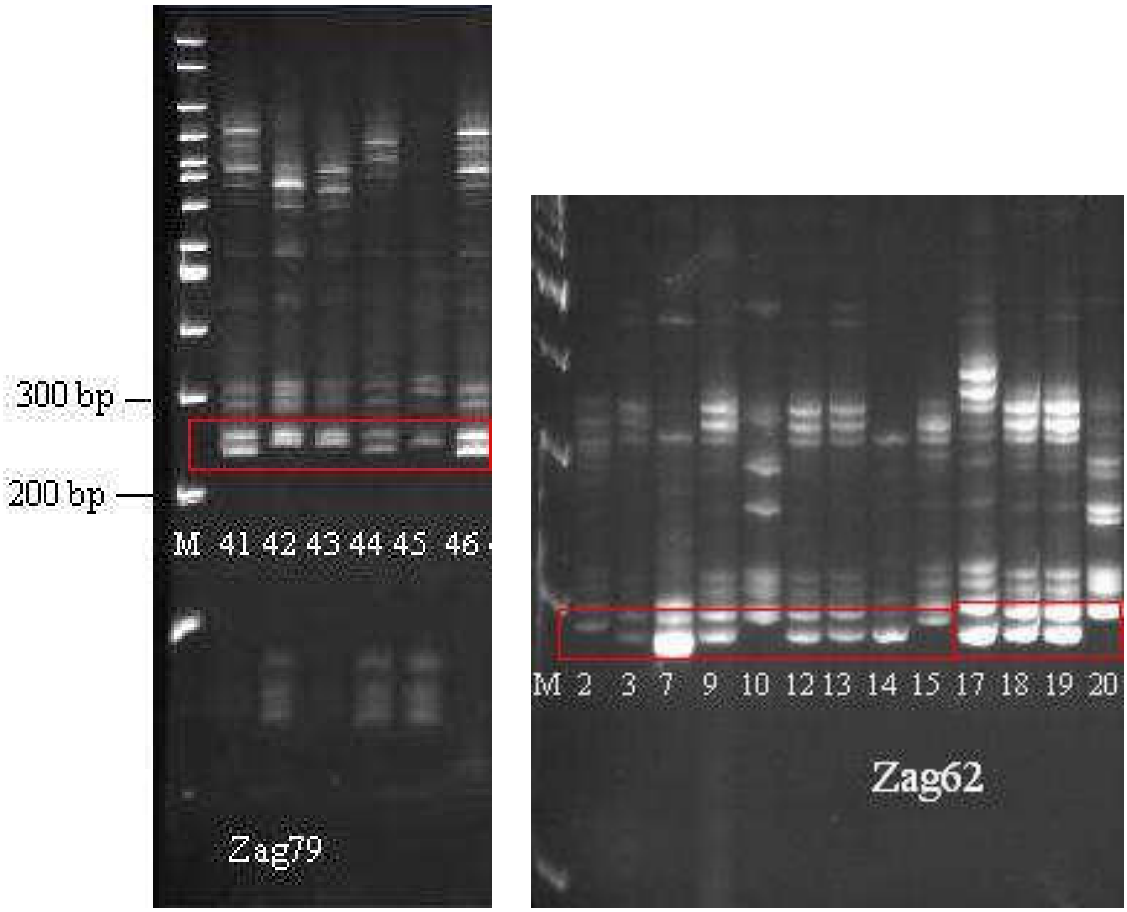


Figure 6. Red rectangular highlights the genotyped bands. GeneRuler DNA Ladder Mix is used as the marker (M) (Ferments) **a.** *left* 7% polyacrylamide / 8 M urea gel was used to separate the PCR products of the SSR – marker Zag79. **b.** *right* 7% polyacrylamide / 8 M urea gel was used to separate the PCR products of the SSR – marker Zag62.

Table 8. Genotyped data of all the samples analyzed during the study with SSR – markers Zag79 and Zag62.

	Zag79			Zag62		
No	**top**	**middle**	**bottom**	**top**	**middle**	**bottom**
1	-	-	+	-	+	+
2	-	+	-	-	+	-
3	+	-	+	+	-	+
4	-	-	-	+	+	-
5	-	-	-	+	+	-
6	-	+	-	-	-	-
7	+	+	-	+	-	+
8	-	+	+	-	-	-
9	+	-	-	+	-	+
10	-	+	-	+	-	-
11	-	-	+	+	-	-
12	+	-	+	+	-	+
13	-	+	-	+	-	+
14	+	-	-	-	-	+
15	+	+	-	+	+	-
16	-	-	-	-	-	-
17	+	-	+	+	-	+
18	+	+	-	+	-	+
19	+	+	-	+	-	+
20	+	+	-	+	-	-
21	+	+	-	+	-	+
22	+	+	-	-	-	-
23	+	+	-	+	-	+
24	+	+	-	+	-	+
25	-	+	-	-	+	+
26	+	+	-	-	+	+
27	-	+	-	+	-	+
28	+	+	-	+	-	+
29	-	-	-	+	-	+
30	-	-	-	-	-	-
31	-	+	-	+	-	+
32	-	+	-	-	+	-
33	-	-	-	-	-	-
34	-	+	+	+	-	+
35	+	-	+	+	-	+
36	+	+	-	-	-	+
37	-	+	-	-	-	-
38	+	-	+	-	+	-
39	-	-	-	-	-	-
40	+	-	-	+	-	+
41	+	-	+	-	+	+
42	+	-	-	-	-	+
43	+	+	-	+	-	+
44	+	-	+	-	+	-
45	-	+	-	-	-	-
46	+	-	+	-	+	-
47	-	+	-	+	+	-
48	+	-	-	+	+	-

	Zag79			Zag62		
No	top	middle	bottom	top	middle	bottom
49	+	+	-	+	+	-
50	-	-	-	-	+	-
51	-	-	-	-	-	-
52	-	-	-	-	+	-
53	-	-	-	-	-	-
54	-	-	-	-	-	-
55	-	-	-	-	-	-
56	-	-	-	-	-	-
57	-	-	-	-	-	-
58	-	-	-	-	-	-
59	-	-	-	-	-	-
60	-	-	-	+	+	-
61	+	-	-	-	-	-
62	+	-	+	-	-	-
63	-	-	-	-	-	+
64	-	-	-	-	-	-
65	-	-	-	-	-	-
66	-	-	-	-	-	-
67	-	-	-	-	-	-
68	-	+	-	-	+	-
69	+	-	+	+	-	+
70	-	+	-	+	-	+
71	+	+	-	-	-	-
72	-	+	-	+	-	-
73	-	-	-	+	+	-
74	-	+	-	-	-	-
75	-	-	-	-	-	+
76	-	-	-	-	-	-
77	+	-	-	-	-	-
78	-	-	-	-	-	+
79	+	-	+	-	-	-
80	-	+	-	+	-	-
81	+	-	-	-	+	-
82	+	-	-	-	+	-
83	+	+	-	+	-	-
84	-	-	-	-	-	-
85	+	-	+	+	+	-
86	-	+	+	+	-	-
87	+	-	+	-	+	-
88	-	-	-	+	+	-
89	+	-	-	-	+	-
90	+	+	-	-	+	-
91	-	+	-	-	+	+
92	+	-	+	-	+	+
93	-	+	-	+	+	-
94	-	+	-	+	+	-
95	+	+	-	-	-	+
96	-	-	-	+	+	-
97	-	+	-	+	+	-
98	+	-	-	+	-	+

4.1.3 SSR marker VVMD27, VVS2 and VVMD5

All SSR – markers did not show polymorphic band pattern with all analyzed grape accessions. SSR marker VVMD5 was the marker that only gave a PCR product with 26 grape accessions. After agarose gel electrophoresis, these samples were loaded to PAGE (Figure 7). During the analyses of the SSR marker VVMD5, presence of 3 bands was investigated as done with the other three SSR markers. However, for the SSR marker VVMD27 and VVS2, presence of two polymorphic bands was investigated for, as shown in the Figure 7 with a red rectangular. Banding patterns of these three SSR markers were translated to Microsoft Excel^{TM} data as present (+) and absent (-) (Table 9).

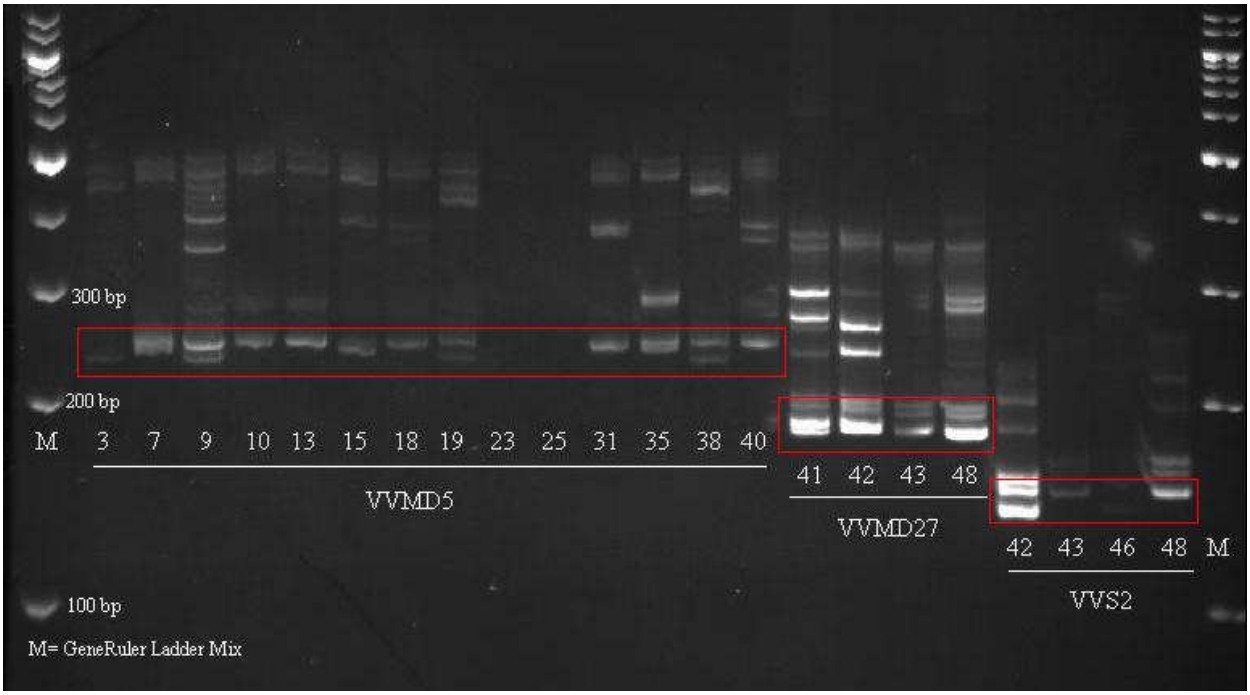


Figure 7. 7% polyacrylamide / 8 M urea gel was used to separate the PCR products of the SSR – markers VVMD27, VVS2, and VVMD5. Red rectangles highlight the genotyped bands.

Table 9. Genotyped data of all the samples analyzed during the study with SSR – markers VVMD27, VVS2, and VVMD5.

	VVMD27		VVS2		VVMD5		
No	**top**	**bottom**	**top**	**bottom**	**No**	**middle**	**bottom**
1	-	+	+	+	1	-	-
2	+	+	+	-	2	-	-
3	-	+	+	-	3	-	+
4	-	-	+	+	4	-	-
5	-	-	-	-	5	-	-
6	-	-	-	+	6	-	-
7	+	+	+	+	7	+	-
8	-	-	-	-	8	-	-
9	-	-	+	+	9	-	+
10	-	+	+	-	10	-	-
11	-	-	-	+	11	-	-
12	+	+	+	+	12	-	-
13	+	-	-	-	13	-	-
14	-	-	+	-	14	-	-
15	+	-	+	+	15	+	-
16	+	-	-	-	16	-	-
17	+	+	-	+	17	-	-
18	+	+	+	+	18	-	-
19	+	-	+	+	19	-	+
20	+	+	-	-	20	-	-
21	+	-	-	+	21	-	-
22	-	-	-	-	22	-	-
23	-	-	+	-	23	-	-
24	-	-	-	-	24	-	-
25	-	+	+	-	25	-	-
26	-	-	-	+	26	-	-
27	+	-	-	-	27	-	-
28	+	-	+	-	28	-	-
29	-	-	+	-	29	-	-
30	-	-	+	+	30	-	-
31	+	-	+	-	31	+	-
32	-	-	+	-	32	-	-
33	-	-	-	-	33	-	-
34	+	+	-	-	34	-	-
35	+	+	+	-	35	+	-
36	-	-	-	-	36	-	-
37	+	+	-	-	37	-	-
38	-	-	-	-	38	-	+
39	-	-	-	-	39	-	-
40	-	+	+	+	40	-	-
41	+	+	+	+	41	+	-
42	+	+	+	+	42	-	-
43	-	+	+	-	43	-	-
44	-	-	+	+	44	-	-
45	-	-	-	-	45	-	-
46	+	+	+	+	46	-	-
47	-	-	-	-	47	-	-
48	-	+	+	-	48	-	+

	VVMD27		VVS2		VVMD5		
No	top	bottom	top	bottom	No	middle	bottom
49	-	+	+	+	49	-	-
50	+	-	+	+	50	-	-
51	-	-	-	-	51	-	-
52	-	-	-	-	52	-	-
53	+	-	+	+	53	-	-
54	-	+	+	-	54	-	-
55	-	-	-	-	55	-	-
56	+	-	+	+	56	-	-
57	-	-	-	-	57	-	-
58	-	-	-	-	58	-	-
59	-	-	-	-	59	-	-
60	-	-	-	-	60	-	-
61	-	+	+	-	61	-	-
62	+	+	+	+	62	+	-
63	-	-	-	-	63	-	-
64	-	-	-	-	64	-	-
65	-	-	-	-	65	+	-
66	-	-	-	-	66	-	+
67	+	-	+	+	67	-	-
68	-	+	-	-	68	-	-
69	+	-	+	-	69	-	-
70	+	-	+	+	70	-	-
71	+	-	+	+	71	-	-
72	-	-	-	-	72	-	-
73	-	-	+	-	73	-	-
74	+	-	+	+	74	-	-
75	-	-	-	-	75	-	-
76	-	-	-	-	76	-	-
77	-	-	-	-	77	-	-
78	-	-	-	-	78	-	-
79	+	+	+	+	79	-	-
80	+	-	+	-	80	+	-
81	+	-	+	-	81	+	-
82	-	+	+	-	82	+	+
83	+	+	+	+	83	-	-
84	-	-	-	-	84	-	-
85	+	+	-	+	85	-	-
86	+	-	-	-	86	-	-
87	-	+	-	+	87	-	+
88	-	-	-	-	88	-	-
89	+	-	+	-	89	+	-
90	+	-	-	+	90	+	-
91	-	+	+	-	91	+	-
92	+	+	+	+	92	+	-
93	+	+	-	+	93	-	+
94	+	+	-	+	94	-	+
95	+	-	+	+	95	-	+
96	+	+	-	-	96	-	+
97	-	-	-	-	97	-	-
98	+	+	+	+	98	-	+

4.1.4 Data Analysis

After translation of polymorphic banding patterns of the SSR markers into Microsoft Excel^{TM} data, present (+) and absent (-) signs were changed as 1 for the former, and 0 for the latter. The MVSP software package version 3.1 was used to calculate Jaccard's similarity coefficients among the genotypes to construct the dendogram of Unweighted pair – group method with arithmetic averaging (UPGMA) (Figure 8). Table 10 summarizes the grape accessions that exhibit close relationship to the Urla grape accessions using Jaccard's similarity coefficient. These 14 grape accessions were further compared with the Urla grape accessions by AFLP – analysis.

Table 10. Similarity matrix data was obtained *via* Jaccard's similarity coefficients between Urla grape accessions and the grape samples that have a higher similarity value than 0.5.

UK1&2	0,545	0,467	0,385	0,333	0,571	0,538	0,417	0,364	0,4	0,5	0,538	0,417	0,3	0,538
UK3	0,308	0,692	0,636	0,538	0,571	0,538	0,545	0,25	0,077	0,286	0,429	0,545	0,083	0,667
UK4	0,5	0,333	0,333	0,286	0,429	0,385	0,25	0,3	0,333	0,6	0,385	0,25	0,222	0,385
UK5	0,5	0,214	0,182	0,154	0,417	0,25	0,333	0,667	0,8	0,3	0,364	0,333	0,6	0,25
UK6	0,308	0,692	0,8	0,667	0,467	0,667	0,545	0,154	0,077	0,5	0,429	0,417	0,083	0,667
	2	**7**	**9**	**12**	**15**	**18**	**21**	**32**	**47**	**48**	**49**	**71**	**72**	**83**

PCA analysis of the samples was calculated by the help of the MVSP software in order to reduce multidimensional data sets to lower dimensions (Figure 9). As a result of the PCA analysis, samples were divided into four distinct clusters (Table 11).

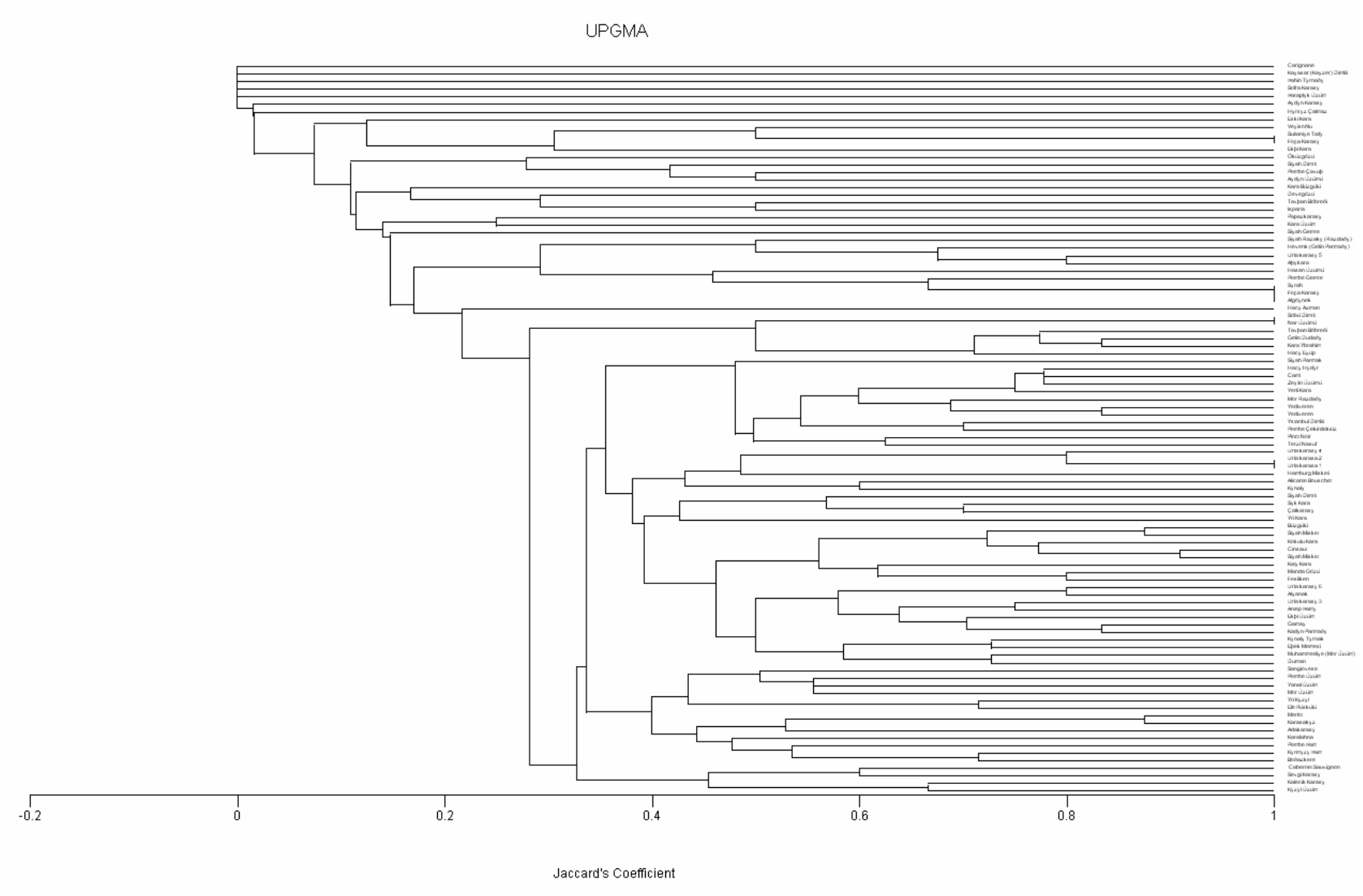


Figure 8. Dendrogram obtained by cluster analysis of Jaccard's similarity coefficients among grape cultivars using SSR markers by polyacrylamide electrophoresis (PAGE). The scale bar represents simple matching distance.

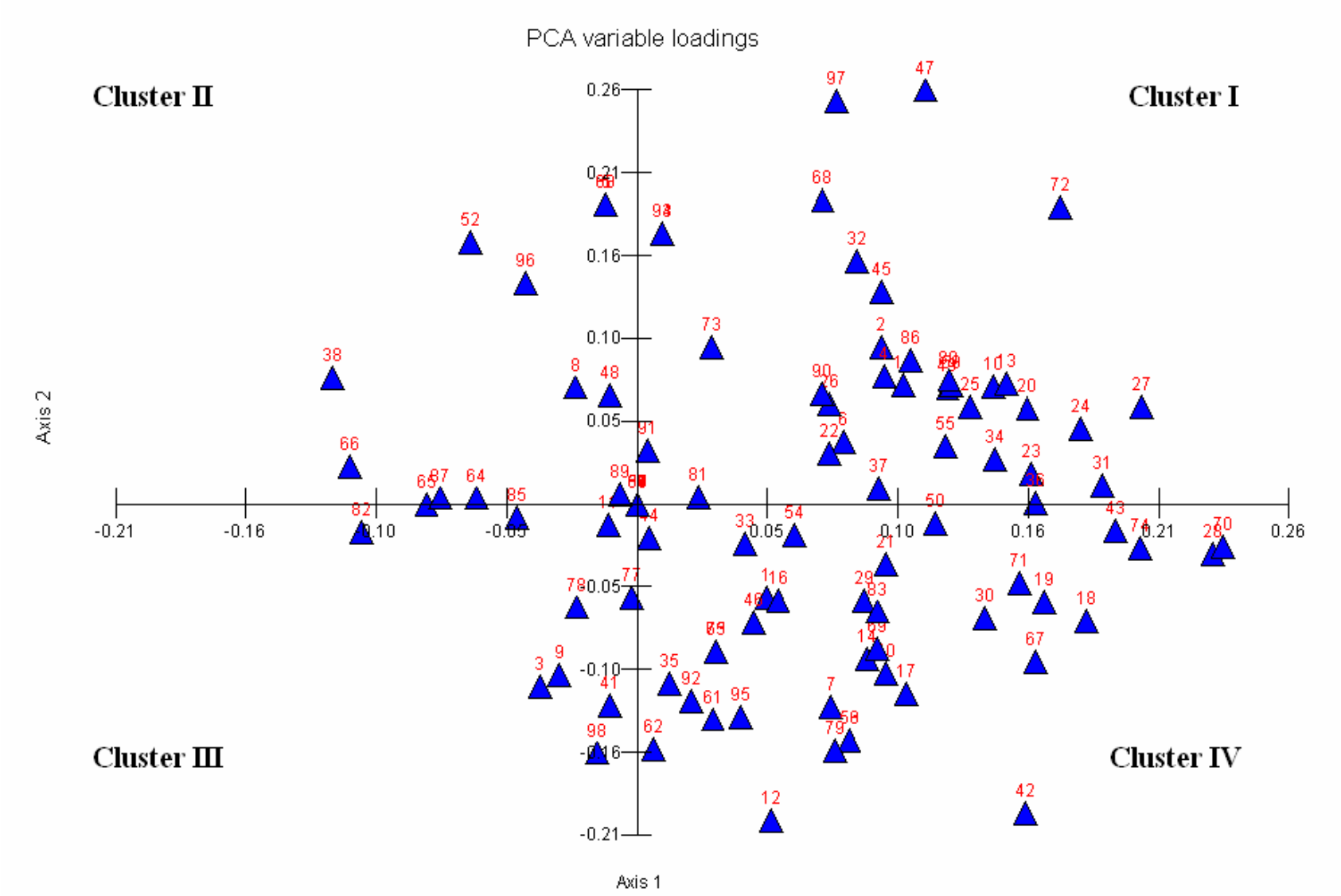


Figure 9. Principal components analysis of all the grape accessions.

Table 11. PCA analysis yielded a scatter plot that produces four distinct clusters.

Cluster I	Cluster II	Cluster III	Cluster IV
Boğazkere	Algöynek	Duman	Kızıl Üzüm
İrikara	Öküzgözü	Alyanak	Kadın Parmağı
Aydın Üzümü	Kınalı	Siyah Gemre	Muhammediye (Mor Üzüm)
Çalkarası	Siyah Dimrit	Siyah Misket	Efe Püskülü
Terzi Nasuf	Hasan Üzümü	Hırsız Çalmaz	Kara Üzüm
Ekşi Üzüm	Foça Karası	Devegözü	Pembe Çekirdeksiz
Siyah Parmak	Eski Kara	Veyisoğlu	Fesliken
Siyah Dimrit	Aydın Karası	Sevgi Karası	Antep Şamı
İri Kızıl	Alicante Bouschet	Hamburg Misketi	Mor Üzüm
Yediveren	Syrah	Urla Karası 6	Zeytin Üzümü
Kalecik Karası	Merlot		Ekşi Kara
Yanal Üzüm	Urla Karası 4		Hacı Eyüp
Yerli Kara			Isparta
Cami			Eşek Memesi
Kırmızı Şam			Manda Gözü
İstanbul Dimriti			Katı Kara
Yediveren			Mor Razdağı
Papazkarası			Adakarası
Şaraplık Üzüm			Siyah Misket
Pembe Çavuş			Kara İbrahim
Aşıkara			Mor Üzümü
Sık Kara			Pembe Şam
Sofra Karası			Söbü Dimrit
Tavşan Böbreği			Hacı Azman
Şahin Tırnağı			Kokulu Kara
Kayasar Dimliti			Foça Karası
Kara Büzgülü			Gelin Dudağı
Siyah Razakı			Kınalı Tırnak
Hevenk			Hacı Hıdır
Pembe Gemre			Pembe Üzüm
Karalahana			Tavşan Böbreği
Karasakız			Sultaniye Tatlı
Carignane			Büzgülü
Pinot Noir			Gamay
Sangiovese			Cinsaut
Cabernet Sauvignon			Urla Karası 3
Urla Karası 1			
Urla Karası 2			
Urla Karası 5			

4.2 AFLP

IRDye® Fluorescent AFLP Kit for Large Plant Genome Analysis (Li-Cor) was used to analyze the samples that have a close relationship with the 5 Urla grape accessions as a result of SSR – analysis. Total 20 grape accessions were analyzed. Figures 10 and 11 demonstrate the AFLP gel picture with the *Eco*RI primers that labeled with IRDye 800 nm (E-ACG, E-ACT, E-AGC, and E-AGG). Figures 12 and 13 show the AFLP gel picture with the *Eco*RI primers that labeled with IRDye 700 nm (E-AAC, E-AAG, E-ACA, and E-ACC). Samples were loaded in the following order; marker (50-350 bp sizing standard), SE, MB, MK, UK1, UK2, S, 2, 7, 9, 12, 15, 18, 21, 32, 47, 48, 49, 71, 72, and 83. Polymorphic AFLP bands were genotyped manually, and total 131 polymorphic bands were analyzed by the MVSP software package version 3.1. Jaccard's similarity coefficient was selected to obtain the UPGMA graph (Figure 14). In addition, PCA scatter graph was combined with the data obtained from UPGMA into two – dimensions (Figure 15).

Table 12. Similarity coefficients of the grape accessions analyzed by AFLP analysis

UPGMA ~ Jaccard's Coefficient						
Similarity matrix						
	se	**mb**	**mk**	**uk1**	**uk2**	**S**
se	1					
mb	0,69	1				
mk	0,468	0,423	1			
uk1	0,398	0,474	0,42	1		
uk2	0,611	0,546	0,433	0,489	1	
s	0,748	0,584	0,411	0,446	0,667	1
2	0,708	0,712	0,409	0,454	0,588	0,693
7	0,758	0,637	0,458	0,429	0,63	0,702
9	0,793	0,669	0,475	0,457	0,595	0,68
12	0,607	0,571	0,427	0,451	0,545	0,556
15	0,805	0,678	0,491	0,46	0,658	0,703
18	0,653	0,581	0,481	0,434	0,615	0,593
21	0,521	0,482	0,406	0,461	0,51	0,509
32	0,539	0,514	0,396	0,358	0,457	0,5
47	0,611	0,518	0,448	0,426	0,505	0,545
48	0,664	0,589	0,456	0,465	0,594	0,661
49	0,729	0,629	0,4	0,405	0,565	0,684
71	0,535	0,509	0,389	0,427	0,51	0,523
72	0,686	0,643	0,418	0,438	0,591	0,685
83	0,578	0,57	0,443	0,421	0,544	0,613
	se	**mb**	**mk**	**uk1**	**uk2**	**s**

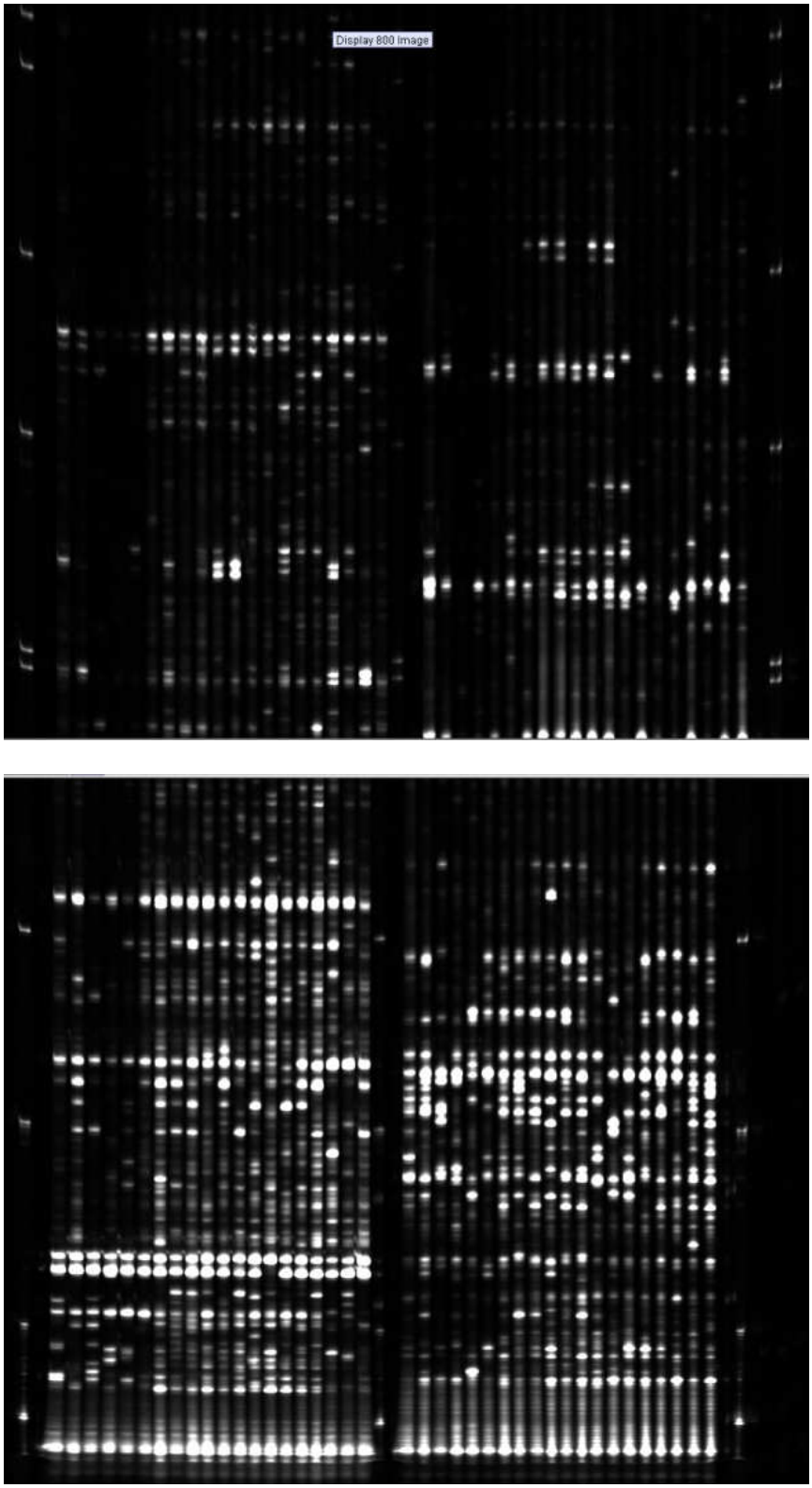


Figure 10. AFLP amplification pattern of the 20 grapevine accessions with using the E-ACG, E-ACT (800 nm) / M-CTC primer combinations.

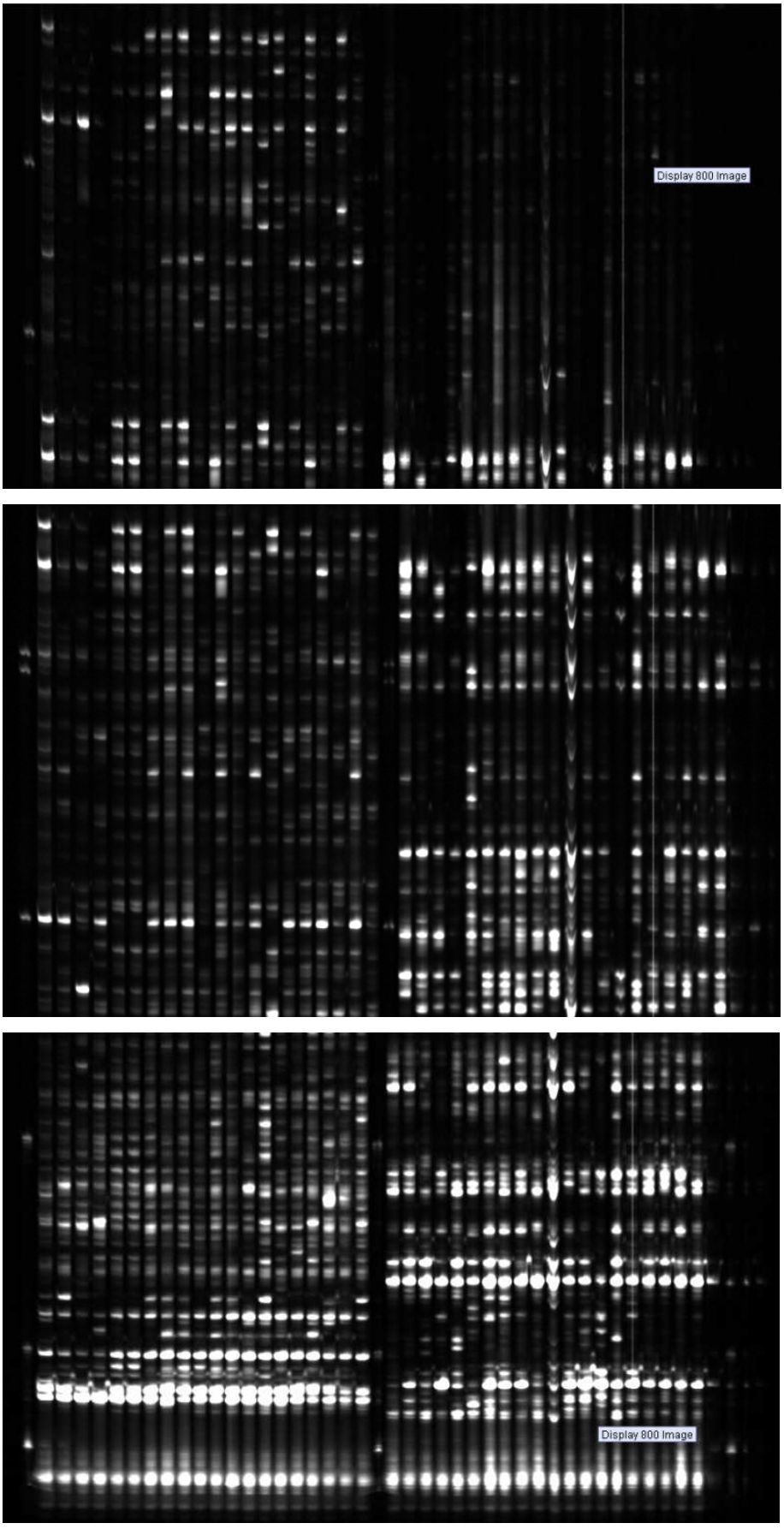


Figure 11. AFLP amplification pattern of the 20 grapevine accessions with using the E-AGC, E-AGG (800 nm) / M-CTC primer combinations.

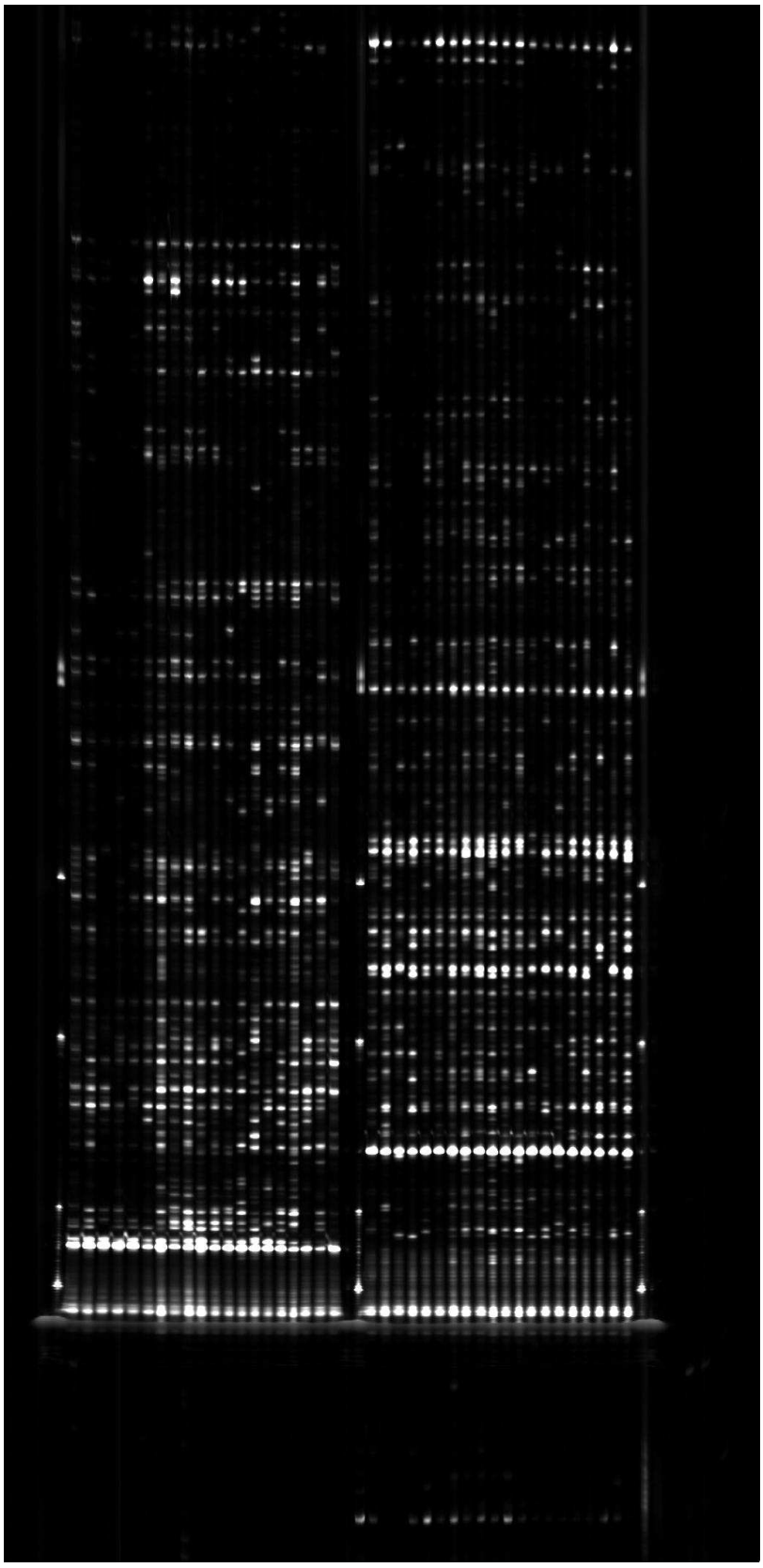


Figure 12. AFLP amplification pattern of the 20 grapevine accessions with using the E-AAC, E-AAG (700 nm) / M-CTC primer combinations.

Figure 13. AFLP amplification pattern of the 20 grapevine accessions with using the E-ACA, E-ACC (700 nm) / M-CTC primer combinations.

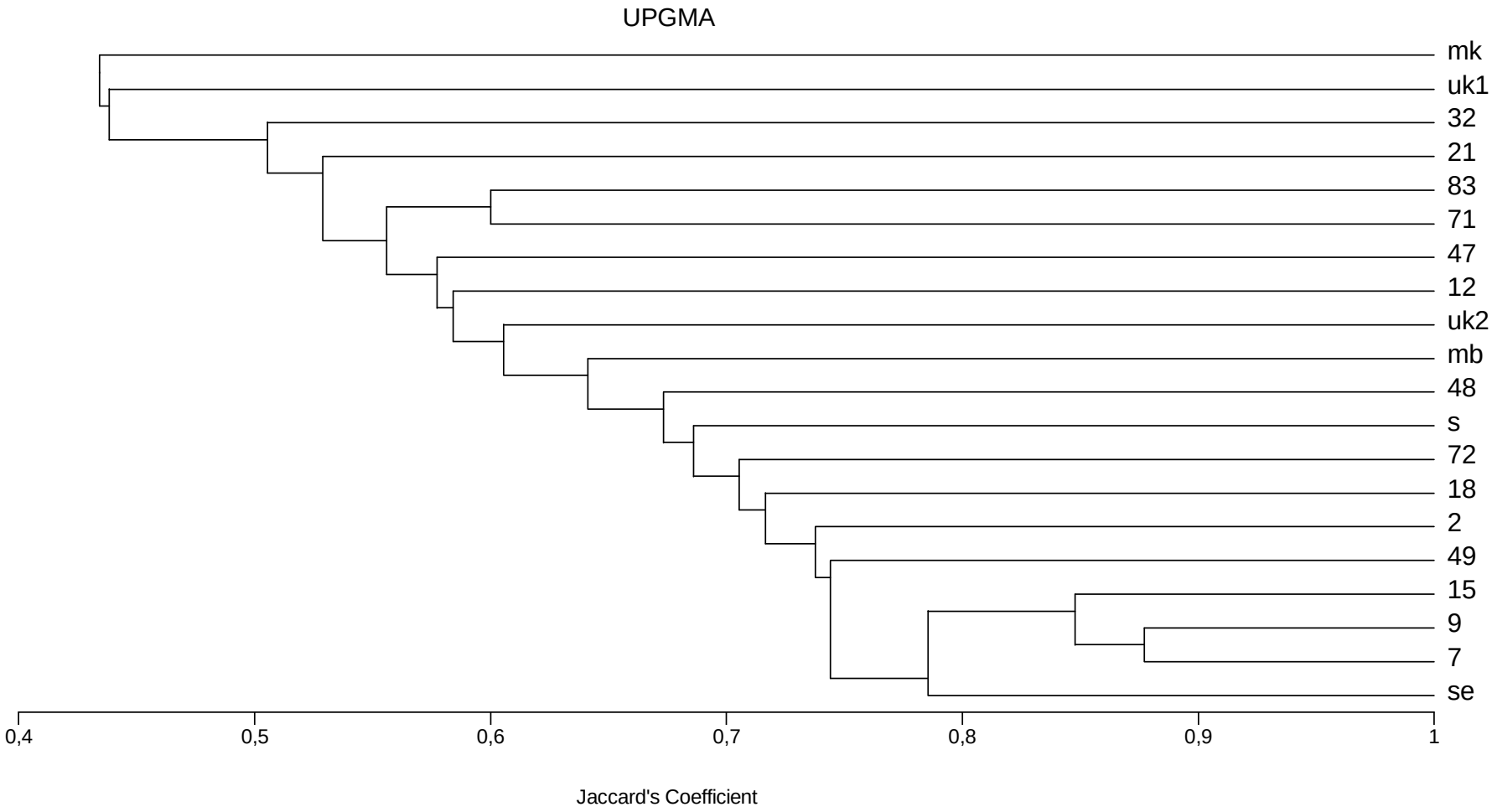


Figure 14. Dendrogram representing the genetic similarity among grapevine accessions. The dendrogram was constructed applying the UPGMA clustering method to the Jaccard's similarity coefficients of genetic similarities based on AFLP analysis with two primer combinations.The scale bar represents simple matching distance.

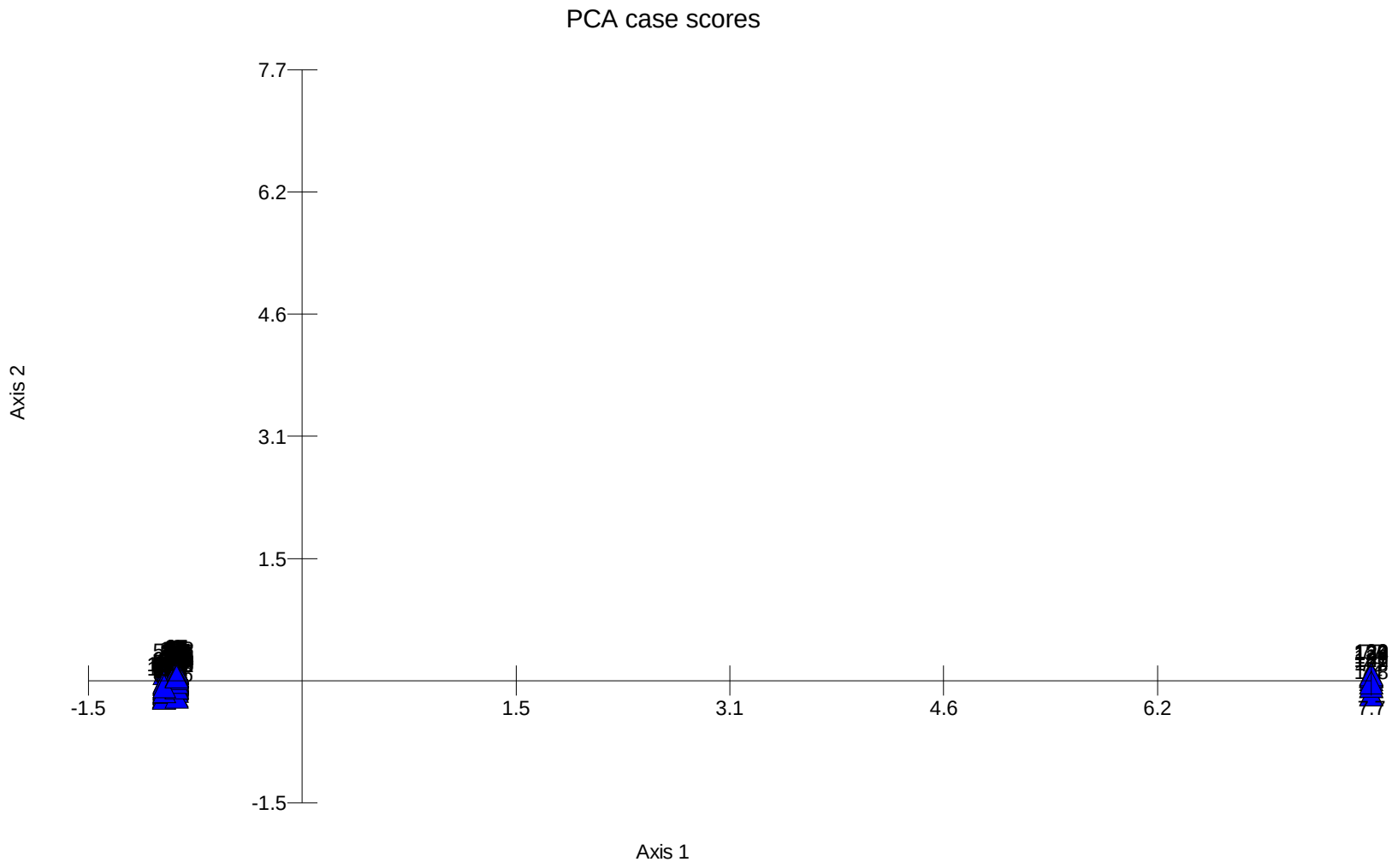


Figure 15. Principal components analysis of the AFLP results.

4.3 Rooting of the Grape Cuttings

Rooting percentage in different grape accessions ranged from 17% to 90%. Best rooting efficiency was observed in Urla Karası 2. Table 13 summarizes the rooting capacity of the grape cuttings.

Table 13. After two and half month (6^{th} March- 22^{nd} July) each of the grape cuttings of the Urla grapevine accessions exhibited distinct rooting capacity.

ID	Number of grape cuttings that rooted	Percentage of rooting
Urla Karası 1	2/12	17% (0,16667)
Urla Karası 2	9/10	90% (0,9000)
Urla Karası 3	6/16	38% (0,375)
Urla Karası 4	5/6	83% (0,833)
Urla Karası 5	8/9	89% (0,88889)

5 DISCUSSION

The total vineyard area of Turkey was 560.000 ha and total grape production was 3.650.000 ton in 1998 according to FAO. For the former one, Turkey was the 4th county after Spain, Italy, and France; and for the latter, it was in the 6th place after Italy, Spain, France, USA, and China. In Turkey, Aegean region comprised the biggest portion in both vineyard area (33%) and grape production (43%). Therefore, grape production, for purpose of both wine and table, is very priceless in Turkey [56].

Molecular marker database for grapevine accessions was established in the European countries, such as Greece. However, in Turkey, molecular characterization of the grapevine accessions has been just started. Few articles, published in 2006, target the characterization of the grapevine accessions endemic to Turkey; especially, the ones special to Anatolian region. This study is the first research on grapevine accession of Aegean region and aimed to comprehend the possibility of finding historically important grapevine accessions.

Scope of the study comprised the SSR and AFLP analyses of the 5 Urla grape accessions, assumed to be the local historical red wine grape varieties, and the Aegean zone black and red wine grape varieties that are also most known red wine grape varieties of Turkey and of Europe. A total of 98 grapevine accessions were analyzed (sample number 76 was out of the study). The GENRES#081 project provides the knowledge required for the selection of the SSR – primers used during the study. VVMD5, VVMD7, VVMD27, VVS2, VrZAG62, and VrZAG79 were the markers of choice as a consequence of this project. However, SSR – analyses of the samples is not enough to conclude a true and reliable result; because, although SSRs have been used for fingerprinting and for differentiation of closely related cultivars, AFLP analysis has the possibility of screening a larger number of anonymous loci than any other tool available [9, 36]. Therefore, AFLP is an inevitable tool for identification of closely related individuals. After the SSR analysis of the 97 grapevine

accessions, LI-COR 4300S DNA Analyzer system was chosen to handle the AFLP part of the study. This system provides advantage over classical AFLP method which requires radioactively labeled primers, as it is designed to perform AFLP analysis with specific infrared dyes labeled primers (IRDye labeled) to give better and accurate results.

The genetic similarity among the different accessions, based on the presence or absence of the amplified fragments, was calculated by Jaccard's similarity coefficients [54]. Using the SSR data of genetic similarity, grapevine accessions were grouped in clusters as shown in Figure 8.

In the UPGMA graph, if there is a line combining at least two samples, in fact, the samples are labeled as "same" with this line. Therefore, as a consequence of the SSR – analysis of the 97 grapevine accessions, it was realized that Urla Karası 1 (UK1) and UK2 are the samples of the same grapevine accession (Figure 8). In addition, it is clearly observed in the UPGMA graph that there are 3 distinct lines apart from the one between UK1 and UK2. First of these three lines combines the grapevine accessions of Sultaniye tatlı (75) and Foça Karası (63); next one is between the grapevine accessions of Syrah (88), Foça Karası (60), and Algöynek (5); and the grapevine accessions of Söbü Dimriti (53) and Nar Üzümü (56) are last group combined with the line. However, it is not concluded that the grapevine accessions that these 3 distinct groups include are the synonymies of each other; because they did not produce same number of polymorphic banding patterns as the other samples. Therefore, their SSR profiles are not enough to make a reliable conclusion. Moreover, UK1 and UK2 are the closest samples to the UK4 with the 0.8 similarity coefficient. The important outcome of the study is that UK3 and UK4 belong to different cluster groups from the rest of the UKs in the PCA scatter graph (Table 11).

Table 10 summarized the samples exhibited a similarity coefficient value higher than 0.5. AFLP analysis of these samples and the grapevine accessions of Urla demonstrated a different result from SSR analysis. Although UK1 and UK2 could be considered as two samples of the same grapevine accessions as a result of SSR data, after AFLP analysis, it is clear that these two samples belong to distinct grapevine accessions (Table 12, Figure 14).

Figure 14 provides the data for genetic relatedness of the Urla samples with the other 14 samples. UK3 (SE) has the highest genetic similarity coefficient value (0,805) with the

sample number 15 (Ekşi Üzüm) (Table 12). Also, their closeness in the UPGMA graph confirms this result. Sample number 9 (Alyanak) (0,793) and 7 (Kadın Parmağı) (0,758) follow the sample number 15 (Ekşi Üzüm). It is also interesting that sample number 9 (Alyanak) and number 7 (Kadın Parmağı) possess a high genetic similarity coefficient value (0.877), and this genetic similarity coefficient is the highest one among whole similarity coefficient data of the analyzed samples. These two grapevines belong to Muğla region of Turkey. However, as they have similar but not same banding pattern with the AFLP markers (Figure 10, 11, 12 and 13); it can be concluded as they are different, but very close grapevine accessions.

Genetic similarity coefficient values of MK (UK4) and UK1 in the Table 12 are not higher than 0.5. This indicates that these samples belong to grapevine accessions certainly different from the analyzed samples. UPGMA graph also confirms this result. MK and UK1 were obviously less similar samples to other analyzed ones.

Table 14 explains the nodes and the groups that construct the UPGMA graph. Construction begins with the most similar samples and end with the least similar samples. Therefore, Table 14 is another way showing the genetic similarities of the analyzed samples.

Table 14. Groups and the nodes of the UPGMA graph of AFLP

Node	Group 1	Group 2	Simil.	Objects in group
1	Kadın Parmağı	Alyanak	0,877	2
2	Node 1	Ekşi üzüm	0,848	3
3	se	Node 2	0,786	4
4	Node 3	Sık Kara	0,744	5
5	Node 4	Boğazkere	0,737	6
6	Node 5	Fesliken	0,716	7
7	Node 6	Hevenk	0,705	8
8	Node 7	s	0,686	9
9	Node 8	Siyah Dimrit	0,673	10
10	Node 9	mb	0,641	11
11	Node 10	uk2	0,605	12
12	Pembe Üzüm	Gamay	0,6	2
13	Node 11	Muhammadiye	0,584	13
14	Node 13	Aşıkara	0,577	14
15	Node 14	Node 12	0,556	16
16	Node 15	Mor Üzüm	0,529	17
17	Node 16	Kırmızı Şam	0,505	18
18	Node 17	uk1	0,438	19
19	Node 18	mk	0,434	20

6 CONCLUSION

Molecular marker techniques, AFLP and SSR were successfully used to characterize grapevine accessions found in Urla region invastigated in this study. Our data suggested that two grapevine accessions of Urla region, MK (UK4) and UK1, possess a lower genetic similarity value from 0.5, therefore they can be considered as different grapevine genotypes from the analyzed grapevine accessions selected from the Gene Bank. Moreover, although SSR analysis concluded UK1 and UK2 as a sample of the same grapevine accessions; AFLP analysis demonstrated that these two genotypes certainly belong to distinct grapevine accessions.

In summary, SSR and AFLP analysis of the total 97 grapevine accessions resulted in relatedness of the already known grapevine accessions and five grapevine varieties that are found in Urla, which could be as new red wine grapevines. As a consequence of the study, new vineyards might be established for local cultivars with historical and economical values. However, they first should be propagated and further studied as to their wine quality features. These new varieties then might have a positive impact on the local and regional viniculture sector which might provide an alternative to the farmers, who are in difficult situation because of the decrease in the tobacco cultivation in the region. Moreover, growing of historical grapevine varieties may have a positive effect on the tourism in Urla region, besides the economical impact from the production of new chateau wines from those cultivars.

7 REFERENCES

1. Zohary, D. and Hopf, M. (2000), Domestication of Plants in the Old World. New York: Oxford University Press.

2. Unwin, T. (1991), Wine and the Vine: An Historical Geography of Viticulture and the Wine Trade. Routledge, London.

3. Fossati, T., Labra, M., Castiglione, S., Failla, O., Scienza, A., Sala, F. (2001), The use of AFLP and SSR molecular markers to decipher homonyms and synonyms in grapevine cultivars: the case of the varietal group known as “Schiave”. Theor Appl Genet, 102:200–205.

4. Grassi, F., Labra, M., Imazio, S., Spada, A., Sgorbati, S., Scienza, A., Sala, F. (2003), Evidence of a secondary grapevine domestication centre detected by SSR analysis. Theor Appl Genet., 107:1315–1320.

5. McGovern, P.E., Glusker, D.L., Exner L.J., Voigt, M.M. (1996), Neolithic resinated wine. Nature (London), 381: 480-481.

6. McGovern, P.E. (2003), Ancient wine, the search for the origins of viticulture, Princeton University Press, Princeton, N.J.

7. Garcia, R.A. *et al.* (2006), Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms, Molecular Ecology, 15:3707–3714.

8. Allaby, R.G., O'Donoghue, K., Sallares, R., Jones, M.K., Brown, T.A. (1997), Evidence for the survival of ancient DNA in charred wheat seeds from European archaeological sites. Ancient Biomol, 1:119-129.

9. Bowers, J.E., Boursiquot, J.M., This, P., Chu, K., Johansson, H., Meredith, C. (1999), Historical genetics: the parentage of Chardonnay, Gamay, and the other wine grapes of north-eastern France. Science, 285:1562-1565.

10. Zohary, D., Spiegel-Roy, P. (1975), Beginnings of fruit growing in the old world. Science 187:319–327.

11. Ergül, A., Kazan, K., Aras, S., Çevik, V., Çelik, H., Söylemezoğlu, G. (2006), AFLP analysis of genetic variation within the two economically important Anatolian grapevine (*Vitis vinifera* L.) varietal groups. Genoma, 49: 467-475.

12. Negrul, A.M. (1938), Evolution of cultivated forms of grapes. C. R. Acad. Sci.U.S.S.R., 18:585-588.

13. Levadoux, L. (1956), Les populations sauvages et cultivées de *Vitis vinifera* L. Ann. Amelior. Plant, 1:59-118.

14. Alleweldt, G., Possingham, JV. (1988), Progress in grapevine breeding. Theor Appl Genet, 75:669-673.

15. Forni, G. (1990), Gli albori dell'agricoltura. REDA, Roma, Italy.

16. Olmo, H.P. (1976), Grapes. In: Simmounds NW (ed) Evolution of crop plants. London, Longman, 294–298.

17. Ağaoğlu, Y.S., Çelik, H. (1986), Conservation of *Vitis vinifera* L. germplasm in Turkey. Vignevini, 13(Suppl. 12): 40–42.

18. Ergül, A., Marasali, B., Ağaoğlu, Y.S. (2002), Molecular discrimination and identification of some Turkish grape cultivars (*Vitis vinifera* L.) by RAPD markers. Vitis, 41(3): 159–160.

19. Alleweldt, G., Spiegel-Roy, P., Reisch, B. (1990), Genetic resources of temperate fruits and nut crops. Grapes (*Vitis*). In: Moore JN, Ballington JR (eds).Vol 1. Int Soc Hort Sci, Wageningen, The Netherlands, 291–327.

20. Ye, G. N., G. Soylemezoglu, N. F.Weeden, W. F. Lamboy, R. M. Pool, B. I. Reisch, (1998), Analysis of the relationship between grapevinecultivars, sports and clones *via* DNA fingerprint. Vitis, 37:33-38.

21. Debener, T., Janakiram T., Mattiesch, L. (2000), Sport and seedlings of rose varieties analysed with molecular markers. Plant Breeding, 119: 71-74.

22. Tessier, L. C., David, J., This P., Boursiquot, J. M., Charrier, A. (1999), Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera*. Theor Appl Genet, 98: 171-177.

23. Imazio1, S., Labra, M., Grassi1, F., Winfield, M., Bardini1, M., Scienza A. (2002), Molecular tools for clone identification: the case of the grapevine cultivar *Traminer*. Plant Breeding, 121: 531-535.

24. Russel, J., Fuller, J., Young, G., Thomas, B., Taramino, G., Macaulay, M., Waugh, R., Powell, W. (1997), Discrimination between barley genotypes using microsatellite markers. Genome, 40: 442-450.

25. Kijas, J. M. H., Fowler, J. C. S., Thomas, M. R. (1995), An evaluation of sequence tagged microsatellite site markers for genetic analysis within Citrus and related species. Genome, 38:1244-1248.

26. Grzebelus, D., Senalik, D., Jagosz, B., Simon, P. W., Machalik, B. (2001), The use of AFLP markers for the identification of carrot breeding lines and F1 hybrids. Plant Breeding, 120:526-528.

27. Botta, R., Scott, N. S., Eynard, I., Thomas, M. R. (1995), Evaluation of microsatellite sequence-tagged site markers for characterizing *Vitis vinifera* cultivars. Vitis, 2:99-102.

28. Sefc, K. M., Regner, F., Glossl, J., Steinkellner, H. (1998), Genotyping of grapevine and rootstock cultivars using microsatellite markers. Vitis, 37:15-20.

29. Thomas, M. R., Matsumoto, S., Cain, P., Scott, N. S. (1993), Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theor. Appl. Genet., 86:1-8.

30. Bourquin, J. C., Sonko, A., Otten, L., Walter, B. (1993), Restriction fragment length polymorphism and molecular taxonomy in *Vitis vinifera* L. Theor. Appl. Genet., 87:431-438.

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

32. Cipriani, G., Lot, G., Huang, W.G., Marrazzo, M.T., Peterlunger, E., Testolin, R. (1999), AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. Theor Appl Genet, 99:65–72.

33. Sefc K.M., Steinkellner, H., Glössl, J., Kampfer, S., Regner, F. (1998b) Reconstruction of a grapevine pedigree by microsatellite analysis. Theor Appl Genet, 97:227–231.

34. Bowers J.E., Meredith, C.P. (1997), The parentage of a classic wine grape, Cabernet Sauvignon. Nature Genet, 16:84–87.

35. Lamboy, W.F., Alpha, C.G. (1998), Using simple sequence repeats (SSRs) for DNA fingerprinting germplasm accessions of grape (*Vitis* L.) species. J Am Soc Hort Sci, 123:182–188.

36. Sefc, K. M., Lopes, M. S., Lefort, F., Botta, R., Roubelakis- Angelakis, K. A., Ibanez I., Pejié, H. W., Wagner, J., Glossl, H., Steinkellner, H. (2000), Microsatellite variability in grapevine cultivars from different European region and evaluation of assignment testing to assess the geographic origin of cultivar. Theor. Appl. Genet., 100:489-505.

37. Benin, M., Gasquez, J., Mahfoudi, A., Bessis, R. (1988), Caractérisation biochimique des cé pages de v*itis vinifera* L. par électrophorése d'isoenzymes foliaires: essai de classiffcation des variétés. Vitis, 27 : 157-172.

38. This, P. *et al*. (2004), Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor Appl Genet, 109: 1448–1458.

39. Aradhya, M.K., Dangl, G.S., Prins, B.H., Boursiquot, J.M., Walker, A.M., Meredith C.P., Simon, C.J. (2003), Genetic structure and differentiation in cultivated grape *Vitis vinifera* L. Genet Res (Camb), 81:179–192.

40. Delseny, M., Laroche, M., Penon, P. (1983), Detection of sequences with Z-DNA forming potential in higher plants. Biochem. Bioph. Res. Commun., 116: 113-20.

41. Sefc, K.M., Lefort, F., Grando, M.S., Scott, K.D., Steinkellner, H., Thomas, M.R (2001), Microsatellite markers for grapevine: a state of the art, Molecular Biology and Biotechnology of Grapevine, Kluwer Publishers, Amsterdam, 407-438.

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

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

44. Bowers, J.E., Dangl, G.S., Meredith, C.P. (1999b), Development and characterization of additional microsatellite DNA markers for grape. Am J Enol Viticult, 50:243–246.

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

46. Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. (1995), AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23:4407-4414.

47. Sensi, E., Vignani, R., Rohde, W., Biricotti, S. (1996), Characterization of genetic biodiversity with *Vitis vinifera* L. Sangiovese and Colorino genotypes by AFLP and ISTR DNA marker technology. Vitis, 35:183-188.

48. Cervera, M.T., Cabezas, J.A., Sancha, J.C., Martinez de Toda, F., Martinez-Zapater, J.M. (1998), Application of AFLPs to the characterization of grapevine *Vitis vinifera* L. genetic resources, a case study with accessions from Rioja (Spain). Theor. Appl. Genet., 97: 51-59.

49. Savelkoul, P. H. M., Aarts, H. J. M., De haas, J., Dijkshoorn, L., Duim, B., Otsen, M., Rademaker, J.L.W., Schouls, L., Lenstra, J.A. (1999), Amplified-Fragment Length Polymorphism Analysis: the State of an Art. Journal of Clinical Microbiology, 37:3083–3091.

50. Anil, Ş. CIHEAM - Options Mediterraneennes, TURKEY

51. Hamamcioglu-Turan,M. (2005), Representation of Historical Stratification in a Church Converted into A Mosque, CIPA 26 September – 01 October 2005 XX International Symposium, Torino, Italy.

52. Yıldırım,B., Gates, M. (2007), Archaeology in Turkey. American journal of archaeology, 2004-2005, 111: 275-356.

53. Nei, M., and W.H. Li. (1979), Mathematical-Model for studying genetic-variation in terms of restriction endonucleases. Proc Natl Acad Sci U S A, 76:5269-5273.

54. Jaccard, P. (1908), Nouvelles rescherches sur la distribution floral. Bull. Soc. Vaud. Sci. Nat., 44:223-270.

55. Kovach, W.L. (1999), MVSP—A Multivariate Statistical Package for Windows, Ver. 3.1, Kovach Computing Services, Pentraeth, Wales, UK.

56. Çelik, H., Çelik, S., Kunter, B.M., Söylemezoğlu, G., Boz, Y., Özer, C., Atak, A., 2005. Bağcılıkta Gelişme ve Üretim Hedefleri. VI. Türkiye Ziraat Mühendisliği Teknik Kongresi, 3-7 Ocak, Ankara.

APPENDIX A

List of Grapevine Accessions

No	Örnek Kodu	Çeşit Adı
1	646-48	Kızıl Üzüm
2		Boğazkere
3	492-45	Duman
4	454-45	İri Kara
5	734-03	Algöynek
6	791-64	Aydın Üzümü
7	639-48	Kadın Parmağı
8		Öküzgözü
9	593-48	Alyanak
10	588-20	Çalkarası
11	491-45	Siyah Gemre
12	549-20	Muhammediye (Mor Üzüm)
13	786-64	Terzi Nasuf
14	824-35	Efe Püskülü
15	849-35	Ekşi Üzüm
16	850-35	Kara Üzüm
17	592-48	Pembe Çekirdeksiz
18	552-20	Fesliken
19	446-45	Antep Şamı
20	693-03	Siyah Parmak
21	784-64	Mor Üzüm
22	502-20	Siyah Dimrit
23	536-20	İri Kızıl
24	500-20	Yediveren
25	626-06	Kalecik Karası
26	547-20	Yanal Üzüm
27	651-48	Yerli Kara
28	848-35	Zeytin Üzümü
29	539-20	Ekşi Kara
30	821-64	Hacı Eyüp
31	484-45	Cami
32	440-45	Kırmızı Şam
33	779-03	Isparta
34	638-48	İstanbul Dimliti
35	538-20	Eşek Memesi
36	825-35	Yediveren
37		Papazkarası
38	589-20	Kınalı
39	438-45	Şaraplık Üzüm
40	696-03	Manda Gözü
41	488-45	Siyah Misket
42	648-48	Katı Kara
43	782-64	Mor Razdağı
44		Adakarası
45	641-48	Pembe Çavuş
46	489-45	Siyah Misket
47	543-20 (534-20)	Aşıkara
48	594-48	Siyah Dimrit

No	Örnek Kodu	Çeşit Adı	
49	852-35	Sık Kara	
50	647-48	Kara İbrahim	
51	597-48	Sofra Karası	
52	495-45	Hasan Üzümü	
53	452-45	Nar Üzümü	
54	496-45	Pembe Şam	
55	546-20	Tavşan Böbreği	
56	780-03	Söbü Dimrit	
57	785-64	Şahin Tırnağı	
58	599-48	Kayasar (Kayzer) Dimliti	
59	643-48	Kara Büzgülü	
60	857-09	Foça Karası	
61	823-35	Hacı Azman	
62	134-11	Kokulu Kara	
63	142-17	Foça Karası	
64	498-20	Eski Kara	
65	499-20	Hırsız Çalmaz	
66	605-48	Aydın Karası	
67	822-35	Gelin Dudağı	
68	792-64	Siyah Razakı (Razdağı)	
69	556-20	Kınalı Tırnak	
70	487-45	Hacı Hıdır	
71	485-45 (486-45)	Pembe Üzüm	
72	688-03	Hevenk (Gelin Parmağı)	
73	554-20	Pembe Gemre	
74	650-48	Tavşan Böbreği	
75	542-20	Sultaniye Tatlı	
76	434-45	Siyah Gemre	
77	555-20	Devegözü	
78	702-03	Veyisoğlu	
79	553-20	Büzgülü	
80	236-17	Karalahna	KL
81	138-17	Karasakız	KS
82	179-31	Sevgi Karası	SK
83	Özel Koleksiyon Bağı	Gamay	G
84	Özel Koleksiyon Bağı	Carignane	C
85	Özel Koleksiyon Bağı	Hamburg Misketi	HM
86	Özel Koleksiyon Bağı	Pinot Noir	P
87	Özel Koleksiyon Bağı	Alicante Bouschet	A
88	Özel Koleksiyon Bağı	Syrah	S
89	Özel Koleksiyon Bağı	Merlot	M
90	Özel Koleksiyon Bağı	Sangiovese	SG
91	Özel Koleksiyon Bağı	Cabernet Sauvignon	CT
92	Özel Koleksiyon Bağı	Cinsaut	Ct
93	UK1	Urla karasa 1	
94	UK2	Urla karasa 2	
95	SE	Urla karası 3	
96	MK	Urla karası 4	
97	MB	Urla karası 5	
98	S	Urla karası 6	

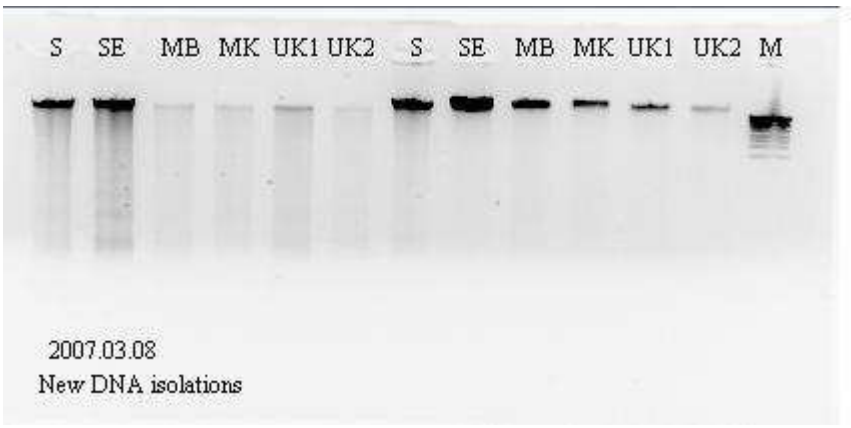
APPENDIX B

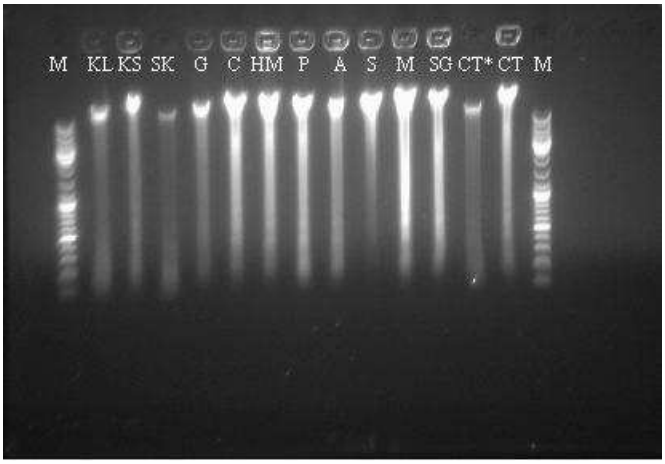
Equipments

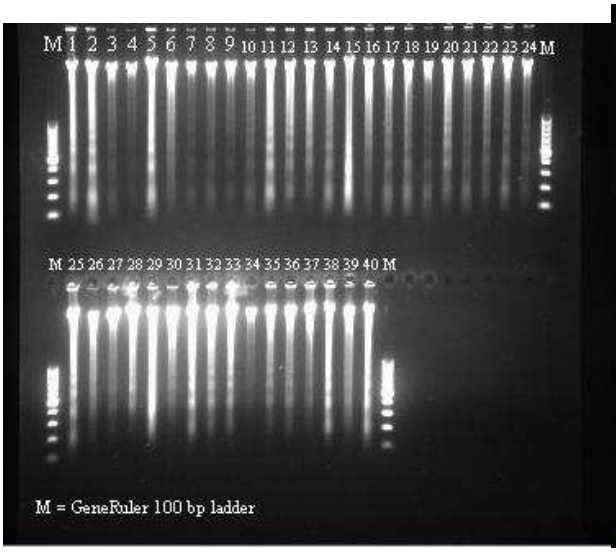
Autoclave	Hirayama, Hiclave HV-110, Japan Certoclav, Table Top Autoclave CV-EL-12L, Austria
Micro Centrifuge	Eppendorf, 5415D, Germany Hitachi, Sorvall RC5C Plus, USA
Deepfreeze	-20 ºC, Bosch, Turkey
Distilled Water	Millipore, Elix-S, France
Electrophoresis Apparatus	Biogen Inc., USA Biorad Inc., USA
Gel Documentation	Biorad GelDoc EQ System, USA
Heater	Thermomixer Comfort, Eppendorf, Germany
Ice Machine	Scotsman Inc., AF20, USA
Magnetic Stirrer	VELP Scientifica, ARE Heating Magnetic Stirrer, Italy
Microliter Pipettes	Gilson, Pipetman, France Eppendorf, Germany
Microwave Oven	Bosch, Turkey
pH Meter	WTW, pH540 GLP MultiCal, Germany
Refrigerator	Bosch, Turkey
Spectrophotometer	Nanodrop, ND-1000, USA
Thermocycler	Eppendorf, PTC-100 Mastercycler Gradient, Germany
Vortex	Velp Scientifica, Italy
Tissue Lyser	Retsch

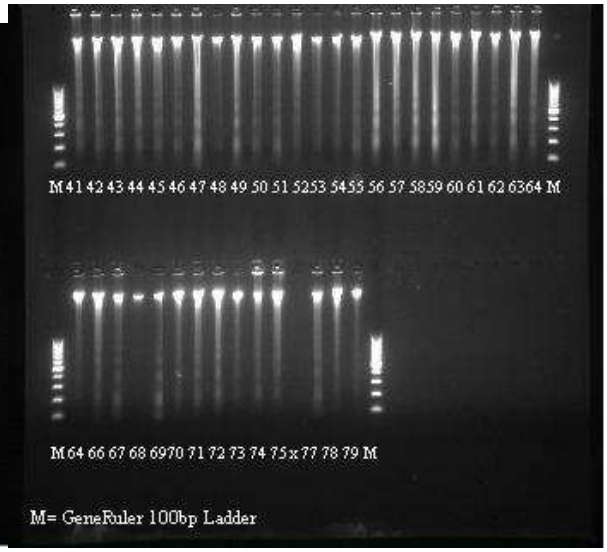
APPENDIX C

DNA Isolations









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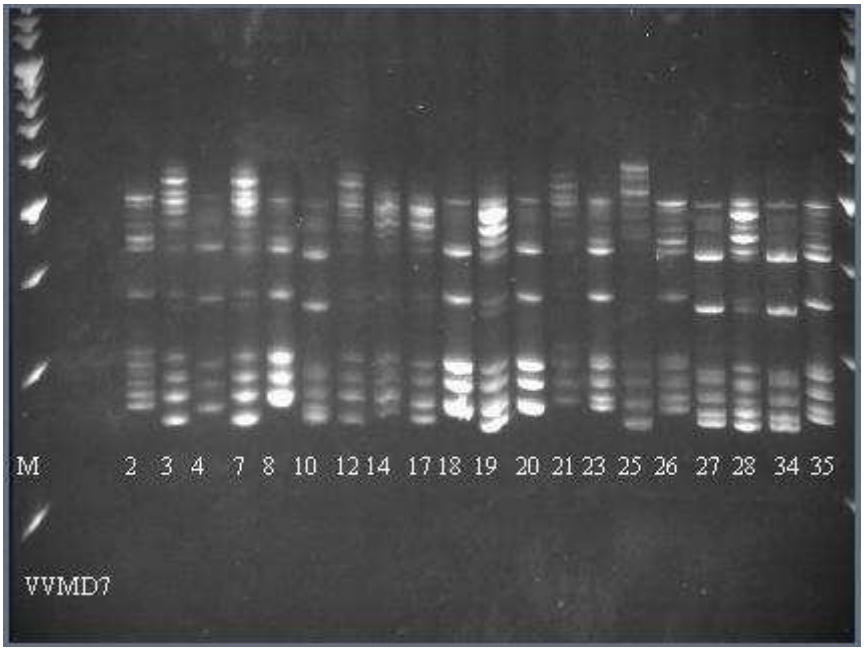
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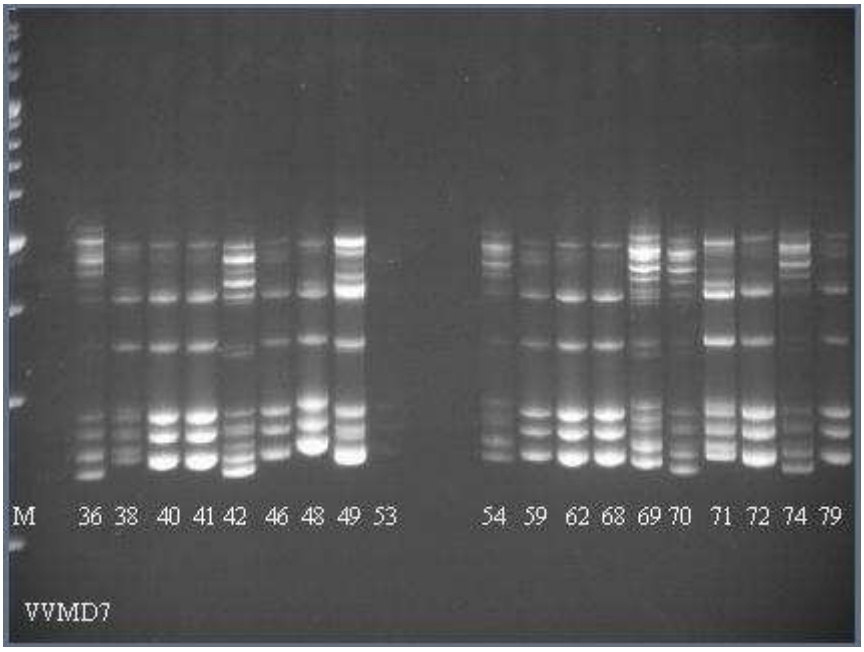
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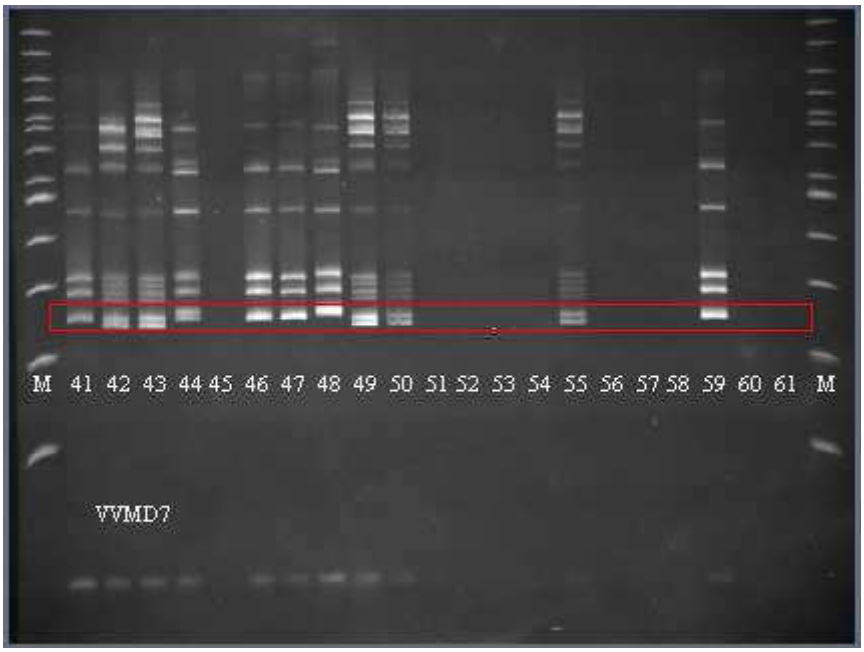
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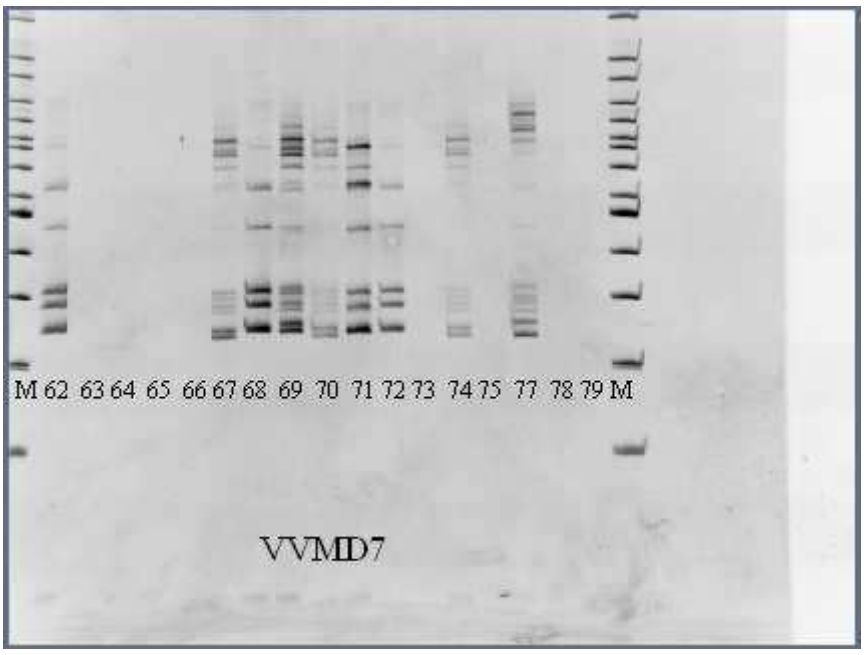
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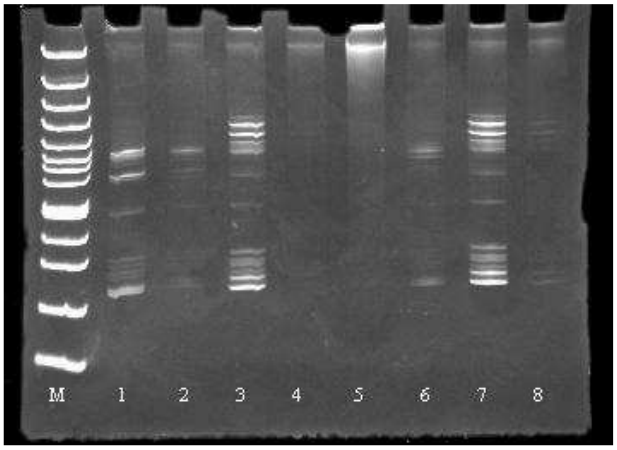
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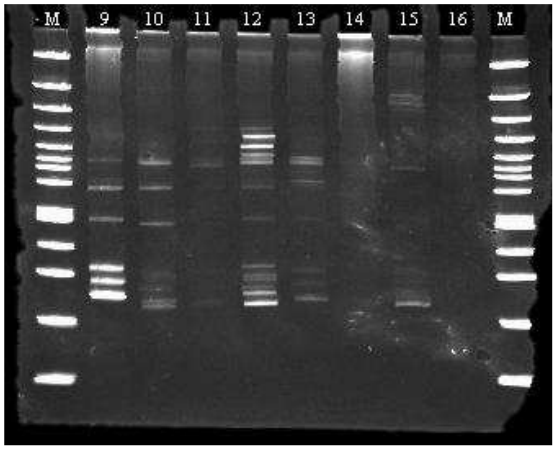
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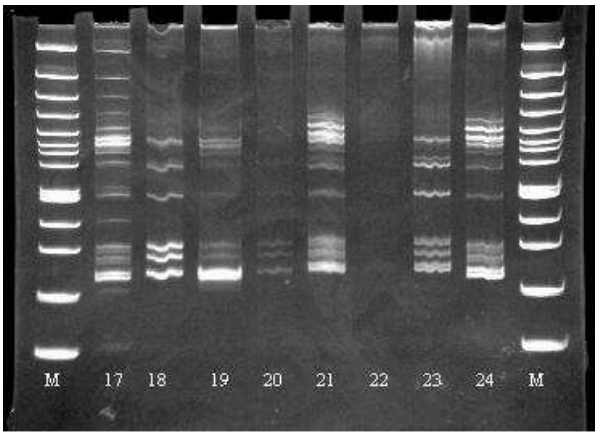
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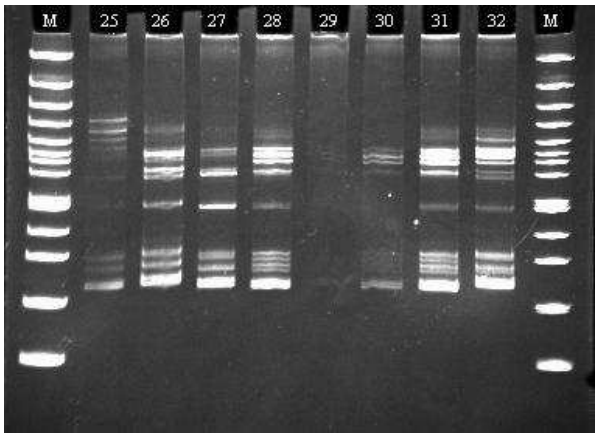
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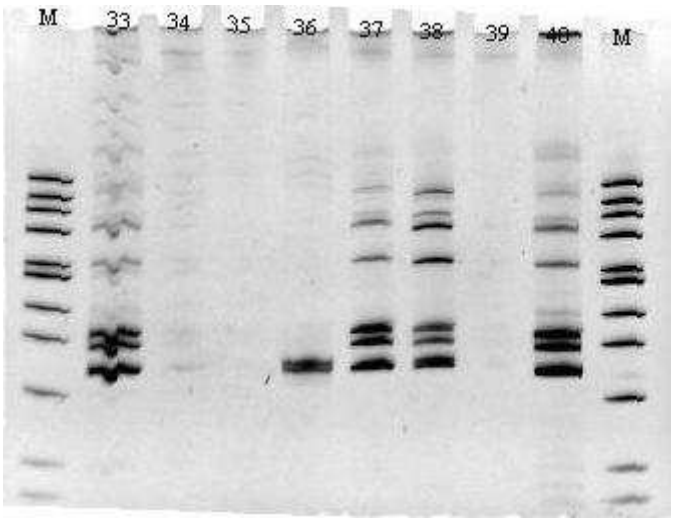
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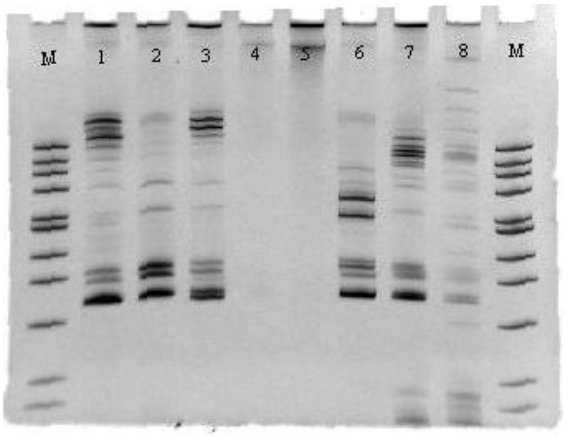
Zag79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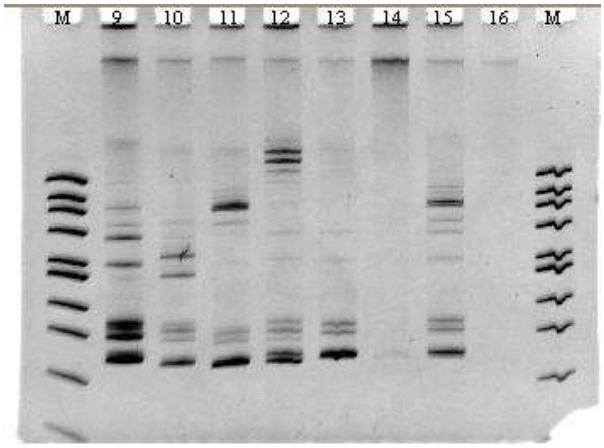
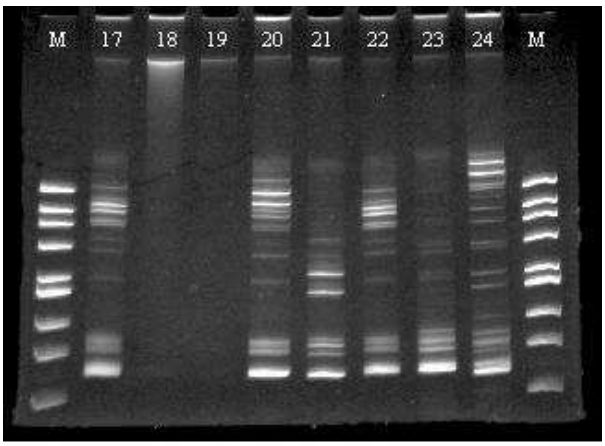
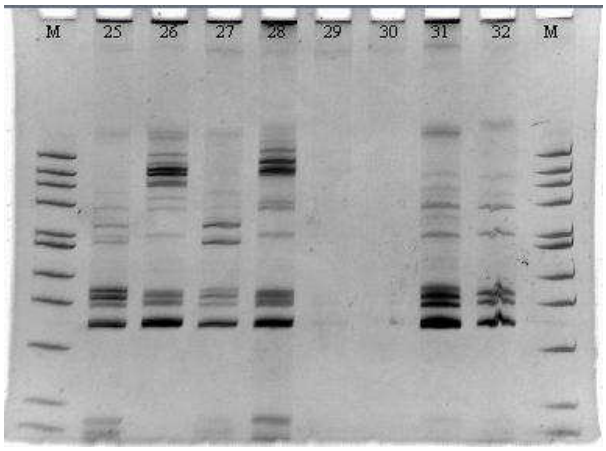
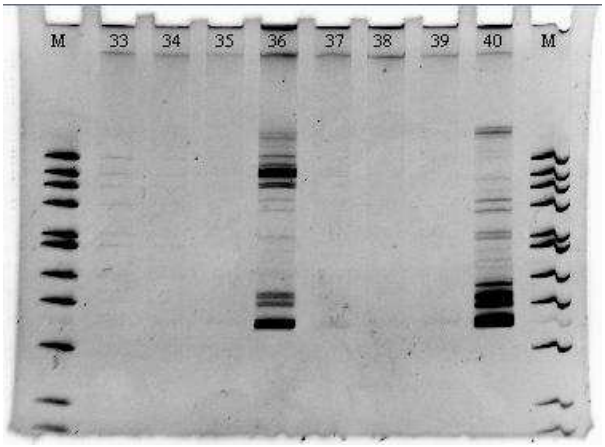
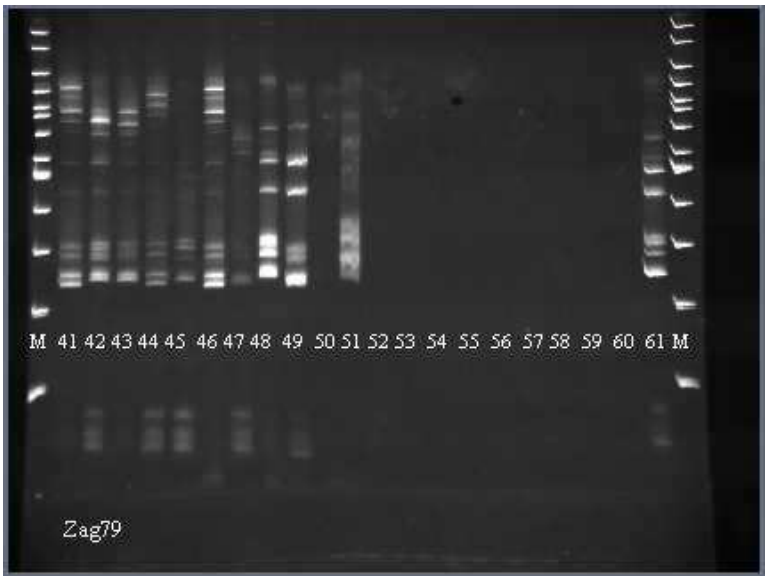
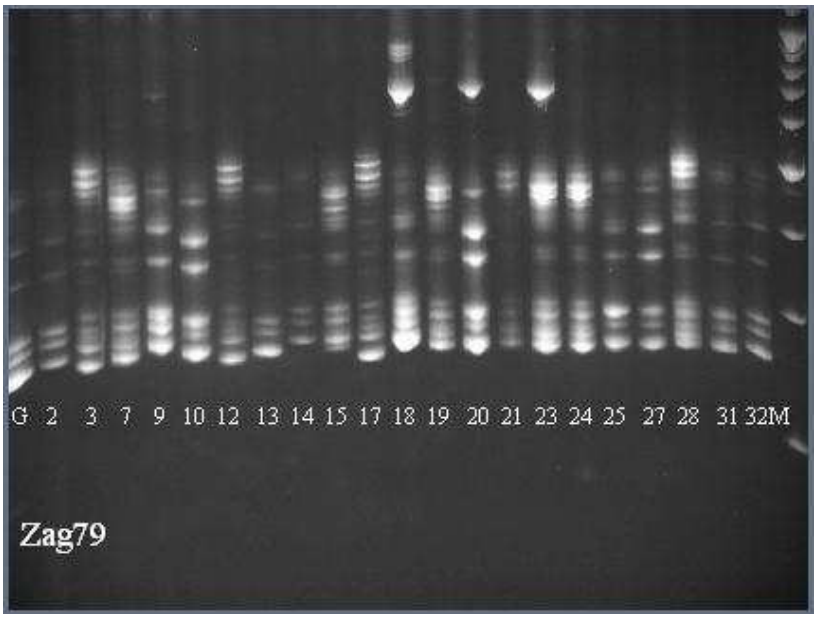

M 9 10 11 12 13 14 15 16 M

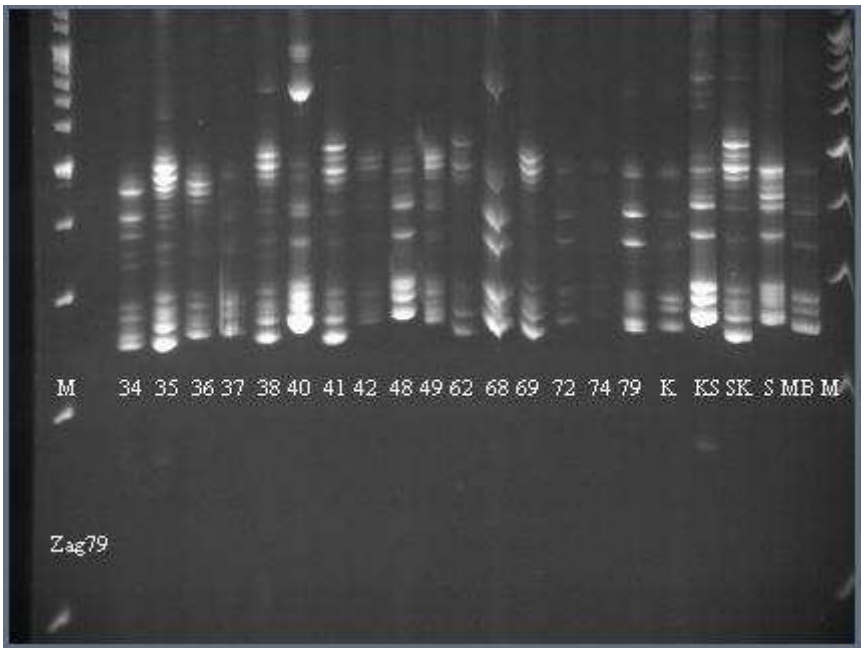

M 17 18 19 20 21 22 23 24 M

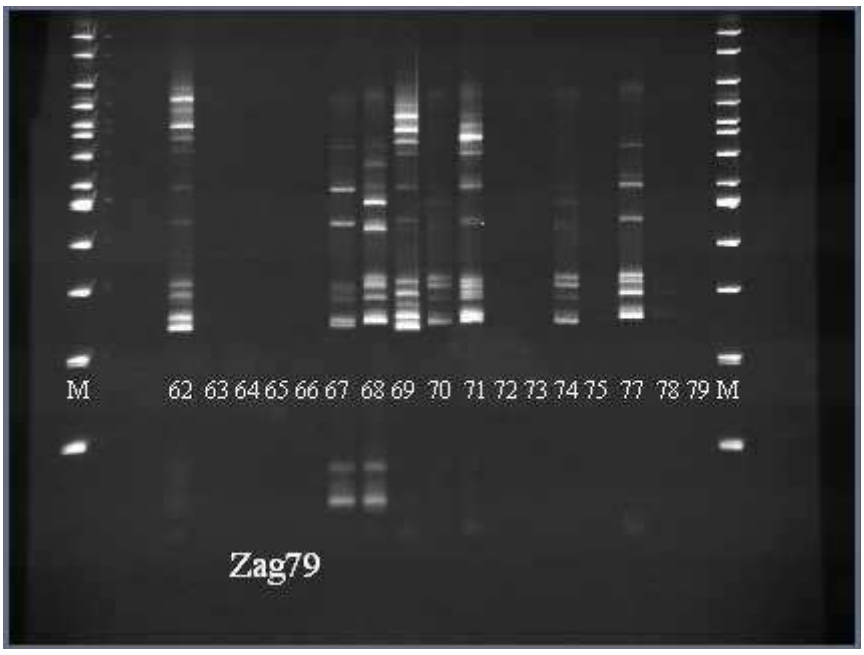

M 25 26 27 28 29 30 31 32 M


M 33 34 35 36 37 38 39 40 M

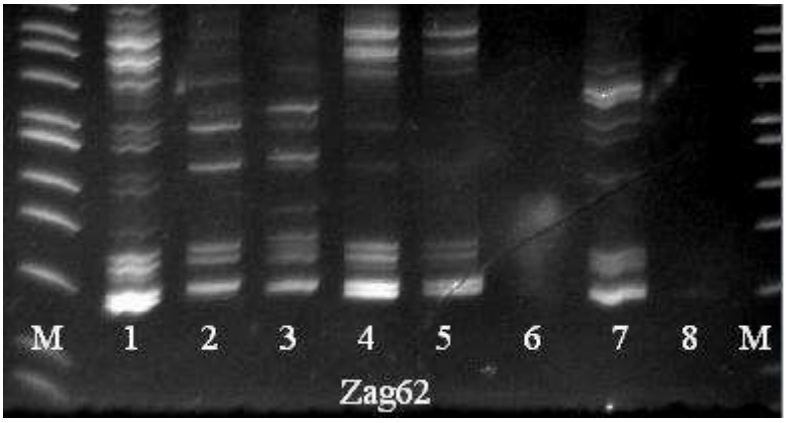

M 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 M
Zag79

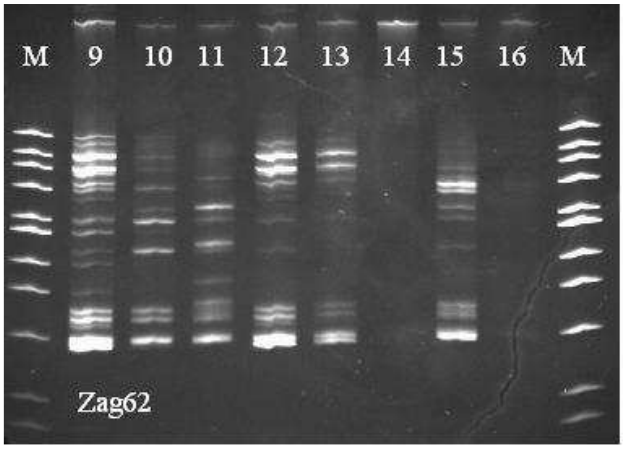

G 2 3 7 9 10 12 13 14 15 17 18 19 20 21 23 24 25 27 28 31 32M
Zag79

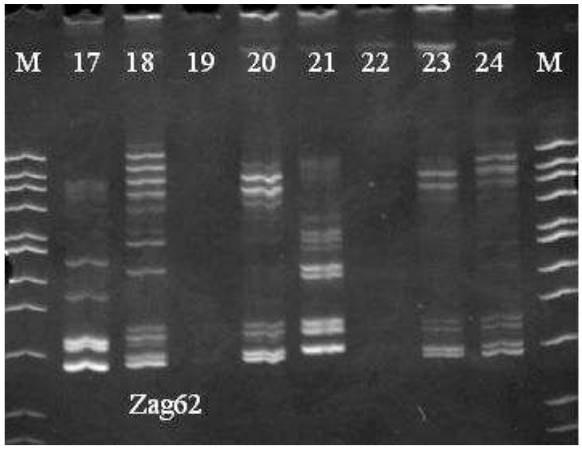

M 34 35 36 37 38 40 41 42 48 49 62 68 69 72 74 79 K KS SK S MB M
Zag79

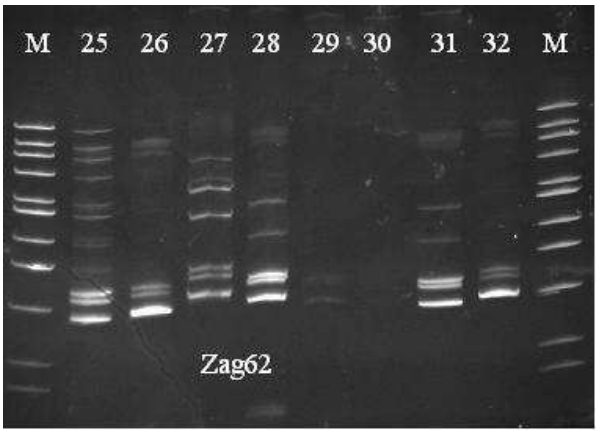

M 62 63 64 65 66 67 68 69 70 71 72 73 74 75 77 78 79 M
Zag79

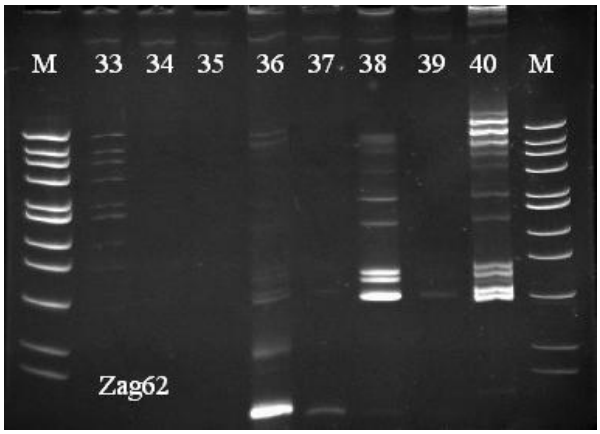
Zag62

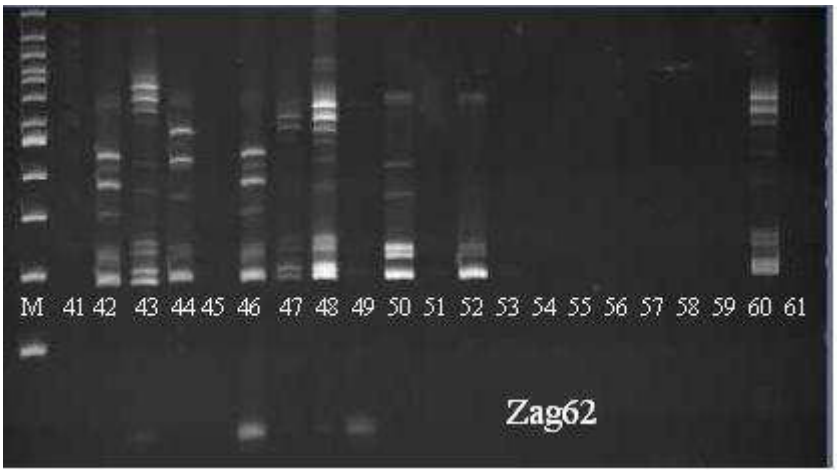

M 1 2 3 4 5 6 7 8 M
Zag62

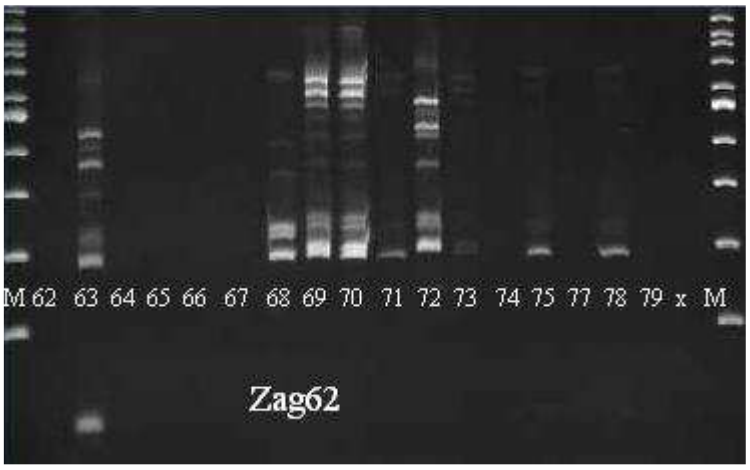

M 9 10 11 12 13 14 15 16 M
Zag62

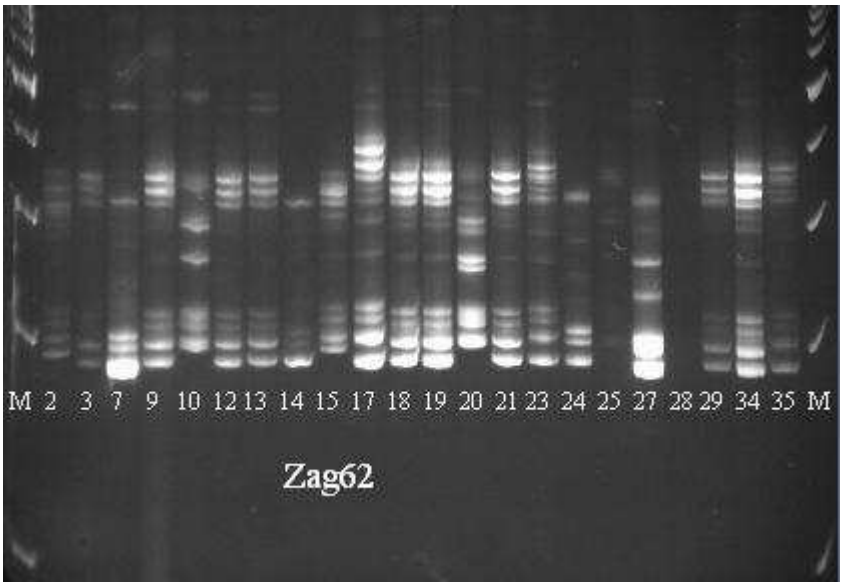

M 17 18 19 20 21 22 23 24 M
Zag62


M 25 26 27 28 29 30 31 32 M
Zag62

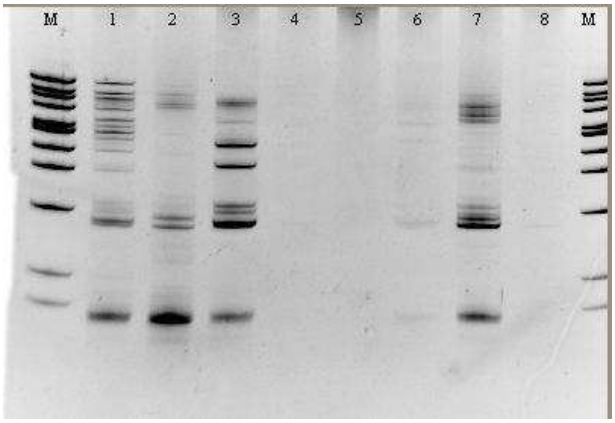

M 33 34 35 36 37 38 39 40 M
Zag62

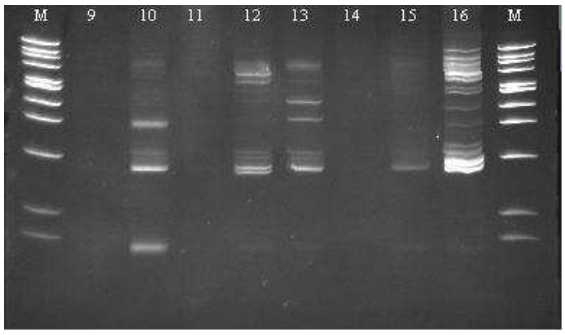

M 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61
Zag62

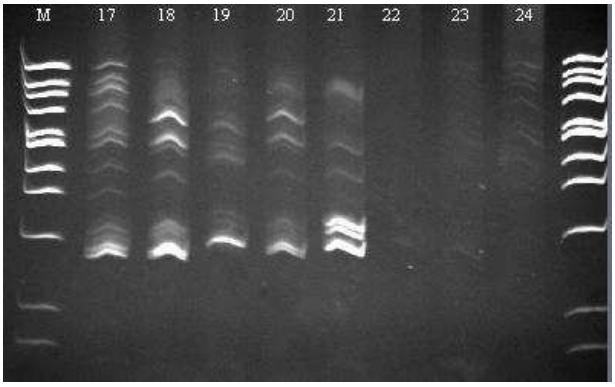

M 62 63 64 65 66 67 68 69 70 71 72 73 74 75 77 78 79 x M
Zag62

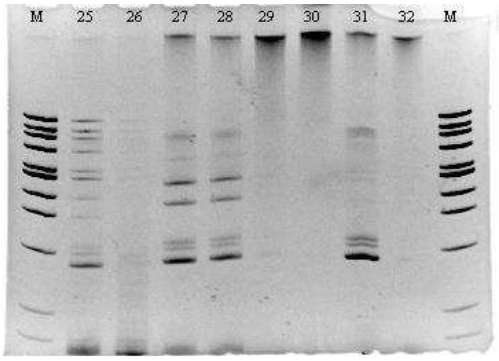

M 2 3 7 9 10 12 13 14 15 17 18 19 20 21 23 24 25 27 28 29 34 35 M
Zag62

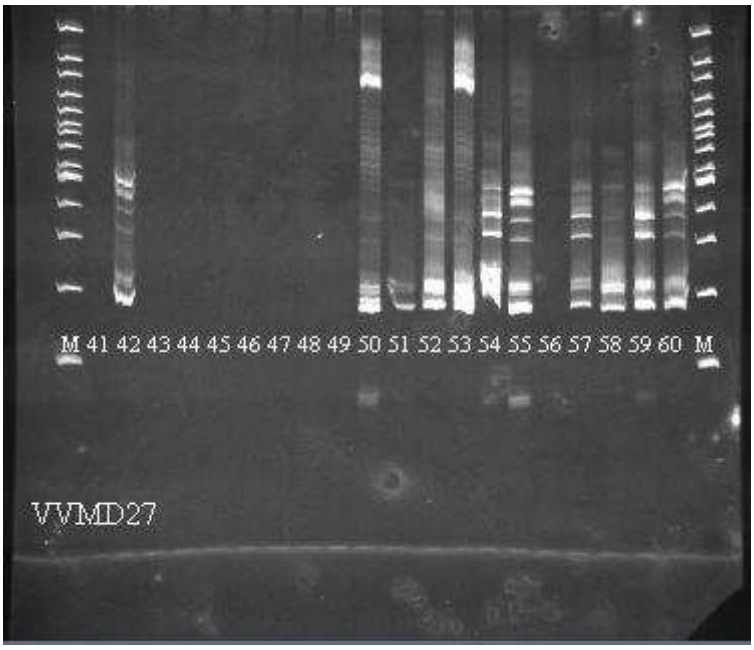
VVMD27

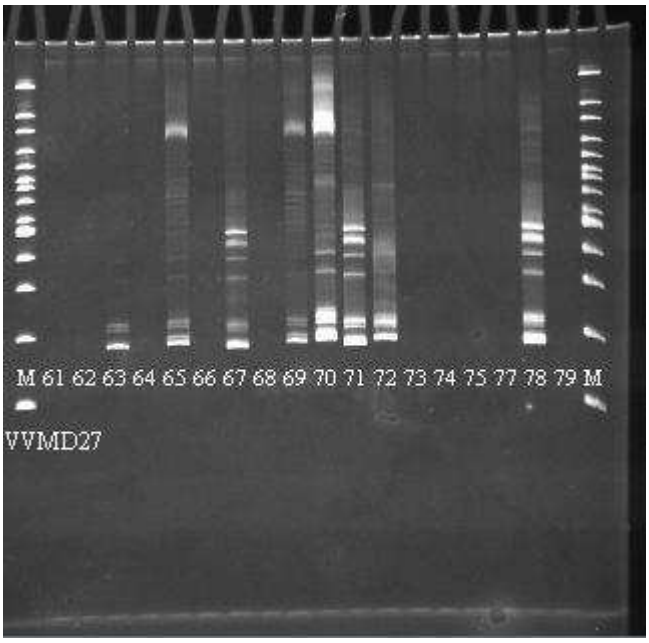

M 1 2 3 4 5 6 7 8 M

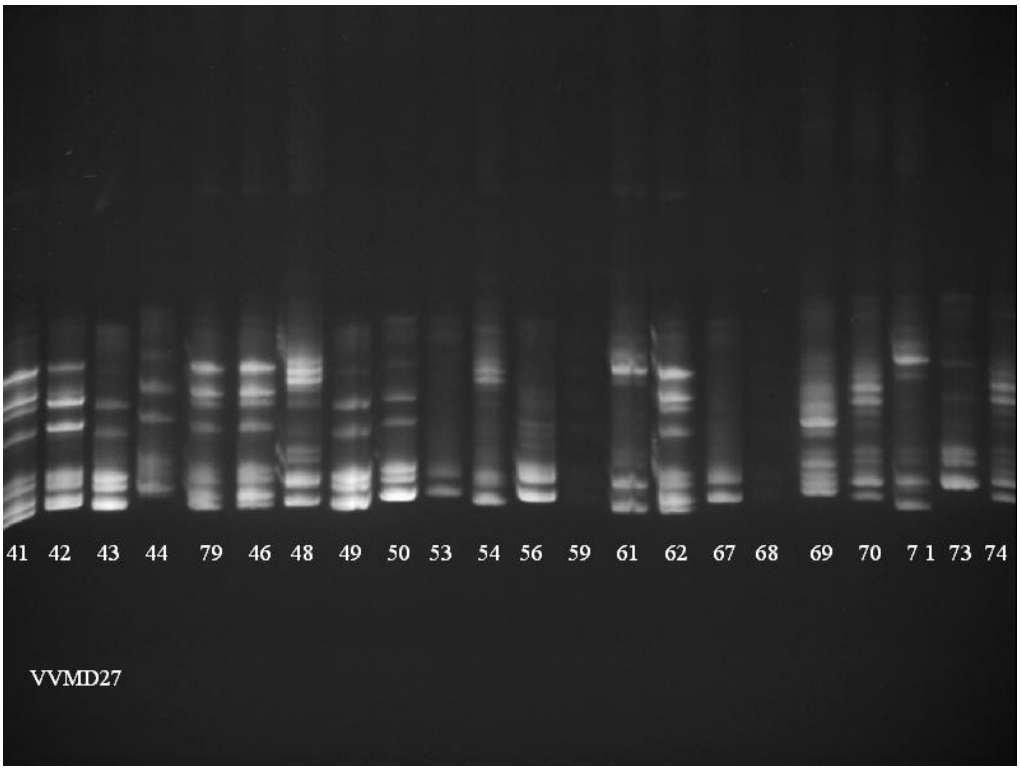

M 9 10 11 12 13 14 15 16 M


M 17 18 19 20 21 22 23 24

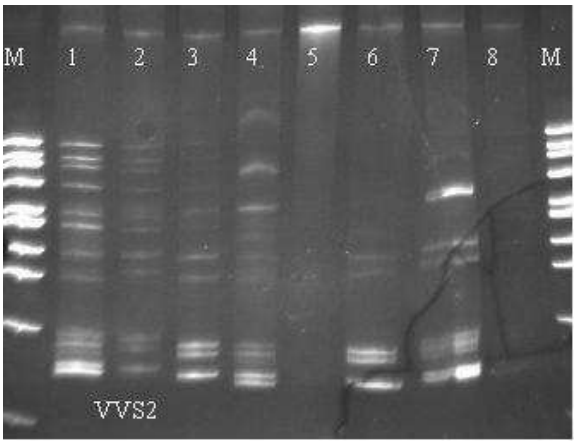

M 25 26 27 28 29 30 31 32 M

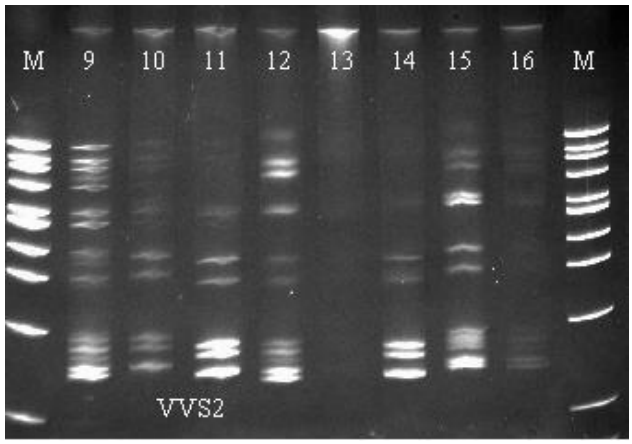

M 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 M
VVMD27

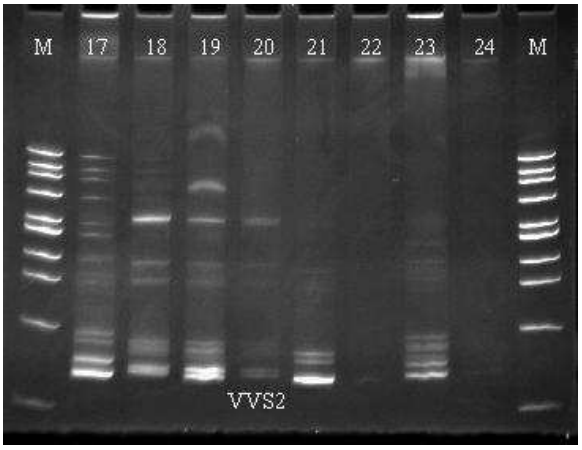

M 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 77 78 79 M
VVMD27

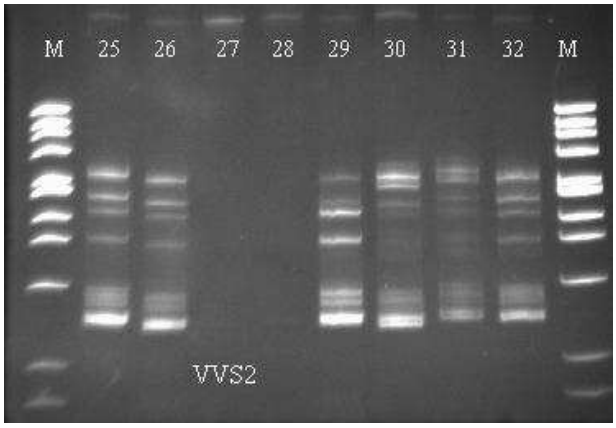

41 42 43 44 79 46 48 49 50 53 54 56 59 61 62 67 68 69 70 7 1 73 74
VVMD27

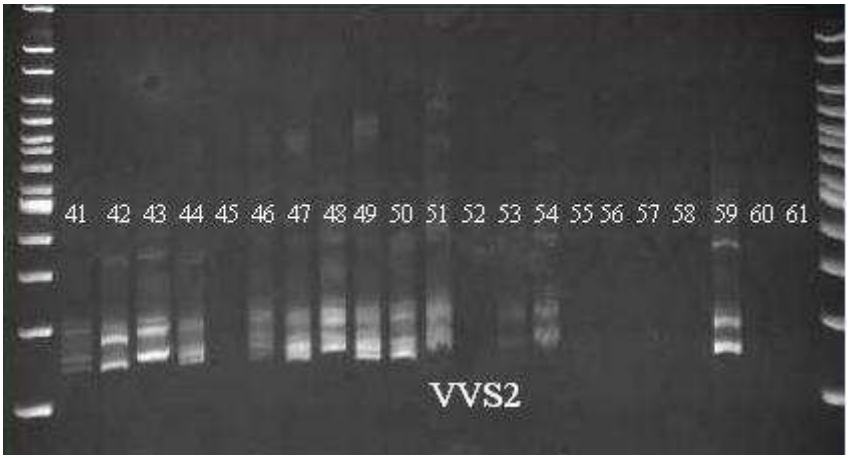
VVS2

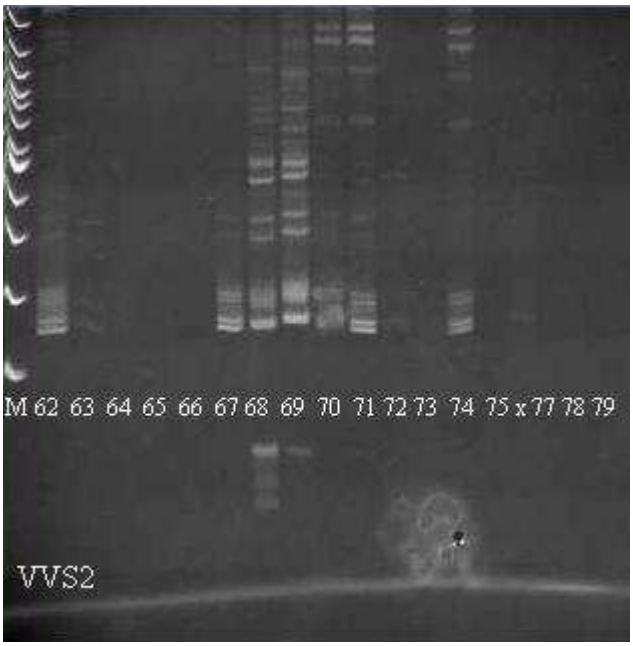

M 1 2 3 4 5 6 7 8 M
VVS2

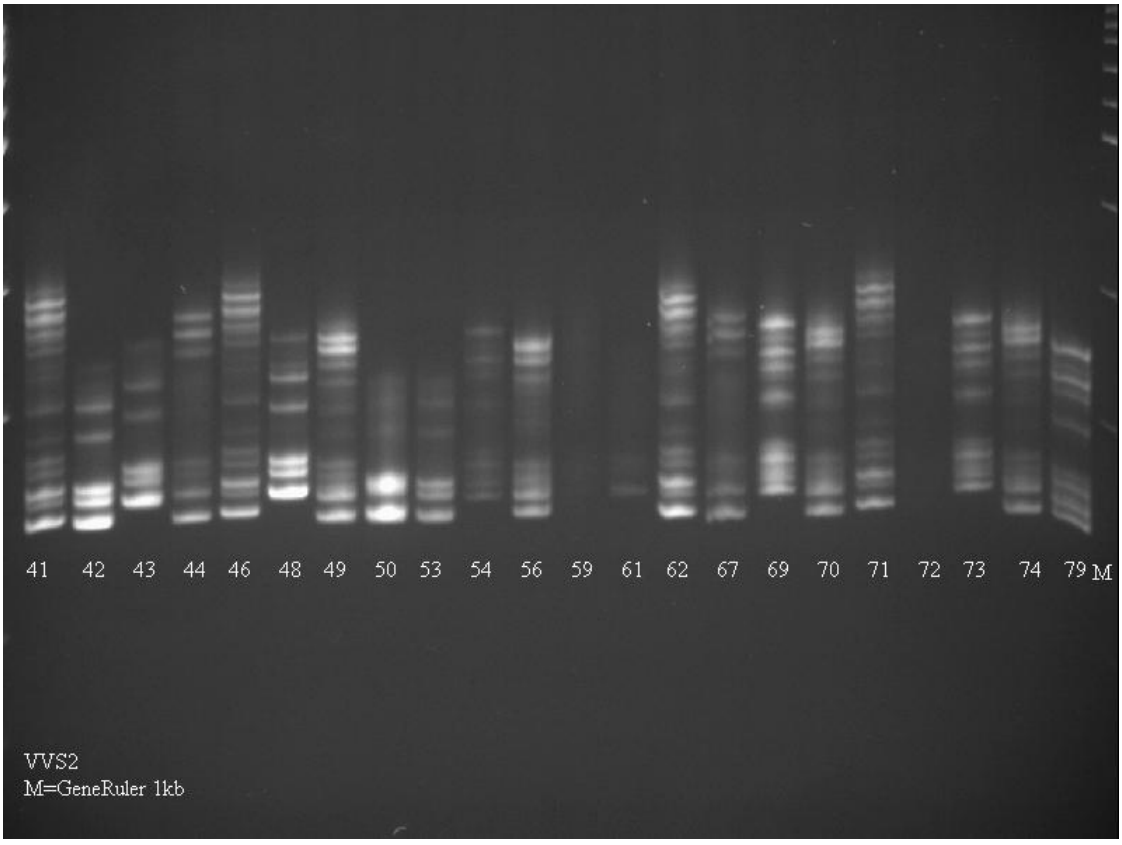

M 9 10 11 12 13 14 15 16 M
VVS2

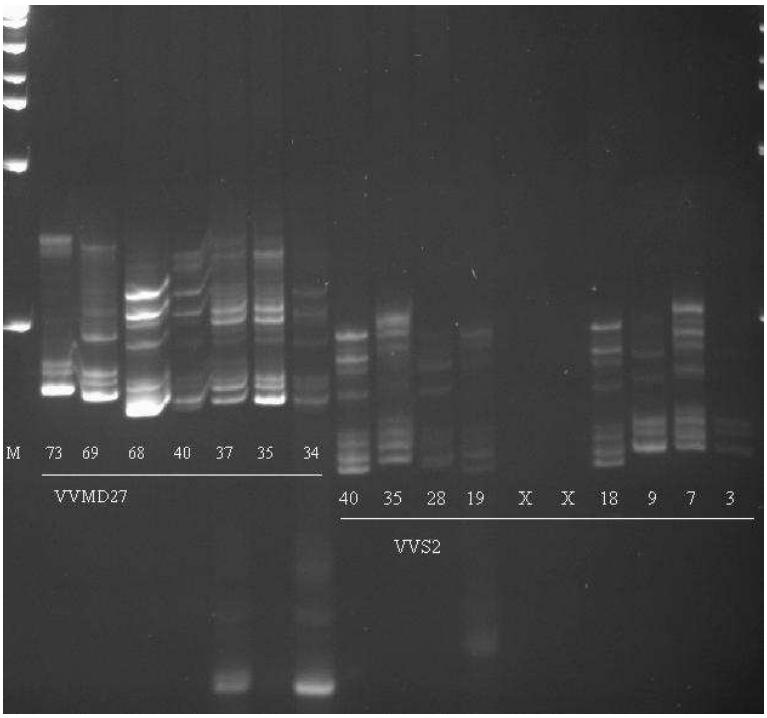

M 17 18 19 20 21 22 23 24 M
VVS2


M 25 26 27 28 29 30 31 32 M
VVS2

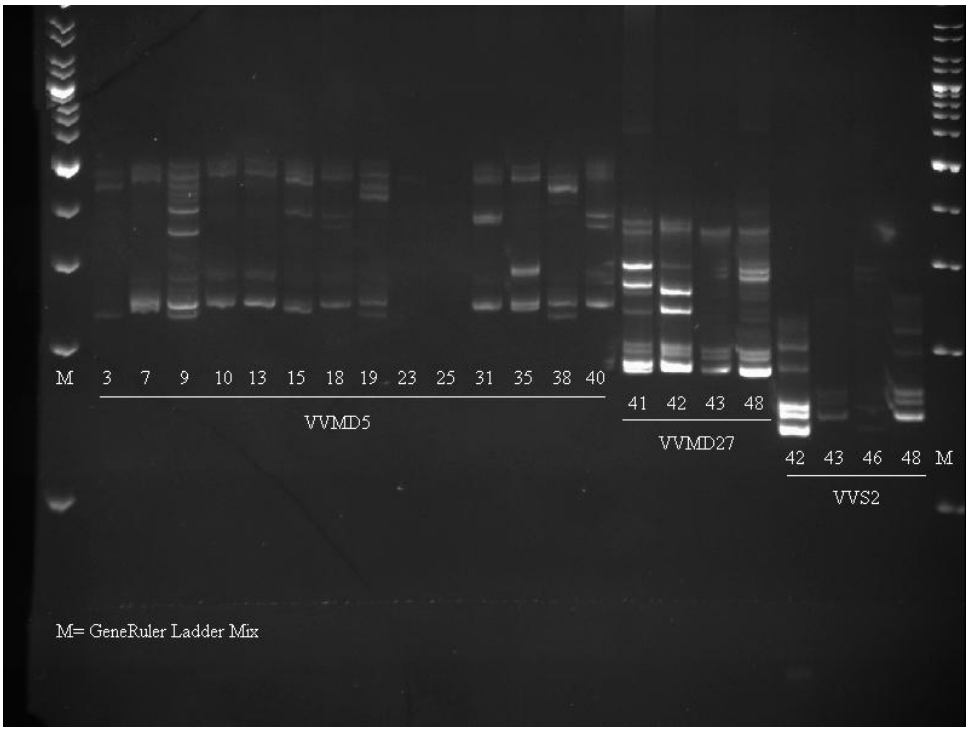

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61
VVS2

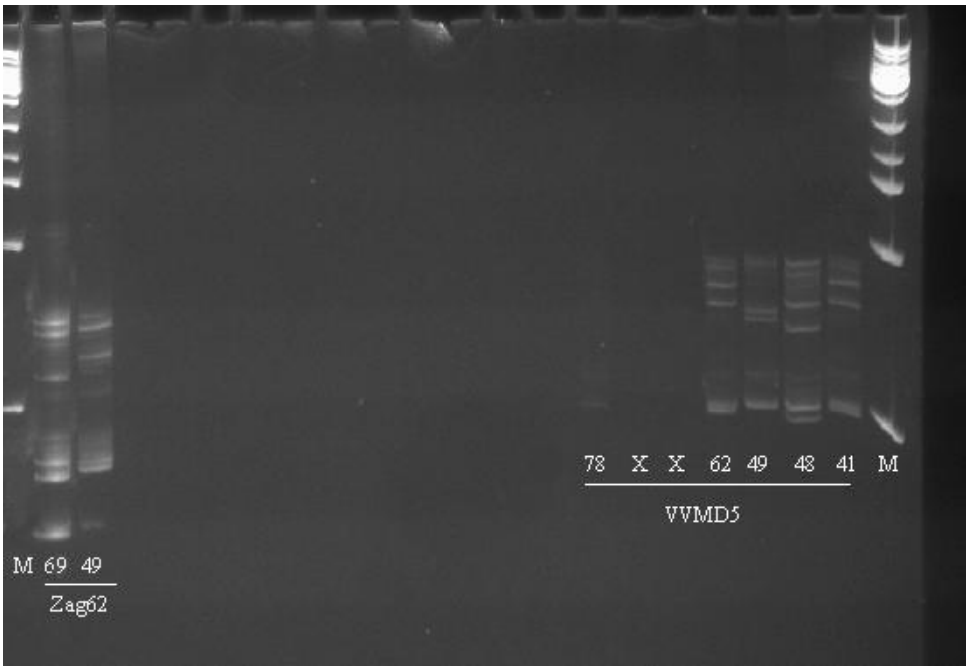

M 62 63 64 65 66 67 68 69 70 71 72 73 74 75 x 77 78 79
VVS2


41 42 43 44 46 48 49 50 53 54 56 59 61 62 67 69 70 71 72 73 74 79 M
VVS2
M=GeneRuler 1kb

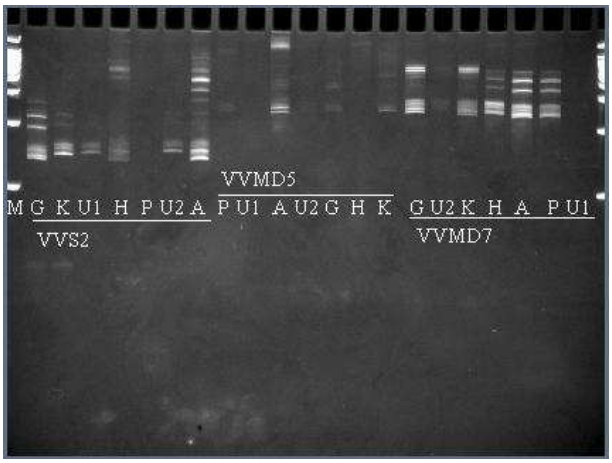

M 73 69 68 40 37 35 34
VVMD27
40 35 28 19 X X 18 9 7 3
VVS2

VVMD5


M 3 7 9 10 13 15 18 19 23 25 31 35 38 40
VVMD5
41 42 43 48
VVMD27
42 43 46 48 M
VVS2
M= GeneRuler Ladder Mix

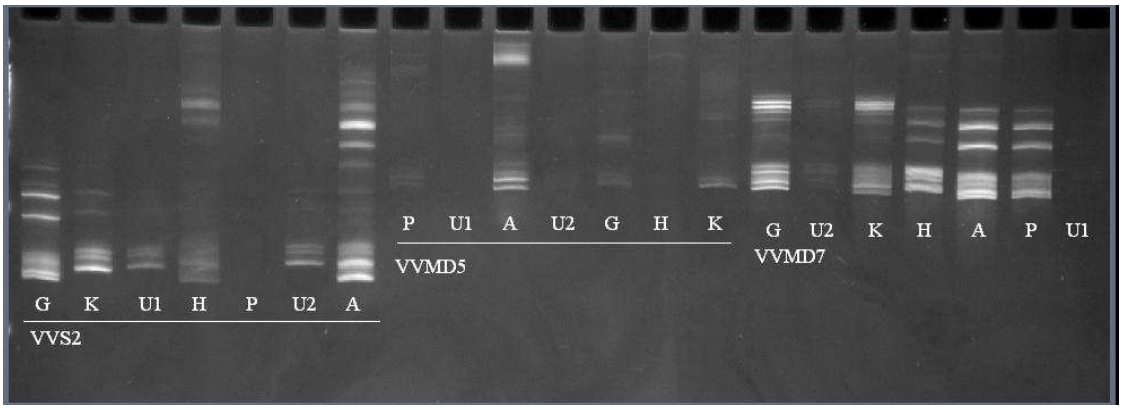


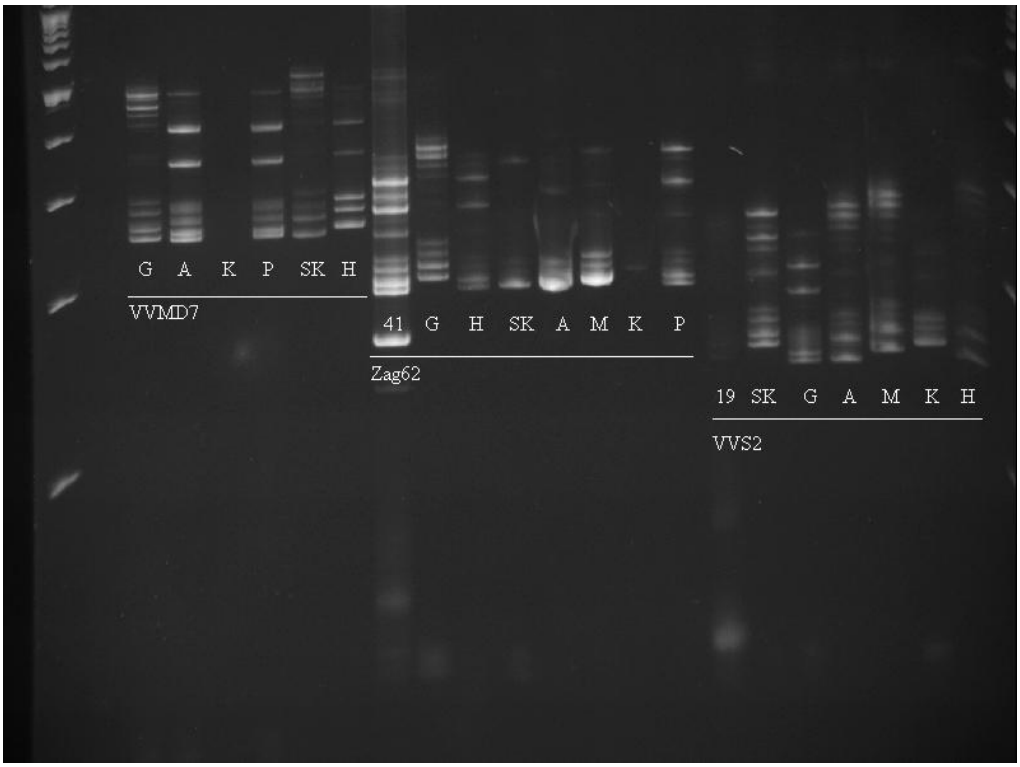
European accessions

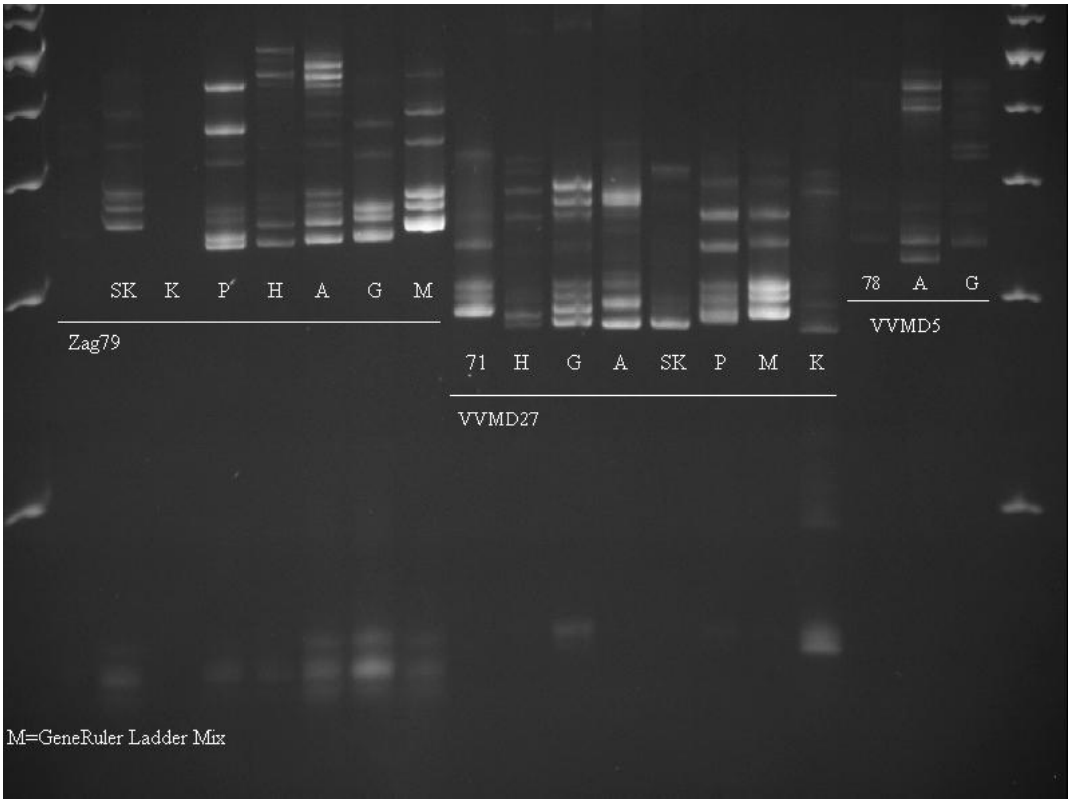

M G K U1 H P U2 A P U1 A U2 G H K G U2 K H A P U1
VVMD5
VVS2
VVMD7

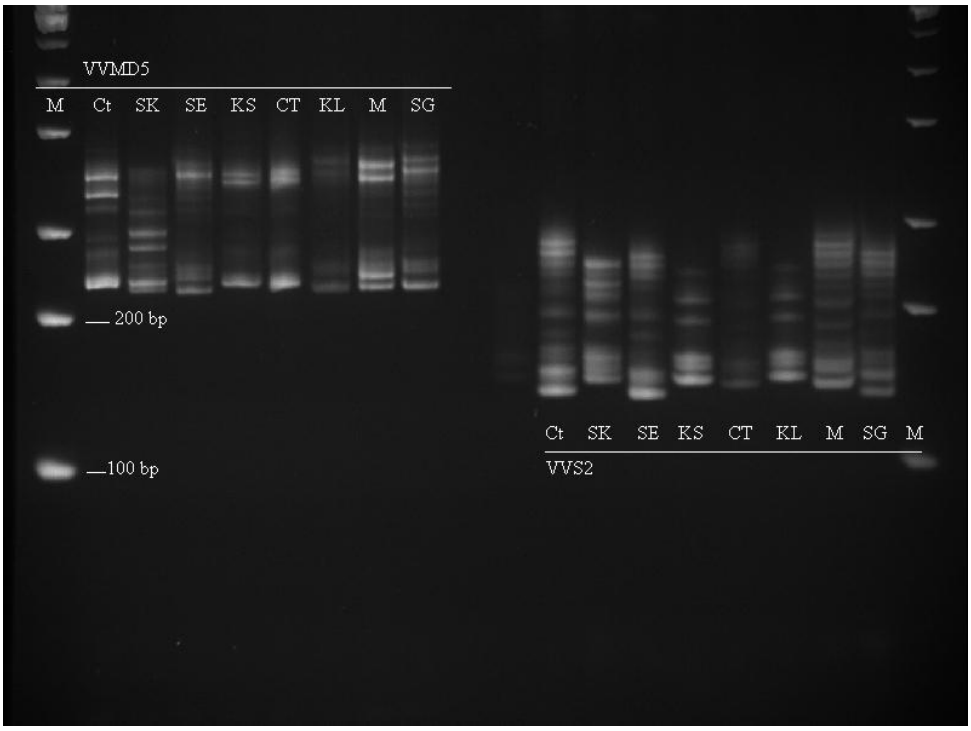

M SK S K SG S M K SK S SG S SK M S K SG S
VVMD5
VVS2
VVMD7

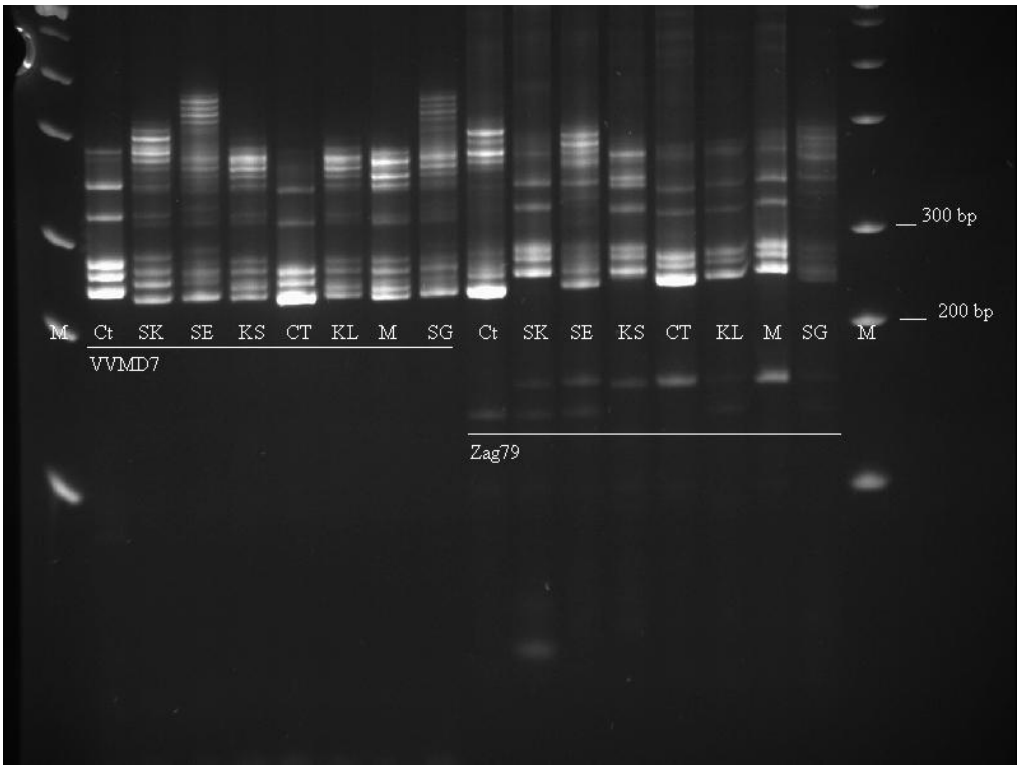

S K SK M SG S S K M SK SG S M S SK K SG S
VVMD27
Zag62
Zag79

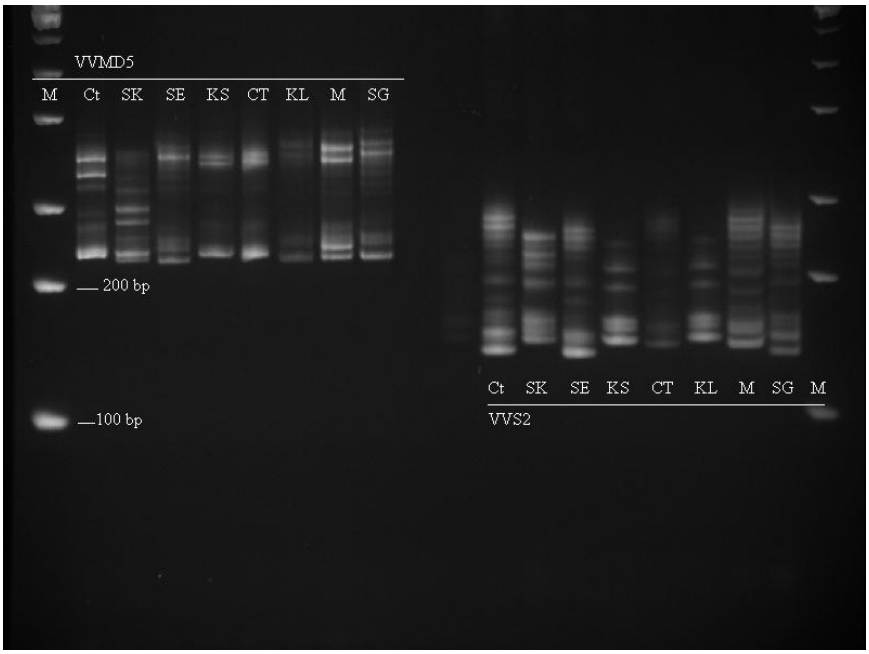

G K U1 H P U2 A
VVS2
P U1 A U2 G H K
VVMD5
G U2 K H A P U1
VVMD7


G A K P SK H
VVMD7
41 G H SK A M K P
Zag62
19 SK G A M K H
VVS2


SK K P H A G M
Zag79
71 H G A SK P M K
VVMD27
78 A G
VVMD5
M=GeneRuler Ladder Mix


VVMD5
M Ct SK SE KS CT KL M SG
200 bp
100 bp
Ct SK SE KS CT KL M SG M
VVS2


300 bp
200 bp
M Ct SK SE KS CT KL M SG Ct SK SE KS CT KL M SG M
VVMD7
Zag79



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