Journal of Agricultural Science; Vol. 8, No. 3; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Genotyping and Phenotyping of the Potential Clones, Biotypes and Variants of Grapevine Cultivar Korinthiaki Staphis (*Vitis vinifera* L.)

Maritina Stavrakaki[1] & Katerina Biniari[1]

[1] Laboratory of Viticulture, Faculty of Crop Science, Agricultural University of Athens, Athens, Greece

Correspondence: Katerina Biniari, Laboratory of Viticulture, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece. Tel: 30-210-529-4632. E-mail: kbiniari@aua.gr; maritina@aua.gr

Received: November 20, 2015 Accepted: January 20, 2016 Online Published: February 15, 2016

doi:10.5539/jas.v8n3p127 URL: http://dx.doi.org/10.5539/jas.v8n3p127

Abstract

This study presents the results regarding the identification and discrimination of twenty seven possible clones of grapevine cultivar Korinthiaki staphis (*Vitis vinifera* L.), three biotypes of Korinthiaki lefki and the related cultivar Staphidampelo using the ampelographic description and the molecular method AFLP. The results from the statistical analysis showed that all the biotypes of cultivar Korinthiaki staphis show small distance and are grouped in the same cluster, depending on their origin, while Staphidampelo and Korinthiaki lefki are neither variants nor biotypes of the cultivar Korinthiaki staphis but different cultivars since they are very distant compared to the other biotypes and moreover, Korinthiaki lefki is in a separate cluster of the dendrogram. The ampelographic description in combination with the molecular method AFLP are effective for the study of the between and within genetic diversity of grapevine cultivars as well as for their identification and discrimination. The results of this study can constitute the base for the implementation of the clonal selection for grapevine cultivar Korinthiaki staphis and the seclusion of the desired clones.

Keywords: AFLP, ampelography, genotyping, grapevine, Korinthiaki staphis, *Vitis vinifera* L.

1. Introduction

The study of the between and within genetic diversity of grapevine cultivars as well as their discrimination and identification are very difficult due to the large number of grapevine cultivars, biotypes and synonyms. It has been estimated that there are more than 8,000 grape cultivars, under 24 000 different names (Viala & Vermorel, 1909). In Greece more than 300 grapevine cultivars (*Vitis vinifera* L.) are grown and classic ampelographic (Krimbas, 1943; Davidis, 1967; Vlachos, 1986; Stavrakakis, 2010), biochemical (Stavrakakis & Loukas, 1983) and molecular methods (Stavrakakis at al., 1997; Stavrakakis & Biniari, 1998; Stavrakaki, 2008) have been used for their discrimination and classification. Among the various polymerase chain reaction (PCR)-based DNA marker techniques available, the Amplified Fragment Length Polymorphism (AFLP) is often used, because it is ideal when the goal is the definition of identity among different clones of the same variety or among genetically close related cultivars, with positive results in differentiating grapevine cultivars and clones (Vignani et al., 2002; Blaich et al., 2007; Stenkamp et al., 2009; Alba et al., 2011).

Korinthiaki staphis, one of the most important Greek grapevine cultivars, is considered as the oldest cultivar of the Greek vineyard and maybe its first cultivation goes back to Greek antiquity. The viticultural areas of Corinth and Egio constituted the first cultivation centers of the variety, from where it was transferred initially (in 1516) to Zante and later (in 1560) to Kefallinia (Logothetis, 1975). Today, in Greece, it is estimated that Korinthiaki staphis is cultivated in approximately 15.000 ha (ranking first in the classification of Greek grape varieties) while its production exceeds 20.000 tons of raisins.

Its long cultivation contributed to the creation of many synonyms, types, variants and mutations. In Greek (Krimbas, 1943, 1944, 1949; Davidis, 1982; Vlachos, 1986; Stavrakakis, 2010 etc.) and International ampelography (Guillon, 1895; Molon, 1906; Viala & Vermorel, 1909; Robinson et al., 2012 etc.) many synonyms are reported, such as Staphidampelos, Korinthiaki staphis, Korinthiaki, Lianorrogi, Black staphis (Greece), Corinthe Noir, Raisin de Corinthe (France), Corinto nero, Passerina nera, Passula di Corinto, Alga Passera (Italy), Black Corinth, Zante Currants, Currant Grape (UK, Australia, USA), Corinto Negro (Spain) etc., while there are also color variations and types, such as Korinthiaki lefki (White Corinth, Passerina bianca, Corinto bianco), Korinthiaki rodini (Passerina rosa), Korinthiaki sxistofilli etc.

As far as its origin is concerned, the ampelographic characters classify Korinthiaki staphis as well as Korinthiaki lefki to *proles pontica*, to which most Greek grapevine varieties belong and to *sub-proles balcanica* (Negrul, 1938, 1946; Levadoux, 1956). This point of view is verified by the results of a study using molecular markers (Aradhya et al. 2003). Because of the exclusive cultivation of Korinthiaki staphis in Greece for many centuries, it has been suggested that it is an indigenous cultivar of the Greek habitat and that it has originated from a local wild grapevine population (Logothetis, 1970). This view, of the existence of one or more secondary genetic centers of grapevine varieties, is enhanced by recent research studies (Grassi et al., 2003; De Andrés et al., 2012). However, there are not sufficient data neither on the time of creation and apparition of Korinthiaki staphis nor on how it was created, if it is a product of natural crossing or the result of mutations.

It is also supported that Greek grapevine cultivar Liatiko constitutes the parent cultivar from which Korinthiaki staphis derived either through mutation (Krimbas, 1930) or through crossing with another cultivar, with grapevine cultivar Staphidampelo being a potential parent cultivar (Myles et al., 2011). In a recent study, biotypes of cultivar Liatiko from Crete and biotypes of cultivar Korinthiaki staphis from Peloponnese where studied with the use of RAPD markers (Stavrakaki & Biniari, 2014). The results showed a low degree of genetic similarity (I = 0.774 – 0.791) and therefore, Liatiko and Korinthiaki staphis are different grapevine cultivars and it seems that Korinthiaki staphis did not derive from Liatiko through mutations.

During the search and selection of the biotypes of Korinthiaki staphis to be studied, there were vines bearing bunches with seeded berries on one arm and normal bunches with seedless berries on the other. Also in the same bunch, there were secondary branches with large seeded berries or with smaller seedless berries. These vines are referenced in the present study as ‘Metallagmeni’ (which means mutant in Greek).

The aim of this study was to identify and to discriminate twenty seven possible clones of Korinthiaki staphis (including two biotypes of Metallagmeni, which is characterized as mentioned by seeded and seedless bunches, and four biotypes of Sxistofilli, which is characterized by different leaves from the typical cultivar), the related cultivar Staphidampelo and three biotypes of Korinthiaki lefki (which is characterized by white skin color in berries) and to determine their phenotypic and genetic similarities using the ampelographic description and the molecular method of AFLP analysis.

2. Materials and Methods

2.1 Grapevine Material

Twenty seven possible clones of Greek grapevine cultivar Korinthiaki staphis, three biotypes of Korinthiaki lefki and Staphidampelo were chosen for identification (Table 1). Leaf material of these vines was obtained from the experimental vineyard in Korakohori, Ilia, where all the possible clones and biotypes from the various cultivation centers of the country were collected and planted by the Laboratory of Viticulture of the Agricultural University of Athens.

Table 1. Studied samples, as obtained from the experimental vineyard in Korakohori, Ilia

N	Abbreviation	Sample Name	Cultivation area
1.	C1	Tsekleni 1	Corinthia-Pyrgos
2.	C2	Tsekleni 2	Corinthia-Pyrgos
3.	C3	Egio 1	Egio
4.	C4	Egio 2	Egio
5.	C5	Egio 3	Egio
6.	C6	Pyrgos 1	Pyrgos
7.	C7	Pyrgos 2	Pyrgos
8.	C8	Pyrgos 3	Pyrgos
9.	C9	Korinthiaki lefki 1	Zante
10.	C10	Korinthiaki lefki 2	Zante
11.	C11	Korinthiaki lefki 3	Egio
12.	C12	Zante ntopio 1	Zante
13.	C13	Zante ntopio 2	Zante
14.	C14	Zante ntopio 3	Zante
15.	C15	Zante proimo 1	Zante
16.	C16	Zante proimo 2	Zante
17.	C17	Zante proimo 3	Zante
18.	C18	Kefallinia 1	Kefallinia
19.	C19	Kefallinia 2	Kefallinia
20.	C20	Kefallinia 3	Kefallinia
21.	C21	Corinth 1	Corinthia
22.	C22	Staphidampelo	Corinthia
23.	C23	Corinth 2	Corinthia
24.	C24	Kefallinia 4	Kefallinia
25.	C25	Kefallinia 5	Kefallinia
26.	C26	Metallagmeni 1	Egio
27.	C27	Metallagmeni 2	Egio
28.	C28	Sxistofilli 1	Egio
29.	C29	Sxistofilli 2	Egio
30.	C30	Sxistofilli 3	Pyrgos
31.	C31	Sxistofilli 4	Pyrgos

2.2 Ampelographic and Molecular Methods

For the ampelographic description, 66 ampelographic characters were used and measured on each grapevine biotype during the years 2011, 2012, 2013 following a list of descriptors developed by the International Organization of Vine and Wine (OIV, 2009) including the preliminary minimal traits relative to shoot, mature leave, bunch etc. (Table 2).

The ampelographic description took place for three consecutive years in the experimental vineyard in Korakohori, Ilia. More specifically, five biotypes from the region of Corinthia (C1, C2, C21, C22, C23), three biotypes from the region of Egio (C3, C4, C5), three biotypes from the region of Pyrgos (C6, C7, C8), six biotypes from the region of Zante (C12, C13, C14, C15, C16, C17), five biotypes from the region of Kefallinia (C18, C19, C20, C24, C25), three biotypes of Korinthiaki lefki (C9, C10, C11), two biotypes of Metallagmeni (C26, C27) and four biotypes of Sxistofilli (C28, C29, C30, C31) were studied.

Table 2. Ampelographic characteristics, based on the OIV descriptors list (OIV, 2009)

Code	Ampelographic characteristic	Notes	Code	Ampelographic characteristic	Notes
001	Young shoot: opening of the shoot tip	1: closed, 3: half open, 5: fully open	011	Shoot: density of erect hairs on nodes	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
002	Young shoot: distribution of anthocyanin coloration on prostrate hairs of the shoot tip	1: absent, 3: piping, 5: overall	012	Shoot: density of erect hairs on internodes	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	013	Shoot: density of prostrate hairs on nodes	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
004	Young shoot: density of prostrate hairs on tip	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	014	Shoot: density of prostrate hairs on internodes	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
005	Young shoot: density of erect hairs on tip	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	015-1	Shoot: area of the anthocyanin coloration on bud scales	1: absent, 2: basal, 3: up to ¾ of bud scale, 4: almost on the whole bud scale
006	Shoot: attitude (before tying)	1: erect, 3: semi-erect, 5: horizontal, 7: semi-drooping, 9: drooping	015-2	Shoot: intensity of anthocyanin coloration on bud scales	1: none or very weak 3: weak, 5: medium, 7: strong, 9: very strong
007	Shoot: color of dorsal side of internodes	1: green, 2: green and red, 3: red	016	Shoot: number of consecutive tendrils	1: 2 or less, 2: 3 or more
008	Shoot: color of ventral side of internodes	1: green, 2: green and red, 3: red	051	Young leaf: color of the upper side of blade (4th leaf)	1: green, 2: yellow, 3: bronze, 4: copper-reddish
009	Shoot: color of dorsal side of nodes	1: green, 2: green and red, 3: red	053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
010	Shoot: color of ventral side of nodes	1: green, 2: green and red, 3: red	054	Young leaf: density of erect hairs between main veins on lower side of blade (4th leaf)	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
055	Young leaf: density of prostrate hairs on main veins on lower side of blade (4th leaf)	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	076	Mature leaf: shape of teeth	1: both sides concave, 2: both sides straight, 3: both sides convex, 5: one side concave, one side convex, 5: mixture between notes 2 & 3
056	Young leaf: density of erect hairs on main veins on lower side of blade (4th leaf)	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	077	Mature leaf: size of teeth in relation to blade size	1: very small, 3: small, 5: medium, 7: large, 9: very large
065	Mature leaf: size of blade	1: very small, 3: small, 5: medium, 7: large, 9: very large	078	Mature leaf: length of teeth compared with their width	1: very short, 3: short, 5: medium, 7: long, 9: very long
067	Mature leaf: shape of blade	1: cordate, 2: wedge-shaped, 3: pentagonal, 4: circular, 5: kidney-shaped	079	Mature leaf: degree of opening / overlapping of petiole sinus	1: very wide open, 3: open, 5: closed, 7: overlapped, 9: strongly overlapped
068	Mature leaf: number of lobes	1: one (entire leaf), 2: three, 3: five: 4: seven, 5: more than seven	080	Mature leaf: shape of base of petiole sinus	1: U-shaped, 2: brace-shaped, 3: V-shaped
069	Mature leaf: color of the upper side of blade	3: pale green, 5: medium green, 7: dark green	081-1	Mature leaf: teeth in the petiole sinus	1: none, 9: present
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	1: absent, 2: only at the petiolar point, 3: up to the 1st bifurcation, 4: up to the 2nd bifurcation, 5: beyond the 2nd bifurcation	081-2	Mature leaf: petiole sinus base limited by veins	1: not limited, 2: on one side, 3: on both sides

071	Mature leaf: area of anthocyanin coloration of main veins on lower side of blade	1: absent, 2: only at the petiolar point, 3: up to the 1st bifurcation, 4: up to the 2nd bifurcation, 5: beyond the 2nd bifurcation	082	Mature leaf: degree of opening / overlapping of upper lateral sinus	1: open, 2: closed, 3: slightly overlapped, 4: strongly overlapped, 5: absence of sinus
072	Mature leaf: goffering of blade	1: absent or very weak 3: weak, 5: medium, 7: strong, 9: very strong	083-1	Mature leaf: shape of base of upper lateral sinuses	1: U-shaped, 2: brace-shaped, 3: V-shaped
073	Mature leaf: undulation of blade between main and lateral veins	1: absent, 9: present	083-2	Mature leaf: teeth in the upper lateral sinuses	1: none, 9: present
074	Mature leaf: profile of blade in cross section	1: flat, 2: V-shaped, 3: involute, 4: revolute, 5: twisted	084	Mature leaf: density of prostrate hairs between the main veins on lower side of blade	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
075	Mature leaf: blistering of upper side of blade	1: absent or very weak 3: weak, 5: medium, 7: strong, 9: very strong	085	Mature leaf: density of erect hairs between the main veins on lower side of blade	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
086	Mature leaf: density of prostrate hairs on main veins on lower side of blade	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	207	Bunch: lignification of peduncle	1: at the base only, 5: up to about the middle, 7: more than the middle
087	Mature leaf: density of erect hairs on main veins on lower side of blade	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	208	Bunch: shape	1: cylindrical, 2: conical, 3: funnel shaped
088	Mature leaf: prostrate hairs on main veins on upper side of blade	1: absent, 9: present	209	Bunch: number of wings of the primary bunch	1: absent, 2: 1-2 wings, 3: 3-4 wings, 4: 5-6 wings, 5: more than 6 wings
089	Mature leaf: erect hairs on main veins on upper side of blade	1: absent, 9: present	222	Berry: uniformity of size	1: not uniform, 2: uniform
090	Mature leaf: density of prostrate hairs on petiole	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	223	Berry: shape	1:obloid, 2: globose, 3: broad ellipsoid, 4: narrow ellipsoid, 5: cylindric, 6: obtuse ovoid, 7: ovoid, 8: obovoid, 9: horn shaped, 10: finger shaped
091	Mature leaf: density of erect hairs on petiole	1: none or very low, 3: low, 5: medium, 7: high, 9: very high	225	Berry: color of skin	1:green yellow, 2:rose, 3: red, 4: grey, 5: dark red violet, 6: blue black
093	Mature leaf: length of petiole compared to length of middle vein	1: much shorter, 3: slightly shorter, 5: equal, 7: slightly longer, 9: much longer	226	Berry: uniformity of skin color	1: not uniform, 2: uniform
094	Mature leaf: depth of upper lateral sinuses	1: very shallow, 3: shallow, 5: medium, 7: deep, 9: very deep	227	Berry: bloom	1: none or very low, 3: low, 5: medium, 7: high, 9: very high
151	Flower: sexual organs	1: fully developed stamens and no gynoecium, 2: fully developed stamens and reduced gynoecium, 3: fully developed stamens and fully developed gynoecium, 4: reflexed stamens and fully developed gynoecium	229	Berry: hilum	1: little visible, 2: visible
204	Bunch: density	1: very loose, 3: loose, 5: medium, 7: dense, 9: very dense	241	Berry: formation of seeds	1:none, 2: rudimentary, 3: complete
206	Bunch: length of peduncle of primary bunch	1: very short, 3: short, 5: medium, 7: long, 9: very long	244	Berry: transversal ridges on dorsal side of seeds	1: absent, 9: present

For the AFLP molecular analysis, a total of five primer combinations with three selective nucleotides and fluorescent dye (in the form of EcoRI[Primer-Axx-Dye] and MseI[Primer-Cxx]) were used to amplify genomic

DNA through the Polymerase Chain Reaction in order to identify and discriminate the selected potential clones and biotypes (Table 3).

Table 3. Primers combination used for AFLP analysis

Primer Code EcoRI-Axx (Dye)	**Primer Code MseI-Cxx**
EcoRI – ACT (FAM)	MseI – CAT
EcoRI – ACC (NED)	MseI – CAG
EcoRI – AGG (JOE)	MseI – CAC
EcoRI – AAG (JOE)	MseI – CAC
EcoRI – AGG (JOE)	MseI – CAT

2.3 DNA Extraction

Grapevine DNA was extracted from young and fully expanded leaves according to Thomas et al. (1993) with minor modifications (Biniari, 2000). One g of leaves from individual vines were frozen in liquid nitrogen and ground to a fine powder, thawed and resuspended in 12.5 mL of buffer A [0.25 M NaCl, 0.2 M TRIS-Cl (pH 8.0), 50 mM EDTA, 0.1 v/v 2-mercaptoethanol, 2.5% w/v polyvinyl-pyrrolidone (MW 40.000)]. A crude nuclei pellet was obtained by centrifugation at 7 000 rpm for 10 min at 4 °C. The pellet was resuspended in 2.5 mL of extraction buffer B [0.5 M NaCl, 0.2 M TRIS-Cl (pH 8.0), 50 mM EDTA, 1% v/v 2-mercaptoethanol, 2.5% w/v polyvinyl-pyrrolidone, 3% sarkosyl, 20% ethanol] and incubated at 37 °C for 45 min. An equal volume of chloroform/isoamyl alcohol (24:1) was then mixed in and the phases were separated by centrifugation at 14 000 rpm for 15 min. The aqueous layer was collected and 0.54 volume of frozen isopropanol (-20 °C) was added to precipitate the DNA. The DNA was fished and resuspended in 300 μL TE (10 mM Tris - HCl, pH 7.4, 1 mM EDTA) containing 15 μg mL^{-1} RNase A and incubated for 15 min at 37 °C. Protein was removed by the addition of a half volume of 7.5 M ammonium acetate, followed by centrifugation and the DNA in the supernatant was precipitated with a 0.25 of cold isopropanol; ca. 120 μg DNA per g FW was obtained.

2.4 Amplification Conditions

AFLP analysis was conducted as reported by Vos et al. (1995), following the AFLP Plant Mapping Protocol by Applied Biosystems (2007), with several modifications.

For the Restriction – Ligation stage, genomic DNA (500 ng) was incubated for 14-16 h (overnight) at 20 °C with the presence of 3 U (units) of enzyme EcoRI (5'..GAATTC..3') and 1 U of enzyme MseI (5'..TTAA..3'). In each sample there were 4 U T4 DNA Ligase, 1μL of EcoRI adaptor and 1 μL MseI adaptor, T4 DNA Ligase buffer, NaCl and BSA, in a final volume of 11 μL. After the incubation, 189 μL of TE buffer were added in each sample, and the products were stored at -20 °C. All pre-amplification and amplification reactions were performed in a Perkin Elmer DNA Thermal Cycler 9600.

For the Preselective Amplification (Preselective PCR), 4 μL of the diluted products of the restriction-ligation stage were used. For the reaction, 15 μL of AFLP Core Mix (Applied Biosystems, USA) and 1.0 μL of AFLP preselective primer pairs (Applied Biosystems, USA) were added in a final volume of 20 μL. The preselective PCR thermal conditions were: 2 min at 72 °C, 20 cycles of 22 s at 94 °C, 33 s at 56 °C and 2 min at 72 °C, with a final step of 30 min at 60 °C. The samples were then stored at 4 °C. After the end of the amplification, 10 μL of the pre-amplified products were checked on 1.5% (w/v) agarose gels, in TAE buffer (40 mM Tris-acetate and 1mM EDTA, pH 8), and stained in ethidium bromide (1 μg mL^{-1}). The gels were photographed on a Gel Doc 1000 (Biorad). The remaining 10 μL of the pre-amplified products were diluted with 190 μL of TE buffer and stored at 4 °C.

For the Selective Amplification (Selective PCR), 1.5 μL of the diluted preselective products were used. For the reaction, 7.5 μL of AFLP Core Mix (Applied Biosystems, USA) and 0.5 μL of each selective primer (Applied Biosystems. USA) were added in a final volume of 10 μL (as mentioned, the primers were in the form of EcoRI[Primer-Axx-Dye] and MseI[Primer-Cxx]) (Table 3). Selective EcoRI primers were labeled with fluorescent dyes to enable detection using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). The selective PCR thermal conditions were: 2 min at 94 °C, 10 cycles of 20 s at 94 °C, 30 s at 66 °C (the annealing temperature was reduced in every cycle by 1 °C) and 2 min at 72 °C, 20 additional cycles of 20 s at 94 °C, 30 s

at 56 °C and 2 min at 72 °C, with a final step of 30 min at 60 °C. The samples were then stored at 4 °C.

For the loading buffer, 13 μL of deionized formamide with 0.5 μL of GeneScan-500 (LIZ) size standard (Applied Biosystems, USA) were added to 1.0 μL of each amplification sample. The samples were first denaturated at 94 °C for 5 min and then separated by capillary electrophoresis on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). AFLP electrophoregrams were acquired and analysed using the GeneMapper v4.0 software (Applied Biosystems, USA), using the Local Southern Method.

2.5 Statistical Analysis

For the statistical analysis, the method UPGMA was used with one distance coefficient and one similarity coefficient. In order to present the morphological relationships between the cultivars, the Euclidean Distances Squared coefficient was used, as implemented in the NTSYS-pc package 2.1 developed by Rohlf (Exeter Software, New York, USA, 1993). The bigger the value of the coefficient for 2 samples, the bigger the distance between them. For the molecular analysis, the degree of genetic similarity (I) detected between each pair of cultivar studied was calculated using the DICE coefficient (Nei & Li, 1979) as implemented in the NTSYS-pc package 2.1.

Relationships among the OIV descriptors (parameters) were studied using the statistical program Jump 8.0 (SAS Institute Inc). Principal Component (PC) analysis was used to evaluate the most important parameters that contributed to the biotype separation into different groups according to their morphological traits (OIV descriptors).

3. Results and Discussion

3.1 OIV Ampelographic Descriptor Evaluation

According to the PC analysis, which transforms the original data set (OIV descriptors) into a smaller set of uncorrelated new variables (Principal Components, where eigenvalues was bigger than 1), 10 components have been produced in a decline series of their importance, explaining 89.72% of the total variability among the different biotypes. All descriptors that are grouped in the same principal component have strong correlation between them. Each component is strongly correlated with a set of the initial OIV descriptors so it could be estimated their contribution to variability. The OIV descriptors strongly correlated with the first 10 components are presented in Table 4 and Figure 1. For example, the OIV descriptors 004 (young shoot: density of prostrate hair on tip), 013 (shoot: density of prostrate hair on nodes), 051 (young leaf: color of the upper side of blade (4th leaf)), 083-1 (mature leaf: shape of base of upper lateral sinuses) contributed better to variability compared to OIV descriptors 068 (mature leaf: number of lobes) or 090 (mature leaf: density of prostrate hairs on petiole).

Table 4. Evaluation of the OIV descriptors and their contribution to the variability of the biotypes studied

Principal Components									
1	2	3	4	5	6	7	8	9	10
% Contribution to variability									
29.12	11.43	9.81	9.62	7.83	6.49	5.03	4.08	3.27	3.05
Eigenvalue									
11.35	4.45	3.82	3.75	3.05	2.52	1.96	1.59	1.28	1.19
Related OIV descriptors									
004	074	015-2	084	093	094	007	227	209	068
013	070	055	086	079	075	204	223	208	090
051	080	015-1	065		082			014	
083-1	071	069	088						
009	076	010	067						
003									

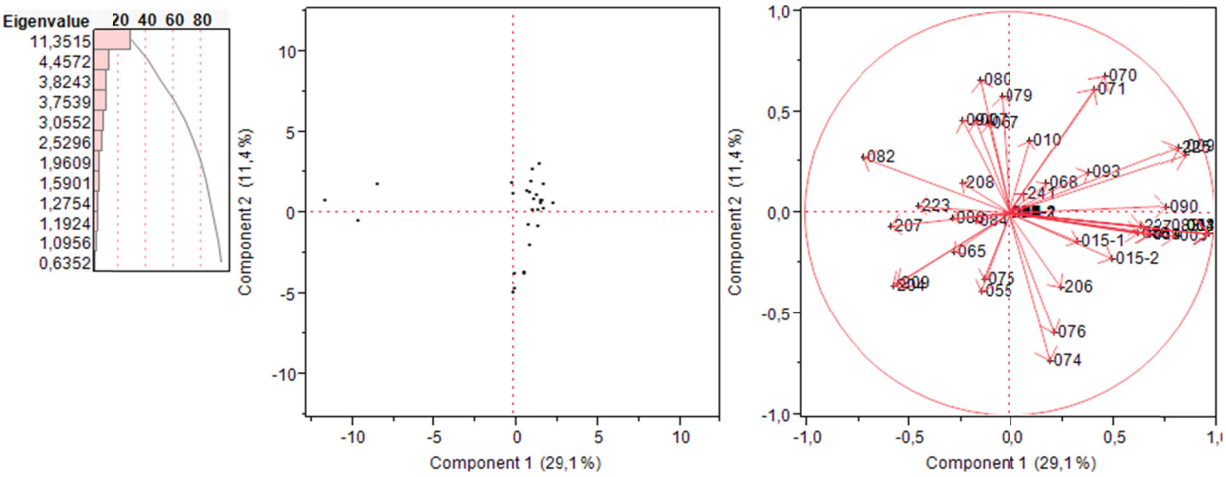

Figure 1. Evaluation of the OIV descriptors and their contribution to the variability of the biotypes studied

It was observed that most biotypes have high density of prostrate hair on tip (young shoot), while biotypes C9, C10, C11 had less prostrate hair. For the same biotypes, absence of prostrate hair on nodes was observed whereas the other biotypes had some prostrate hair. Also, the color of the upper side of blade of the 4th leaf of the young shoot is green-yellow for biotypes C9, C10, C11, whereas for the other biotypes, the color is copper-reddish. Moreover, shape of base of upper lateral sinuses of the mature leaf for biotypes C9, C10, C11 is U-shaped whereas for the other biotypes is V-shaped.

Cluster analysis separated the varieties in particular groups according to their morphological characteristics: the data from the ampelographic description with the 66 ampelographic descriptors of the biotypes studied (Appendix A) were used to create a distance matrix in order to generate a dendrogram (Figure 2), which showed the formation of two main clusters, discriminating all of the samples studied.

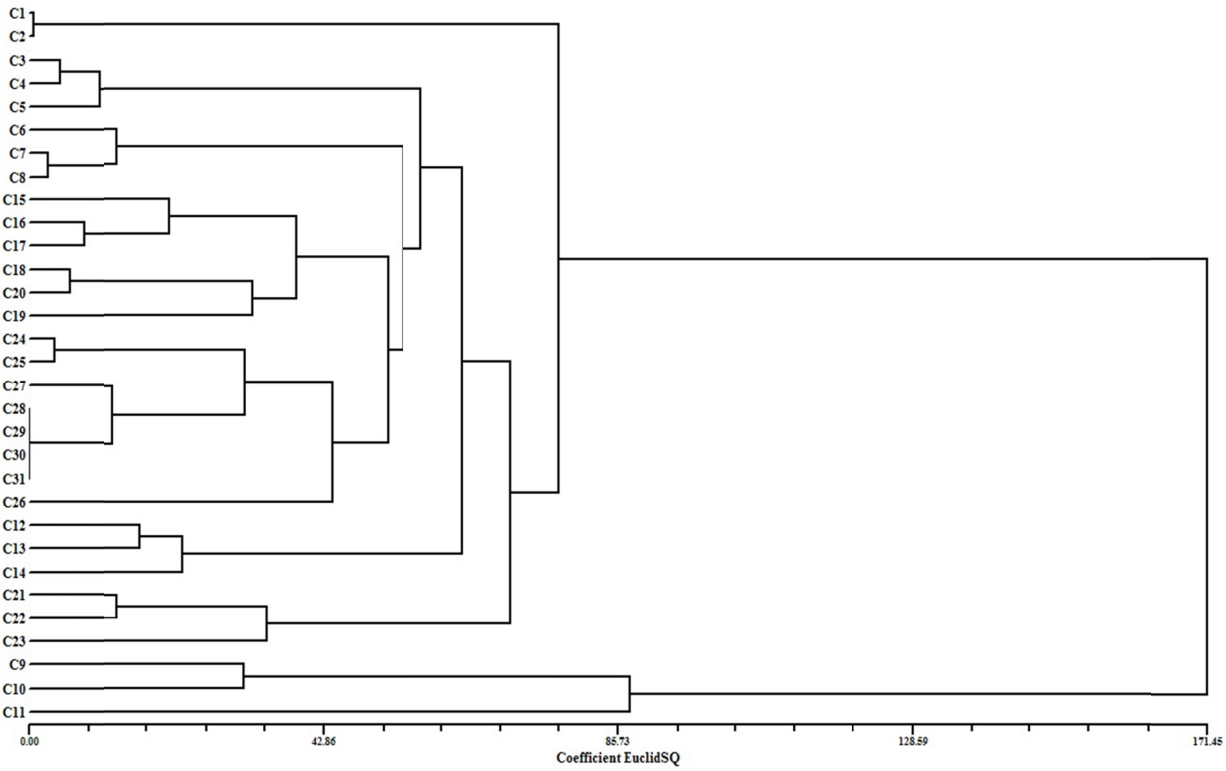

Figure 2. Dendrogram based on ampleographic descriptors showing the relationship among samples studied

In general, and with a few exceptions, the biotypes of Korinthiaki staphis of the same cultivation center show small to very small distance between them, while between biotypes of different cultivation centers, the distance is bigger. The biotypes originating from the cultivation center of Egio show the smallest distances between them but also between most of the biotypes studied, and especially with those of Kefallinia. Also, their distance with biotypes C28, C29, C30, C31 (Sxistofilli) is relatively small.

Moreover, no significant differences were observed between the biotypes C26 and C27. Lastly, the biotypes of Sxistofilli (C28, C29, C30, C31) show identity between them, while relatively small is their distance with the biotypes from Egio. The ampelographic differences between these biotypes are found on the descriptors of the leaves and particularly on the number of lobes, on the bunch size and on the color of the berry skin.

The biotypes of Korinthiaki staphis and Staphidampelo are different cultivars and they are found on a totally different cluster of the dendrogram compared to the biotypes of Korinthiaki lefki. Therefore, and despite the similarities in specific ampelographic descriptors and in the nature of seedlessness, Korinthiaki lefki constitutes a different cultivar and not a color mutation of Korinthiaki staphis.

From the analysis of the above results, (Table 3, Figure 1), it is shown that there is phenotypic fluctuation between the three biotypes of Korinthiaki Lefki studied. In particular, the biotypes coming from Zante (C9, C10) do not differ significantly between them but they show a relatively high distance compared to the biotype coming from Egio (C11).

3.2 Molecular Analysis

For the molecular analysis and the identification of the biotypes and cultivars studied, five primer combinations were used to amplify genomic DNA from the twenty seven possible clones of Greek grapevine cultivar Korinthiaki staphis, cultivar Staphidampelo and the three biotypes of Korinthiaki lefki. They proved to be highly polymorphic and produced a total of more than 210 amplified fragments (Table 5), discriminating all of the samples studied. The molecular analysis data were then used to obtain a genetic similarity dendrogram (Figure 3). Since each similarity and distance coefficient has a different scale, there may be difference in their degree.

Table 5. Primers used and number of amplified fragments

Primer Code **EcoRI – Axx – Dye – MseI – Cxx**	**Number of Amplified Fragments**
EcoRI – ACT FAM – MseI – CAT	34
EcoRI – ACC NED – MseI – CAG	26
EcoRI – AGG JOE – MseI – CAC	33
EcoRI – AAG JOE – MseI – CAC	65
EcoRI – AGG JOE – MseI – CAT	60
Total	**218**

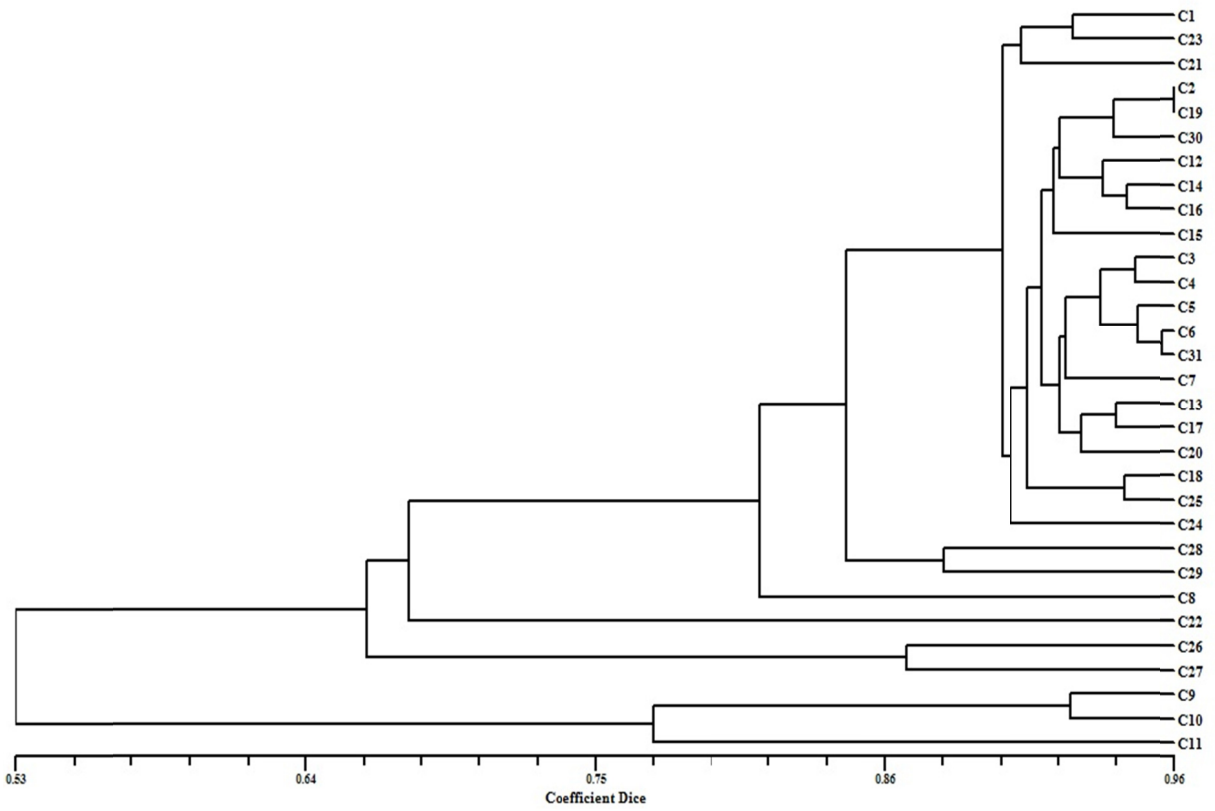

Figure 3. Dendrogram based on AFLP amplification products showing the relationship among samples studied

The biotypes of Korinthiaki staphis showed high degree of genetic similarity between them. Especially biotypes from Egio (C3, C4, C5) show a high degree of genetic similarity between them but also between the biotypes from Ilia (C6, C7), Corinth (C21, C23), Zante (C12, C13, C14) and Kefallinia (C19, C20, C24). In contrast, there is genetic diversity within the biotypes of Sxistofilli and in a smaller degree within the biotypes of Metallagmeni.

The molecular data with the AFLP analysis show that there is genetic variation within the group of biotypes regardless of the cultivation center and this supports the hypothesis that their differences (morphological, biological and physiological) can be attributed more to human selection (mass selection) than to geographical isolation (This et al., 2006). For example, biotypes C1, C2 constitute a product of mass selection with criterion the productivity and the bunch size.

Also, very low degree of genetic similarity was detected between all biotypes of Korinthiaki Staphis, the biotypes of Korinthiaki lefki and the cultivar Staphidampelo (I = 0.670 – 0.757) confirming the results of the ampelographic description. Besides, grapevine cultivar Staphidampelo is described as a different cultivar by Krimbas (1943).

The results also show genetic variation within the biotypes of Korinthiaki lefki. The biotypes C9, C10 show very high degree of genetic similarity (I = 0.945) and they possibly originate from the same parent cultivar through the accumulation of mutations. On the contrary, the biotype C11 significantly differs from C9, C10, so it can be concluded that it is a different cultivar. A similar study but with the use of SSR markers investigated the genetic relationship between the cultivars Korinthiaki staphis (Black Corinth), Korinthiaki lefki (Corinth blanc) and Corinto bianco (Vargas et al., 2007). As mentioned in this study, the samples of the first two cultivars originated from Greece and the samples of the third cultivar from Italy. The results of this study showed that the three cultivars are different with the note that cultivar Corinto bianco originates from the cultivar Pedro Ximenes, through mutation (Vargas et al., 2007).

4. Conclusions

The present study verifies the indispensable role of the ampelographic description and the great effectiveness of the combination of Ampelography and AFLP analysis when it comes to studying the polyclonality of grapevine

cultivars, like Korinthiaki staphis.

The ampelographic description, especially when it takes place for several years and when a great number of descriptors is used, in combination with molecular methods can be extremely effective for the study of the between and within genetic diversity of grapevine cultivars and they can constitute reliable tools for the discrimination of grapevine cultivars, clones and biotypes.

The above mentioned results, aside from the scientific and research interest, can have an immediate implementation in the viticultural practice and in the implementation of programs of clonal selection for the emergence of the most valuable clones of the various biotypes of Korinthiaki staphis.

Acknowledgements

The authors would like to thank the members of S.K.O.S.A.S.E. for their kind help and contribution and for providing the rural area in order for the experimental vineyard to be established as well as for its maintenance.

References

Alba, V., Anaclerio, A., Gasparro, M., Caputo, A. R., Montemurro, C., Blanco, A., & Antonacci, D. (2011). Ampelographic and molecular characterization of Aglianico Accessions (*Vitis vinifera* L.) collected in Southern Italy. *South African Journal of Enology & Viticulture, 32*(2), 164-173.

Applied Biosystems. (2007). *AFLP Plant Mapping Protocol*. USA.

Aradhya, M. K., Dangl, G. S., Prins, B. H., Boursiquot, J. M., Walker, M. A., Meredith, C. P., & Simon, C. J. (2003). Genetic structure and differentiation in cultivated grape *Vitis vinifera* L. *Genetics Research Cambridge, 81*, 179-192. http://dx.doi.org/10.1017/S0016672303006177

Biniari, K. (2000). *Identification and authentication of grapevine cultivars using molecular methods* (Unpublished doctoral dissertation). Agricultural University of Athens, Athens, Greece.

Blaich, R., Konradi, J., Ruhl, E., & Fomeck, A. (2007). Assessing genetic variation among Pinot noir (*Vitis vinifera* L.) clones with AFLP markers. *American Journal of Enology & Viticulture, 58*(4), 526-529.

Davidis, X. O. (1967). *An Introduction to Ampelography*. Privately printed, Athens, Greece.

Davidis, X. O. (1982). *Greek Ampelology* (Vol. III). Elements of Ampelography, Athens, Greece.

De Andrés, M. T., Benito, A., Pérez-Rivera, G., Ocete, R., Lopez, M. A., Gaforio, L., … Arroyo-García, R. (2012). Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. *Molecular Ecology, 21*(4), 800-816. http://dx.doi.org/10.1111/j.1365-294X.2011.05395.x

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. *Theoretical and Applied Genetics, 107*, 1315-1320. http://dx.doi.org/10.1007/s00122-003-1321-1

Guillon, J. M. (1895). *Les cépages orientaux*. G. Carre. Paris.

Krimbas, V. (1930). The origin of Staphidampelos. *Proceeding of the Academy of Athens, 5*, 23.

Krimbas, V. (1943). *Greek Ampelography* (Vol. I). Ministry of Agriculture, Athens, Greece.

Krimbas, V. (1944). *Greek Ampelography* (Vol. II). Ministry of Agriculture, Athens, Greece.

Krimbas, V. (1949). *Greek Ampelography* (Vol. III). Ministry of Agriculture, Athens, Greece.

Levadoux, L. (1956). Les populations sauvages et cultivées de *Vitis vinifera* L. *Annales de l' Amélioration des Plantes, 6*, 59-118.

Logothetis, B. X. (1970). *The development of the vine and of viticulture in Greece, based on archeological findings in the area*. Aristotle University of Thessaloniki, Thessaloniki, Greece.

Logothetis, B. X. (1975). Contributions de la vigne et du vin à la civilisation de la Grèce et de la Méditerranée orientale. *Annuaire de la Faculté Agronomique de l'Université de Thessaloniki, 17*, 1-286.

Meneghetti, S., Costacurta, A., Morreale, G., & Calò, A. (2011). Study of intra-varietal genetic variability in grapevine cultivars by PCR-derived molecular markers and correlations with the geographic origins. *Molecular Biotechnology, 50*(1), 72-85, http://dx.doi.org/10.1007/s12033-011-9403-9

Molon, G. (1906). *Ampelografia* (Vol. II). Ulrico Hoepli, Milano.

Myles, S., Boyko, A. R., Owens, C. L., Brown, P. J., Grassi, F., Aradhya, M. K., … Buckler, E. S. (2011).

Genetic structure and domestication history of the grape. *Proceedings of the National Academy of Sciences, USA, 108*(9), 3530-3535. Retrieved from http://www.pnas.org/cgi/doi/10.1073/pnas.1009363108

Negrul, A. M. (1938). Evolution of cultivated forms of grapes. *Comptes Rendus de l'Académie des Sciences*, *USSR, 18*, 585-588.

Negrul, A. M. (1946). Origin of the cultivated grapevine and its classification. In A. Baranoc, Y. F. Kai, M. A. Lazarevski, A. M. Negrul, T. V. Palibin & N. N. Prosmoserdov (Eds.), *Ampelographia of USSR* (Vol. I, pp. 159-216). Moscow, Russia.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences, USA, 76*, 5273-5296. http://dx.doi.org/10.1073/pnas.76.10.5269

OIV. (2009). *OIV Descriptor List for grape varieties and Vitis species* (2nd ed.). Organisation Internationale de la Vigne et du Vin. Retrieved from http://www.oiv.int/oiv/info/enplubicationoiv

Robinson, J., Harding, J., & Voulliamoz, J. (2012). A complete guide to 1.368 vine varieties, including their origins and flavours. *Wine grapes*. Penguin books Ltd. London.

Rohlf, F. J. (1993). *NTSYS-pc. Numerical taxonomy and multivariate analysis system* (Version 1.80). Exeter Software, Setauket, New York.

Stavrakaki, M. (2008). *Genetic study of grapevine varieties (Vitis vinifera L.) using molecular markers RAPDs and SSRs* (Unpublished master's thesis). Agricultural University of Athens, Athens, Greece.

Stavrakaki, M., & Biniari, K. (2014). *Is grapevine cultivar Korinthiki staphis a mutation of grapevine cultivar Liatiko?* Paper presented at the 15th Panhellenic Conference of the Hellenic Scientific Society for Genetics and Plant Breeding, Larissa, October 15-17, Greece.

Stavrakakis, M. N. (2010). Ampelography. *Tropi Publications* (in Greek).

Stavrakakis, M. N., & Biniari, K. (1998). Genetic study of grape cultivars belonging to the muscat family by random amplified polymorphic DNA markers. *Vitis, 37*(3), 119-122.

Stavrakakis, M. N., & Loukas, M. (1983). The between and within grape cultivars genetic variation. *Scientia Horticulturae, 19*, 321-334. http://dx.doi.org/10.1016/0304-4238(83)90080-8

Stavrakakis, M. N., Biniari, K., & Hatzopoylos, P. (1997). Identification and discrimination of eight Greek grape cultivars (*Vitis vinifera* L.) by random amplified polymorphic DNA markers. *Vitis, 36*, 175-178.

Stenkamp, S. H. G., Becker, M. S., Hill, B. H. E., Blaich, R., & Fomeck, A. (2009). Clonal variation and stability assay of chimeric Pinot Meunier (*Vitis vinifera* L.) and descending sports. *Euphytica, 165*, 197-209. http://dx.doi.org/10.1007/s10681-008-9807-1

This, P., Lacombe, T., & Thomas, M. R. (2006). Historical origins and genetic diversity of wine grapes. *Trends in Genetics, 22*(9), 511-519. http://dx.doi.org/10.1016/j.tig.2006.07.008

Thomas, M. R., Matsumoto, S., Cain, P., & Scott, N. S. (1993). Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. *Theoretical & Applied Genetics, 86*, 173-180. http://dx.doi.org/10.1007/bf00222076

Vargas, A. M., Vélez, M. D., de Andrés, M. T., Laucou, V., Lacombe, T., Boursiquot, J. M., … Ibáñez, J. (2007). Corinto bianco: a seedless mutant of Pedro Ximenes. *American Journal of Enology & Viticulture, 58*(4), 540-543.

Viala, P., & Vermorel, V. (1909). *Traité Général d'Ampélographie* (Vol. 7). Maison éd., Paris.

Vignani, R., Scali, M., & Masi, E. (2002). Genomic variability in *Vitis vinifera* L. "Sangiovese" assessed by microsatellite and non-radioactive AFLP test. *Electronic Journal of Biotechnology, 5*(1), 1-11. http://dx.doi.org/10.2225/vol5-issue1-fulltext-2

Vlachos, M. (1986). Ampelography. *Thessaloniki* (in Greek).

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Home, M., … Zabeau, M. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research, 23*, 4407-4414. http://dx.doi.org/10.1093/nar/23.21.4407

Appendix

Appendix A. Ampelographic characterization of the biotypes studied, based on the 66 OIV descriptors used (OIV descriptors list, 2009)

OIV	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31
001	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
002	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
003	2-3	2-3	2-3	2-3	2-3	2	2	1	1-3	1-3	1-3	5	5	5	5	5	5	5	5	5	3-5	3-5	5-7	5	5	5	5	5	5	5	5
004	7	7	7	7	7	7	7	7	5-7	5-7	5-7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
005	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
006	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
007	2-3	2-3	2-3	2-3	2-3	2	2	2	2	2-3	3	3	3	3	2	2	2	2	2	2	2-3	2-3	2-3	2-3	2-3	2-3	2-3	2-3	2-3	2-3	2-3
008	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
009	2	2	2-3	2-3	2-3	3	3	3	1	1	1	2-3	2-3	2-3	2	2	2	2-3	2-3	2-3	3	3	2-3	2	2	2-3	2-3	2-3	2-3	2-3	2-3
010	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2-3	-3	1	1	1	1	1	1	1	1	1
011	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
012	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
013	3	3	3	3	3	3	3	3	1	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
014	3	3	3	3	3	3	3	3	1-3	1-3	1-3	3	3	3	3	3	3	3	3	3	3	3	3-5	1-3	1-3	3	3	3	3	3	3
015-1	2	2	3	3	3	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	2	3	2	3	3	2-3	2-3	2-3	2-3	2-3	2-3
015-2	5	5	5	5	5	3	3	3	3	3	3	5	5	5	5	5	5	3-5	3-5	3-5	3-5	3-5	3-5	5	5	5	5	5	5	5	5
016	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
051	4	4	4	4	4	4	4	4	1-2	1-2	1-2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
053	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
054	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
055	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5-7	5-7	5-7	7	7	7	7	7	7	7	7
056	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
065	7	7	5	5	5	5	5-7	5	5-7	5-7	5-7	5-7	5-7	5-7	5	5	5	5	5-7	5	5	5	5	5	5	5	5-7	5-7	5-7	5-7	5-7
067	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2-3	2-3	2-3	2-3
068	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4
069	7	7	7	7	7	5	5	5	3	3	3	7	7	7	5	5	5	7	7	7	5	5	5	5	5	7	7	7	7	7	7
070	1-2	1	2	2	3	3	3	3	1	2	1	2	2	3	1	1	1	2	2	2	3	3	3	2	2	2	2	2	2	2	2
071	1-2	1-2	1-2	1-2	1-2	3	2	2	1	2	1	2	2	2	1	1	1	2	1	2	2	2	2	2	2	2	2	2	2	2	2
072	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
073	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
074	3	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
075	3	3	1-3	1-3	1-3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

OIV	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31
076	5	5	5	5	5	5	5	5	3	3	3	3	3	3	5	5	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3
077	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
078	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
079	3	3	5	5	5	3-5	5-7	5-7	5	5	5	5	3	5	5	3	3	5	5	5	3-5	5	3-5	5-7	5-7	5	5	5	5	5	5
080	2	2	3	3	3	3	3	3	3	3	3	3	3	3	1	1	1	2	2	2	2	2	2	3	1	3	3	3	3	3	3
081-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
081-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
082	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
083-1	3	3	3	3	3	3	3	3	1	1	1	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3
083-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
084	5	5	3	3	3	5	5	5	5	5	5	5	5	5	5	5	5	5	3-5	5	5	5	5	3-5	3-5	5	5	5	5	5	5
085	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
086	5	5	3	3	3	3	3	3	5	5	5	5	5	5	5	5	5	3-5	3-5	3-5	5	5	5	5	5	5	5	5	5	5	5
087	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
088	9	9	1	1	1	9	9	9	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
089	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
090	3	3	3	3	3	3-5	3	3	1	1	1	3	3	3	3	3	3	3	3	3	3	3	3	5	5	3	3	3	3	3	3
091	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
093	3	3	3-5	3-5	3-5	5	5	5	3	3	3	7	7	7	5	5	5	5	5	5	5	5	5	3	3	3-5	3-5	3-5	3-5	3-5	3-5
094	1-3	1-3	3	3	3	3	3-5	3-5	3-5	3-5	3-5	5-7	5-7	5-7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
151	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
204	7	7	5	5	5	5	5	5	7	5-7	7	7-9	5-7	5	5-7	5-7	5	5-7	5	5-7	5	5	5	5	5	5	5	5	5	5	5
206	5-7	5-7	1-3	3	1-3	5	5	5	1-3	1-3	3	1-3	3	5	3	3	3	3	3-5	1-3	3	3	3	3	3	3	3	3	3	3	3
207	1	1	1	1	5	1	1	1	5	1	7	1-5	5	1	5	1-5	1	1	1	1-5	1	1-5	1	1	1	1	1	1	1	1	1
208	2	2	1-2	1	2	2	2	2	1	3	3	2	1-3	1	3	1	1	3	3	1-3	2	3	2	2	2	2	2	2	2	2	2
209	3	3	3	2	2	2	2	2	4	3	3	2	3	2	3	2	2	2-3	3-4	3	2	2	3	2	2	2	2	2	2	2	2
222	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
223	2	2	3	3	2	2	2	2	1	2	7	3	2	2	2	3	2	2-3	1-2	3	2-3	2	2	2	2	2	2	2	2	2	2
225	2-3	2-3	5	5	5	5	5	5	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6
226	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
227	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
229	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
241	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
244	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Appendix B. Electrophoregrams of the amplified fragments using the AFLP method, as shown in the ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA)