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Genetic fingerprinting and potential grape quality of old *Vitis vinifera* genotypes

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1. CHAPTER: INTRODUCTION

1.1. Importance of identification, recovery and valorization of *Vitis* germplasm

Viticulture and enological industry have a relevant importance in Italy. In the year 2014, the national wine production was 41 million hl, that is, +3% than the average production of the last five years. Considering just wines, musts excluded, the Apulia is the third main producing Italian region (5.6 thousands hl), after Veneto and Emilia Romagna (http://www.inumeridelvino.it/2015/04/ produzione-di-vino-in-italia-aggiornamento-2014-stat.html). The Daunia area, that is, the Foggia province, in Northern Apulia, has a considerable relevance: with 27,000 ha devote to viticulture and more that 2 millions hl of wine, it is the main Apulian viticultural area in terms of surface and production. It is also very important at a national level, since it normally occupies the fifth position among all the Italian provinces that produce musts and wines.

Viticulture is one of the most ancient agricultural activities and has been practiced for thousands of years. The grapevine varieties presently grown derive from domestication, from crosses between cultivated varieties and from crosses between domesticated grapevines and cultivated varieties most of which now are disappeared. After many multiplications, any genotype is prone to produce and fix some genetic mutations, generating several "variants" of the initial genotype. Virgilio, in the *Georgiche* wrote that it was not possible to know how many grapevine varieties exist, since they are as numerous as the grains of sand raised by the Zephyrus wind in the Libya plain are. The grapevine names often derive from their morphology (e.g. "Pinot" comes from the pine-cone form of the bunch), behavior (e.g. "Primitivo" comes from the early grape maturation period), presumed site of origin (e.g. "Uva di Troia" was thought to come from the mythical site of the Asia Minor) etc. With the commercial exchanges many genotypes have been naturalized in places different from the original ones where they have been often were called with a different name/s. Hence, presently, many genotypes have several synonyms and homonyms that make difficult their cataloging and identification (Zohary and Hopf, 2000; Silvestroni and Virgili, 2005; Storchi and Lelli, 2005).

As summarized by Storchi and Lelli (2005), approximately 2000 grapevine cultivars are estimated to be grown in Italy in the past. This richness of variety lasted about until the XIX century. This huge number of varieties, and related wines, although useful for helping to preserve the biodiversity within the grapevine species, created a great confusion and degenerated often into poor quality products; for this reason, it was seen as an obstacle to the development of a modern oenological industry. At the beginning of the XX century, many technicians suggested to reduce the number of cultivated varieties, maintaining just the best ones. Unfortunately, over the years, the reduction of the cultivar number was dramatic and caused the loss of a significant part of the previous grapevine germplasm accumulated over the centuries. Presently just over 350 varieties are enrolled in the Italian Catalogue of Grapevine Varieties, and, according to the national statistics, just ten varieties occupy more than 44% of the Italian vineyard surface. This tendency involved all the Italian regions, Apulia included, leading to a certain leveling of the enological products, fact that has a negative effects on both the internal and the foreign wine markets.

Today there is an agreement on the opinion that the local varieties have a multiple importance within the Italian grapevine germplasm. This is due to their ability to determine typical sensory and hedonistic characteristics of wine, as well as to evocate historical and cultural values. For these reasons, the regional varieties attract the interest of producers, operators, consumers and researchers. In facts, in the recent years, either scientific Institutions or grapevine producers have focused their attention on the recovery of old grapevine germplasm, typical of any growing area, and on the valorization of these genotypes by evaluating their enological potential.

Generally speaking, the recovery and valorization of genetic resources typical of a specific growing area, maintaining the genotype diversity of cultivated crops, is fundamental to preserve the species genetic pool that certainly includes several under-utilized but potential useful characters, and presently it is thought as a strategy to promote the territorial identity and the diversification of the local food products. This approach contrasts the tendency that, in the past decades, privileged the growing of few very efficient genotypes, resulting in a diffuse genetic erosion (FAO, 2011). As

concerns grapevine, limiting the loss of varieties is thought as a strategy to defend the national wine production, given the strong competition of the world markets and the wide diffusion of "international" varieties, and their wines, common to all the viticultural Countries.

The local germplasm includes well-known "major" varieties, the so called "minor" varieties, and genotypes still unknown. The major varieties, represent a solid reference for the traditional and famous wine production, although new knowledge may derive from investigating them by using the newest technologies. As concerns the minor varieties, the knowledge about their true identity, morphology, viticultural performance oenological potential is still limited and fragmentary; as for the unknown genotypes, they are still not coded and characterized.

The Apulia region is one of the ancient grapevine-growing areas, thus, it should have a rich heritage of grapevine varieties. Nevertheless, this region is thought to have one less "specialized" variety assortment among the Italian regions (http://www.inumeridelvino.it/2013/11/puglia-principali-vitigni-aggiornamento-istat-2010.html).

In order to contribute at refraining the trend toward the impoverishment of the grapevine germplasm, the University of Foggia undertaken a surveys aimed at individuating old grapevine accessions in Apulia, with particular attention to the Daunia area (de Palma *et al*., 2011). Within this research, a dozen accessions were identified in the "Alto Tavoliere Dauno" and twenty in the "Gargano" promontory (de Palma *et al*., 2013).

Generally speaking, after the individuation of the old accessions it is necessary to ascertain if they are not already known with another name/names in other growing areas; if not, each of them may be indicated as an "unique genotypes".

1.2. Identification and characterization of the grapevine genotypes

The characterization and identification of grapevine varieties is obtained by "ampelographic" studies. The world "ampelography" comes from the Greek ampelos (grape) and grafo (to describe) and

literally means “description of grapevine”. Ampelographic studies combine several approaches, such as:

- ampelographic description, that utilizes morphological descriptors of grapevine organs in order to characterize their morphological traits at proper phenological stages;
- ampelometric measurements, that consist in measuring the main parameters of the vine organs such as leaves, bunches, berries, seeds, in order to obtain a “profile” to utilize for the variety identification;
- biochemical and biomolecular analysis, based on the study of some metabolite or of some DNA specific regions giving “objective” information for genotype characterization and identification.

Using more than one method allows the obtaining of most accurate and significant results.

The ampelographic study is a fast and quite inexpensive method for variety characterization, based on the observation of morphological descriptors of the phenotype. However, it is not absolutely probative since the morphological traits are influenced by several agro-environmental factors. Moreover, their assessment and interpretation is quite subjective, thus a reliable discriminations based only on vine morphology is often difficult to realize. This source of error frequently leads to mislabeling of individuals and rise of homonyms and/or synonyms for a same accession. Furthermore, genotypes related to each other may have similar traits, thus it is difficult to distinguish among them using only a morphological approach (Aradhya *et al*., 2003).

The ampelometric study may be also not expensive, but is time consuming, since it requires to take a huge number of measurements and elaborate mathematically the results. This is why, a specific informatics programs have been created. These programs allow also to obtain a graphic representation of the studied vine organs of any varieties. Leaves are the most studied organs since they are easy to take, store, analyze and compare. The mathematical elaboration of the basic measurements aimed to obtain some numerical indices that are not influenced by the agro-environmental factors. However, the discriminant power of this method is not very high.

The study of molecular DNA markers is expensive, but the interpretation of their results is more objective. Among them, Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polyphorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) are used. These markers, being direct expression of genotype, are not affected by the effect of environmental factors (soil, climate, methods of cultivation, diseases, etc.).

Presently, SSRs are the favorite type of DNA markers because their properties make them suitable for a wide range of applications, from phylogenetic, parentage testing and pedigree reconstruction, to cultivar discrimination and identification and management of germplasm collections (Thomas *et al*., 1993; Bowers *et al*., 1996; Thomas *et al*., 1998; Bowers *et al*., 1999; Sefc *et al*., 2000). SSRs (microsatellites) are arrays of short motifs that consist of 1 to 4 pairs of DNA bases. This locus-specific type of marker is characterized by abundance, hypervariability, high reproducibility, Mendelian inheritance and codominant nature. Microsatellite are suitable for discriminating between homozygosis and heterozygosis, and among closely related taxa (Scott *et al*., 2000; Meredith, 2001). Due to all these positive traits, SSRs are widely used for cultivar identification and genetic diversity studies on grapevine. The massive use of this type of marker since 1990s allowed to get a wide range of information as concerns also many "synonym and homonym ambiguities" that were still unknown or that had been only hypothesized by the ampelographers, but non demonstrated. However, it is known that SSRs are not useful to distinguish among clones of a same variety (Crespan, 2010).

About 300 microsatellites have been developed, mainly by the *Vitis* Microsatellite Consortium, and adopted at international level for grapevine genotyping. They are typically based on di-nucleotide repeats. Standardization and exchange of information concerning grapevine genetic resources using di-nucleotide repeats reference microsatellite markers were also proposed (This *et al*., 2004). However, in order to avoid allele miscalling, these markers require a very accurate and reliable protocols for allele separation and identification. Weeks and coll. (2002) reported that 83% of discrepancies between laboratories in scoring di-nucleotide alleles are due to arbitrary decisions in

binning, the process that converts raw allele lengths into allele classes and express the size as an integer.

In order to study the grapevine germplasm of the Italian regions, the *Fondazione in Rete per la Ricerca Agroalimentare-AGER* supported the national research project "An Italian *Vitis* database with multidisciplinary approach, for exploitation and valorization of the regional genotypes" (2011-2014). To study and characterize a wide range of the accessions individuated in all the Italian regions, a large set of characters were chosen among those coded by the *Organisation Internationale de la Vigne et du Vin* (OIV) to describe the grapevine varieties; moreover, the results of the European research projects that selected the most representative and discriminant descriptors were considered. The most important selected characters were the following: a) 48 morphological traits concerning shoot, young leaf, flower sexual organs, fertility, mature leaf, bunch and berry at maturity, all analyzed under the guidance of ampelographic record cards; b) 18 ampelometric traits, to measure and elaborate by means of a specific software (Superampelo 2.0); c) 9 vegetative and productive traits, to assess by visual inspection or by measuring or weighing; d) 14 microsatellite *loci*, that is, 9 *loci* selected by the European research projects GenRes081 and GrapeGen06 (VVS2, VVMD5, VVMD7, VVMD27, ZAG62, ZAG79, VVMD25, VVMD27), plus other 5 *loci* (VVMD6, VVMD17, VVMD21, VVMD24, VMC1b11) that are recognized as reliable and polymorphic ones.

1.3. The evaluation of grape oenological potential

It is recognized that the composition of grape at harvest is fundamental for determining the future quality of the wine (Carrara *et al*., 2008), hence the assessment of grape characteristics is a basic step requirement in any process aimed to evaluate the oenological potential of grapevine accessions. Chemical parameters that define the grape technological maturity (sugars and organic acids) (Liu *et al*., 2006; Munoz-Robredo *et al*., 2011), together with the grape phenolic compounds, are well-known as a key factor in the wine quality.

Sugars and organic acids are known to be the primary compounds involved in the taste and in the quality assessment of fleshy fruit, included grapes; due to their direct impact on the wine-making process, sugar, acidity and pH levels are often defined as grape "**technological parameters**".

It is well-known hat leaves are specialized organs for producing sugars that, after migration, accumulate in other organs included fruits. The main grape sugars are glucose (8% fresh weight) and fructose (7% f.w.); sucrose is mainly important for transportation (Tucker, 1993). In the berry juice, glucose and fructose account for at least 99% of carbohydrates (Kanellis and Roubelakis-Angelakis, 1993). In wine grapes, the total sugar concentration indicates the potential alcohol level after fermentation. As the grape ripening begins, after veraison, the sugar level increases rapidly reaching an appropriate state of ripeness for a district. Grapes characterized by a very high sugar concentration give wines having a high alcohol level, which may mask the effect of other quality components; it happens especially with some genotypes, but also in districts with warm summers and very low rainfalls (Jackson and Lombard, 1993). Sugar accumulation is considered as the main process of fruit maturation. Sugars are also known as the starting point for obtaining many other fundamental berry compounds, including phenols and aromas; some aromatic compounds are not accumulated in the berry until sugars o not overcome 15 degree. The sugar accumulation, under given growing and environmental conditions, changes according to the grape variety and their typical maturation period: in facts, it is normally easier for early ripening varieties, since the sugar accumulation escapes from unfavourable climatic conditions that may characterize the late season. Sugar accumulation changes from 1-2% in green berries to 16-25% in ripen berry (Fregoni, 2005; Boluin and Guimberteau, 2004).

The main berry acids are tartaric and malic acids, which account for at least 90% of the titratable acidity. Total acidity declines during the ripening, mainly due the acid utilization as respiratory substrate that involves especially the malic acid (Ulrich, 1970). In wine grapes, the acidity is an important quality factor since it influences the perception of the freshness and savouriness of the wine. Cultivars characterized by a higher tartrate/malate ratio give best wines. Nevertheless, a high tartaric content is often related to a low sugar content and gives low quality wines, while a low acidity

may be accompanied by both low or high sugar concentration: in any case, wine result "unbalanced" (Lavee and Nir, 1986; Kanellis and Roubelakis-Angelakis, 1993). As summarized by (Fregoni, 2005), the malic acid is quite easily oxidized during the grape maturation, since it is intensively respired at temperature about 30°C, while the tartaric acid is more stable, since its intense respiration occurs at about 37°C. In the must, the malic acid concentration rages from 3 to 7 g/L, while the tartaric acid concentration ranges from 7 to 10 g/L. The warm climate reduces juice total acidity. Genotypes characterized by "more" tartaric acid have a greater resistance to the lowering of total acidity during grape ripening. A satisfying malic acidity gives freshness to the wine; however, a too high malic acidity gives sour taste: high malic acidity is commonly related to non-well ripen grapes. Aridity is known to reduce the sum of malic and tartaric acids, while rains during grape maturation are known to increase it. Must acidity regulates must pH. The pH is a measure of the "true" free must acidity; it is very important for the evolution of malic-lactic fermentation during the winemaking process, for the final wine taste and color, for the wine biological stability, for the protein and metal stability. In the Potassium rich soils, such as those of Apulia, the root uptake of this element is high and, at cell level, increases the acid salification and hence the pH. As a result, pH tends to have higher values, such as in many growing areas of Southern Italy compared to the Northern ones.

Phenol compounds are considered as the main contributors for important wine organoleptic traits such as color, astringency, and bitterness; moreover, due to their antioxidant and free radical-scavenging proprieties (López-Vélez *et al*., 2003; Ladete, 2012), increasing interest is presently devoted to their support in health benefits.

Although wine phenolics may have origin from microbial and wood sources, most of them originate in grape. They include the nonflavonoid and flavonoid compounds. The former consist of stilbens and phenolic acids. As for grape stilbens, the resveratrol produced by berry skins is the most important compound. It is a powerful antioxidant considered very useful for human health. Its concentration varies according to the variety (red grapes are more provided) but also to the grape exposure to *Botrytis*, *Oidium and Plasmopara* attacks, since it is a product of reaction to biotic stresses;

nevertheless, UV radiation is also a resveratrol elicitor. Trans-resveratrol is the most represented form (maximum 20 µg/g) (Fregoni, 2005). Phenolic acids, present in berry skin and pulp, consist of hydroxybenzoic and hydroxycinnamic acids esterified with tartaric acid. Flavonoids include anthocyanins, flavan-3-ols and flavonols. Anthocyanins gives the color to the skin of the black-berry varieties (varieties having pink, red, purple-violet, blue or almost black berry skin). Flavan-3-ols (tannins) are present, in berry skins and seeds, as monomeric forms (catechins), small oligomeric forms or large proanthocyanidin polymers also known as condensed tannins. Flavonols (glycosides of quercetin, myricetine, campferol) are accumulated in the berry skin throughout the fruit development and protect it from dangerous effects of ultraviolet radiation; they are also very important at healthy level (Glories 1988; Downey *et al*., 2006).

In the white wines, hydroxycinnamic acids and, at a lesser extent, flavan-3-ol monomers, are the most important phenolics: they act on the visual attribute of these wines. In the red wines, anthocyanins and tannins, especially the proanthocyanidins, are the most important compounds. Tannins are responsible for the bitter and the astringent component of the mouthfeel of a wine; condensed tannins are the most involved in the astringency. Moreover, tannins are necessary for the co-pigmentation reaction that stabilize the anthocyanins and the color of the wine during aging (Timberlake and Bridle 1976; Kennedy, 2008). Anthocyanin-flavonol copigments are also formed: although they are less stable than the anthocyani-tannin ones, they may be also important for the wine color stability in the early stages of the winemaking (Boulton, 2001).

As summarized by Kennedy (2008) and by Downey and coll. (2006), the concentration of grape phenolics changes throughout berry development. From the fruit set to berry veraison, hydroxycinnamic acids and tannins, especially seed tannins, increase, when expressed on a berry weight basis. Most of skin and seed tannins are present as proanthocyanidins. Proanthocyanidin composition differs between seeds and skin: seeds have shorter polymers with a similar amounts of catechin and epicatechin units; skin proanthocyanidin polymers are generally longer and epicatechin units prevail in their composition. Moreover, tannins produced by skins seem generally more “ripen”

than those produced by seeds, thus, if it is desired a more developed wine, more skin tannins are needed. The level of extractable tannins decrease, in both seeds and skins, between veraison and harvest: from an evolutionary point of view, this decreasing corresponds to a reduction of the overall bitterness and astringency of the grape and is part of a "seed dispersal strategy", that involves also sugar accumulation in the juice and anthocyanin accumulation in the skin. In facts, form veraison to ripening, anthocyanins concentration increase in the skin and the berry color intensifies becoming more attractive; they decline late in berry development.

As summarized by Fregoni (2005), the hydroxycinnamic acid concentration ranges between 50 and 200 mg/kg of grape in the skin and between 20 and 170 mg/kg of grape in the pulp. Among varieties, these compounds vary about from 80 mg/kg (Claiette) to 367 mg/kg (Muscadet); for example, in grapes of Chardonnay, Pinot noire and Syrah were found about 189, 167 and 108 mg/kg of grape. As concerns flavonols, their concentration ranges between 10 and 100 mg/kg of grape. Anthocyanin concentration ranges from 500 to 3000 mg/kg of grapes; for example, they were found >1200 mg/kg in Cabernet Franc, >1500 in Merlot and Sangiovese and >1800 in Cabernet Sauvignon. At the beginning of their formation (veraison), only cyanidin and peonidin (glucosides of the dihydroxylated anthocyanins) accumulate, followed, during the time, by delphinidin, petunidin, and malvidin (trihydroxylated anthocyanins). Tannins (flavan-3-ols) concentration in the seeds varies in the range 1000-6000 mg/kg of grape.

Within a wide germplasm collection of white-berry accessions (seven varieties, three of which with two biotypes), skin proanthocynidins have been found ranging from 300 mg/kg of grape (Moscato di Terracina, pre-clone 657) to 2400 mg/kg of grape (Procanico, pre-clone 437) (Moretti *et al*., 2007). As summarized by de Palma and coll. (2010), within some of the most important black-berry varieties grown in Apulia, skin proanthocynidins reached 1890 mg/kg of grape in Nero di Troia; further analyses showed values as high as 2.449 mg/kg of grape in Bombino Nero and 2.893 mg/kg of grape in Negroamaro (not published data).

Nevertheless, it is well-known that during the winemaking process, more than half of the amount of phenol accumulated in grapes are lost, due to incomplete extraction and to phenomena of adsorption and precipitation; as a consequence less than half of grape phenols are recovered in wine (Singleton and Trousdale 1992). Hence, in order to produce high quality wine, it is important starting from grapes having a high phenol concentration.

As it has been reported by Fregoni (2005), anthocyanin extractability may vary from 20% to 50%; it is considered low when is 20-35%, intermediate when is 35-45%, and high when is >45%. The extractability depends on skin ability to diffuse the pigment: this trait is influenced by several agro-environmental factors, such as grape maturity level, plant water status etc., but, under same conditions, the variety makes the difference: for example Merlot exhibits higher extractability than Cabernet Sauvignon. Thus, as summarized by Moio and coll. (2007), the amount of extracted phenols depend on phenol concentration in grapes and on the composition of the skin cell wall and of seed cuticle, apart from the winemaking techniques. The anthocyanin extractability and their relationship with the cell wall, particularly with the cellulose and pectic compounds that are about 30-40% of total cell wall polysaccharides, have been largely studied: pectins of cell wall and middle lamella need be largely degraded in order to allow phenols to diffuse easily from the grape into the must. Differences in skin polysaccharide content and in degree of pectin methylation, that is specific of any variety, are highly responsible for different anthocyanin extractabilities.

On the whole, type and quantity of grape and wine phenolics are influenced by the growing conditions, harvesting time and winemaking techniques, but the first variable is the grape variety (Lachman *et al*., 2009). The indices of skin and seen phenol content are considered useful to evaluate the oenological attitude of the grapevine variety. The extraction in an alcoholic solvent at low pH, that is almost drastic, is suitable to assess the variety basic phenol accumulation habit, while the extraction in aqueous solvent, at must pH, is suitable to evaluate the final suitability to produce phenol-rich wines (Di Stefano and Cravero, 1991).

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2. CHARTER: RESEARCH OBJECTIVES AND METHODOLOGIES

2.1. Research objectives

This study is aimed to the characterization of several old grapevine genotypes found in the Daunia province, according two types of approaches:

- using SSR markers to identify the genetic profiles (genetic fingerprinting) and to compare them with those of genotypes already known at a national and international level;
- assessing the main qualitative characteristics of the grapes from a technological and phenolic point of view, in order to evaluate the potential interest of these genotypes for a further oenological use.

In Italy, such as in other Countries of ancient viticultural tradition, a huge number of variety names coexist due to several causes, such as, the movement of plant material from one place to another, the loss of the original name and the arbitrary substitution with a new one often deriving from the farmer, or the growing area, or a particular morphological trait of the vine or of the grape. These facts give rise to a huge number of cases of synonymy and/or homonymy. Genetic characterization and varietal identification are essential to resolve these cases and bring order in the vast ampelographic heritage. The present PhD study is part of a national research project called: AGER- "An Italian Vitis database with multidisciplinary approach, for exploitation and valorization of the regional genotypes" to which the University of Foggia participates. The main objective of this project is to identify and characterize in terms of morphological, physiological, bio-molecular and technological traits the regional genotypes. The results of this PhD work, together with other information already available, has been used to implement the first Italian *Vitis* Database (IVD). For major and minor genotypes found in the Apulia region, many data have been entered in the IVD as concerns selected characters (and relative

descriptors) referred to genetic, morphological, phenological, vegetative-productive and qualitative traits.

2.1.1. The search path

The starting point of this research project was the recovery of grapevine accessions in very old vineyards by now "forgotten". Since 2004, some professors and researchers at the University of Foggia in collaboration with agronomists and growers of the place have identified a group of 35 accessions in the Alto Tavoliere, Gargano and Monti Dauni areas. The accessions were highlighted by local residents (elderly, old farmers, etc.) and nominated with dialect names and typical names of the recovery area. The studied accessions were identified in ancient vineyards, however, while the accessions found in the Gargano and in the Monti Dauni areas were studied *in situ*, those of the Alto Tavoliere area were collected in a field collection at the Cantine Fortore, Torremaggiore (FG) to study the behavior of all the plants in a homogeneou environmental conditions.

Because of the limited information on the identity, the diffusion and confusion of the variety names recovered in each area, the study continued with the genetic characterization for all accessions identified using SSR molecular markers. Then, the genetic profiles were compared with that of other genotypes enrolled in national and international databases in order to identify eventual synonyms and to clarify their identity. Finally, we have studied the qualitative characteristics of the grapes, which are important for the evaluation of their oenological potential.

2.2. Material and methods

2.2.1. Plant material

The 35 accessions analyzed (Table 1) include 13 accessions found in the “Alto Tavoliere” area, 1 from the “Monti Dauni” area and 21 from the “Gargano” area. In order to obtain a correct allele sizing and share information among labs, the cv Sangiovese was included as reference variety.

Table 1 - List studied accessions.

"Alto Tavoliere" area	
white berry varieties (13) Anonimo, Biancoreale, Bombino Bianco (2 accessions), Ciucciuitto, Lunardobello, Malvasia Bastarda, Selvaggio, Squaccianosa (2 accessions), Tuccanese Moscio, Uvarilla, Uva Palomma.	
"Monti Dauni" area	
black berry varieties (1) Tuccanese	
"Gargano" area	
black berry varieties (9) Bombino Nero falso, Malvagia Nera, Moscatiddone Nero, Moscato Tamburro, Sanguinella, Somarello Rosso, Tinturino, Uva della Macchia, Uva Nera Tosta.	**white berry varieties (12)** Bell'Italia, Chiapparone, Lugliese, Moscatello del Vasto, Moscatello di Vico, Moscatiddone Bianco, Moscato Saraceno, Nardobello, Scannapecora, Uva Pane, Uva Sagra, Zibibbo.

DNA was extracted from young leaves in good health status (to minimize contamination) by two methodology: CetylTrimethyl Ammonium Bromide (CTAB) method (Mulcahy *et al*. (1993) modified by Vignani *et al*. (2002)) and DNeasy Plant mini kit (Qiagen S.r.l., Milano), because in each have been found critical points and consequent difficulty of performing. The vegetal material was transported in cool bag at Arboriculture Laboratory of Department of Sciences Agricultural, Food and Environmental, University of Foggia. The samples were stored at -80 °C in order to maintain the integrity of the tissues until DNA extraction.

2.2.2. Analysis of genetic profile

Genetic analysis provides a preliminary step that consists in the DNA extraction from young leaves and in the extracted DNA purification. Obtaining DNA in sufficient quantity and good quality is fundamental for the result, because the DNA is the substrate of all reactions necessary for genetic analysis. The following step was the genetic fingerprinting performed using the "microsatellite" technique based on amplifying, by an enzymatic reaction called PCR, some genome regions of *Vitis vinifera* containing specific microsatellite loci DNA (SSR, Simple Sequence Repeats) constituted by 1 to 6 nucleotide repeated at a tandem of 5 to 100 times. This step involves several stages: SSR choice, PCR analysis, electrophoretic analysis of the amplified products, allele sizing. The last step was the genetic profile comparison.

2.2.2.1. DNA extraction

The DNA extraction with CTAB method allows to obtain a good DNA yield, but not always a good purity, so for some accessions characterized by high concentration of carbohydrates, polyphenols and proteins, it was necessary to use the DNeasy Plant mini kit.

The DNA extraction with DNeasy Plant mini kit has the advantage of a great speed of application, allows us to obtain more DNA pure and it is safer for the health of the operator that is not in contact with hazardou reagents. By contrast, the disadvantages were the low DNA yield and the high cost.

For the DNA extraction, the glassware and plastic material used were sterilized by autoclaving at 120 ° C for 20 minutes and the reagents used were free of DNase, RNase and protease.

CTAB method

Frozen tissue (0.2-0.3 g) was ground in a mortar and pestle in liquid nitrogen. The tissue powder was transferred in 15 ml falcon tube and homogenized in 3 ml of pre-worm (60 °C) CTAB buffer containing: 1.4 M NaCl (pH 8.0); 100 mM Tris-HCl (pH 8.0); CTAB 2% (w/v); 2-Mercaptoethanol 0.4% (w/v). The mixture was incubated for 1 hour in water-bath at 65 °C and mixed 2-3 times.

Samples were cooled at room temperature, homogenized in an equal volume of chloroform/octanol (24/1; v/v) and centrifuged at 4500 rpm (Rotor SX4250, Beckman Coulter, Milano) and 4 °C for 15 minutes. The upper (aqueous) phase was transferred in new 15 ml falcon tube, homogenized in an equal volume of cold iso-propanol (-20 °C), incubated at -80 °C for 30 minutes and centrifuged at 4500 rpm (Rotor SX4250, Beckman Coulter, Milano) and 4 °C for 30 minutes. Then, the aqueous phase was discarded; the pellet was rinsed with 1.5 ml of 76% EtOH/0.2M NaAc (sodium acetate) in 1.5 ml tube, incubated in ice for 30 minutes and centrifuged at 11600 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) and 4 °C for 10 minutes. The aqueous phase was discarded again, the pellet was rinsed in 1.5 ml of 76% EtOH/0.01M NH_4Ac (ammonium acetate), incubated in ice for 30 min, centrifuged at 11600 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) and 4 °C for 10 minutes, after the aqueous phase discarded, and the pellet was dried on lab bench.

Dry pellet was dissolved in TE 500 µl containing 10 mM Tris-HCL (pH 8.0) and 1 mM Na_2EDTA (pH 8.0), then 5.5 µl of RNase (10 mg/ml) was added and it was incubated at 37 °C for 30 minutes mixing the tube 2-3 times. After, an equal volume of phenol/chloroform (1:1) were added, and it was mixed and centrifuged at 11600 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) and 4 °C for 10 minutes. The upper (aqueous) phase was transferred in new 1.5 ml tube, then 1/10 volume of 3 M NaCl and 2 volume of absolute cold EtOH (-20 °C) were added; it was incubated at -80 °C for 30 minutes, centrifuged at 11600 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) and 4 °C for 10 minutes and finally it was discarded. The pellet was dried on lab bench and dissolved in 150-200 µl of ultrapure water and sterile.

DNA extraction with DNeasy Plant mini kit

Frozen vegetal tissue (0.1 g) was ground to a fine powder under liquid nitrogen using a mortar and pestle. The tissue powder was transferred in a 1.5 ml tube; it was homogenized in 400 µl of pre-worm (37 °C) AP1 buffer and incubated for 10 minutes in water-bath at 65 °C. After, 2 µl of RNase (10 mg/ml) were added and it was incubated at 37 °C for 30 minutes. The mixture was homogenized with 130 µl of P3 buffer, and then it was incubated for 5 minutes on ice and centrifuged for 5 minutes at 14400 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl). The supernatant was transferred into the QIAshredder Mini spin column (lilac) placed in a 2 ml collection tube, and then it was centrifuged for 2 minutes at 14800 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl). The flow-through fraction, without pellet, was transferred into a new tube and 1.5 volumes of AW1 buffer was added. The mixture was transferred into the DNeasy Mini spin column placed in a 2 ml collection tube; it was centrifuged for 1 min at 8200 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) and finally, the flow-through was discarded. After, the DNeasy Mini spin column was washed two times with 500 µl of AW2 Buffer:

- the first time, it was centrifuged at 8200 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) for 1 minute;

- the second time, it was centrifuged at 14400 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) for 2 minutes and it was placed on lab bench for dry the membrane.

Finally, 50 µl of ultrapure water and sterile were added directly into the DNeasy membrane, it was incubated for 10 minutes at room temperature (15–25°C), and then it was centrifuged for 1 minute at 8000 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl). This step was repeated two or three times to obtain the maximum DNA yield.

For some accessions, that is, "Tuccanese" of Monti Dauni area, "Bombino Nero falso", "Moscatiddone Nero", "Uva Pane" and "Chiapparone" of the Gargano area, it was necessary to use the Qiagen kit for DNA extraction because the absorbance ratio Abs_{260}/Abs_{280} was very low (about 1.3). For these accessions, the DNA yield was very low.

For all the other accessions, DNA was extracted using the CTAB method. Much more DNA yield was obtained by these samples. It was sufficient to perform several washes the extracted DNA with ethanol (DNA purification) to obtain a DNA samples free from phenol and protein compounds. More purification cycles were needed by the Gargano accessions.

2.2.2.2. DNA quantification

The quality and quantity of extracted DNA were measured by UV spectrophotometry. Each sample was scanned at a wavelength between 220 nm and 320 nm against a blank containing ultrapure water by spectrophometer Smart Spec Plus (Bio-Rad Laboratories S.R.L., Milano), obtaining a graph which trend has allowed us to detect the presence of proteins and other contaminants. Five microliters of DNA dissolved in ultrapure water were diluted in 145 µL of ultrapure and sterile water. The DNA yield was measured at 260 nm: the absorbance of 1 at 260 nm (A_{260}) corresponds approximately to 50 g/ml of DNA, therefore the DNA concentration in the cuvette was calculated according to the formula:

$$\text{DNA concentration (ng/µl)} = A_{260} \times 50 \times \text{dilution factor.}$$

At the same time, a second measurement was performed at 280 nm to verify the absorption of proteins. DNA purity was determined by calculating the absorbance ratio $A_{260/280}$. This ratio was considered valid index of DNA purity (quality). In fact, while the measurement of absorbance at 260 indicates the nucleic acid presence, the measurement of absorbance at 280 indicates the impurity presence (particularly protein). A value between 1.8 and 2 indicates good purity DNA.

After the quantification, the DNA concentration of each sample was brought to 2.5 ng/µl with ultrapure and sterile water. Finally, the samples were stored at -20 °C until the analysis.

2.2.2.3. DNA purification

For some Gargano accessions, which DNA was extracted by the method of CTAB, the $A_{260/280}$ was very low and the graph obtained by spectrophotometer showed the contaminant presence. In this case the DNA was purified by repeating the following steps: the total volume of the DNA extracted was brought to 500 µl using TE, and 1/10 volume of 3 M NaCl and 2 volumes of absolute EtOH at -20 °C were added. It was incubated at -80 °C for 30 minutes and, finally it was centrifuged at 11600 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) at 4 °C for 10 minutes. The aqueous phase was discarded and the pellet was suspended in 1 ml of 70% EtOH, and it was centrifuged at 11600 rpm (Rotor n. 75003424, Thermo Fisher Scientific Milano Srl) at 4 °C for 10 minutes. The aqueous phase was discarded one more time and the pellet was dried on lab bench and, then it was dissolved in 150-200 µl of ultrapure water and sterile.

2.2.2.4. SSR analyses

SSR choice

The variety identification of accessions studied was performed using fourteen SSR molecular markers: VVS2 (Thomas and Scott, 1993), VVMD5, VVMD6, VVMD7 (Bowers *et al.,* 1996), VrZAG62, VrZag79 (Sefc *el al.*, 1999), VVMD17, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32 (Bowers *et al.,* 1999), VMC1b11 (Zyprian *et al.,* 2005). They were chosen partly upon the proposal of the European project GENRES081 (This and Dettweiler, 2003) and

GrapeGen06 (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, VrZAG79, VVMD25, VVMD28, VVMD32) and partly because considered highly polymorphic (VVMD6, VVMD17, VVMD21, VVMD24, VMC1b11). One of each pair of the primers was labeled at the 5' end whit a fluorescent dyes. The fluorescent dyes used are 6-FAM, HEX, NED and PET, which respectively emit blue, green, yellow and orange light. The use of different marking types allows the simultaneous analysis with the sequencer of more amplification products obtained with different primers. The SSR molecular markers were supplied by Life Technologies Italia, Monza (Fil. Life Technologies Europe BV) and Eurofins Genomics S.r.l, Milano (Fil. Eurofins MWG Operon). The table 2 shows the sequence of primer each pair.

Table 2 - List used oligonucleotides.

Name primer	Sequence primer	Fluorescent dyes	Size
VVS2	**5'** CAG CCC GTA AAT GTA TCC ATC **3'**	**6-FAM**	123-165
	3' GTT TAA ATT CAA AAT TCT AAT TCA ACT GG **5'**		
VVMD5	**5'**CTA GAG CTA CGC CAA TCC AA **3'**	**NED**	215-270
	3' GTT TAT ACC AAA AAT CAT ATT CCT AAA **5'**		
VVMD7	**5'**AGA GTT GCG GAG AAC AGG AT **3'**	**HEX**	180-270
	3' GTT TCG AAC CTT CAC ACG CTT GAT **5'**		
VVMD27	**5'** GTA CCA GAT CTG AAT ACA TCC GTA AGT **3'**	**NED**	165-216
	3' GTT TAC GGG TAT AGA GCA AAC GGT GT **5'**		
VrZAG62	**5'** GGT GAA ATG GGC ACC GAA CAC ACG C **3'**	**HEX**	181-220
	3' GTT TCC ATG TCT CTC CTC AGC TTC TCA GC **5'**		
VrZAG79	**5'** AGA TTG TGG AGG AGG GAA CAA ACC G **3'**	**6-FAM**	230-270
	3' GTT TGC CCC CAT TTT CAA ACT CCC TTC C **5'**		
VVMD25	**5'** TTC CGT TAA AGC AAA AGA AAA AGG **3'**	**HEX**	229-275
	3' GTT TGG ATT TGA AAT TTA TTG AGG GG **5'**		
VVMD28	**5'** AAC AAT TCA ATG AAA AGA GAG AGA GAG A **3'**	**PET**	210-283
	3' GTT TCA TCA ATT TCG TAT CTC TAT TTG CTG **5'**		
VVMD32	**5'** TAT GAT TTT TTA GGG GGG TGA GG **3'**	**PET**	228-280
	3' GTT TGG AAA GAT GGG ATG ACT CGC **5'**		
VVMD6	**5'** ATC TCT AAC CCT AAA ACC AT **3'**	**6-FAM**	180-250
	3' GTT TCT GTG CTA AGA CGA AGA AGA **5'**		
VVMD17	**5'** TGA CTC GCC AAA ATC TGA CG **3'**	**HEX**	212-236
	3' GTT TCA CAC ATA TCA TCA CCA CAC GG **5'**		
VVMD21	**5'** GGT TGT CTA TGG AGT TGA TGT TGC **3'**	**6-FAM**	165-280
	3' GTT TGC TTC AGT AAA AAG GGA TTG CG **5'**		
VVMD24	**5'** GTG GAT GAT GGA GTA GTC ACG C **3'**	**NED**	206-225
	3' GTT TGA TTT TAG GTT CAT GTT GGT GAA GG **5'**		
VMC1b11	**5'** CTT TGA AAA TTC CTT CCG GGT T **3'**	**PET**	156-197
	3' GTT TAT TCA AAG CCA CCC GTT CTC T **5'**		

PCR analysis

In order to eliminate or minimize the presence of multiple bands and PCR byproducts, different concentrations of the extracted DNA, Mg^{++} and primer were tried for several loci, as listed below.

1) Loci VVS2, VVMD7, VVMD27, VrZAG62, VrZAG79, VVMD6, VVMD25, VVMD32, VMC1b11, VVMD17, VVMD21 and VVMD24: the amplification mixture was prepared with 10 ng DNA, 0.5 µM of each primer, 200 µM for each dNTP (EuroClone S.p.A., Milano), 4 µL of GoTaq Polymerase reaction buffer 5X (Promega Italia S.R.L., Milano), 1.875 mM of $MgCl_2$ (Promega Italia S.R.L., Milano) and 0.5 units of GoTaq DNA polymerase (Promega Italia S.R.L., Milano) and deionized H_2O.
2) Locus VVMD5: the amplification mixture was prepared with 4 µl the extracted DNA diluted 1:10, 1 µM of each primer, 200 µM for each dNTP (EuroClone S.p.A., Milano), 4 µL of GoTaq Polymerase reaction buffer 5X (Promega Italia S.R.L., Milano), 3.75 mM of $MgCl_2$ (Promega Italia S.R.L., Milano) and 0.5 units of GoTaq DNA polymerase (Promega Italia S.R.L., Milano) and deionized H_2O.
3) Locus VVMD28: the amplification mixture was prepared with 4 µl the extracted DNA diluted 1:10, 0.5 µM of each primer, 200 µM for each dNTP (EuroClone S.p.A., Milano), 4 µL of GoTaq Polymerase reaction buffer 5X (Promega Italia S.R.L., Milano), 1.875 mM of $MgCl_2$ (Promega Italia S.R.L., Milano) and 0.5 units of GoTaq DNA polymerase (Promega Italia S.R.L., Milano) and deionized H_2O.

Amplifications were performed in a final reaction volume of 20 µl in a thermocycler (My Cycler, Bio-Rad Laboratories S.R.L., Milano) using an amplification program for all PCR consisted of an initial denaturation of 2 minutes at 95 °C, followed by 30 cycles of 30 seconds at 92 °C, annealing for 30 seconds at 52 °C and 1 minute at 72 °C and a final extension stage of 5 minutes at 72 °C.

Electrophoretic analysis of amplified products

Aliquots of the amplified products were checked in 2% agarose gel stained with ethidium bromide (10 mg/ml) in TAE buffer 1X containing 40 mM Tris-Acetate and 1 mM Na_2EDTA (pH 8.0). On the gel, 6 µl of each amplified mixed with 2 µl of loading buffer containing 25 mM Na_2EDTA, 60% sucrose and Orange G were loaded. To evaluate the amplified quantity was used a molecular marker (Sharpmass™ 100 Plus, EuroClone S.p.A., Milano) consists of 12 DNA fragments ranging from 100 bp to 3 kb. The electrophoretic run was set at 85 V for about 25-30 minutes. The agarose gels were visualized on UV transilluminator (Gel Doc XR System, Bio-Rad Laboratories S.R.L., Milano) and the bands obtained was compared with the molecular marker.

The remaining PCR products were diluted with ultrapure and sterile water for the analysis to the sequencer.

For each accession, the amplified products were grouped by locus, and divided into two groups (group "A" and group "B"):

- group A, containing the amplified loci VVS2, VrZAG79, VrZAG62, VVMD7, VVMD27, VVMD5 and VMC1b11;
- group B, containing the amplified loci VVMD6, VVMD21, VVMD17, VVMD25, VVMD24, VVMD32 and VVMD28.

Hence, for each accession, two tubes containing the PCR products, diluted basing on the intensity of the bands visualized by the Gel Doc, were prepared. This separation was necessary to minimize the overlapping of the electrophorogram peaks obtained by the locus sequencing.

Allele sizing

The microsatellite fragment separation was performed, using capillary electrophoresis, by the Centro Interdipartimentale di Servizi per le Biotecnologie di Interesse Agrario, Chimico, Industriale (CIBIACI), University of Firenze.

Amplified alleles are represented by peaks on electropherograms; for each peak, the fragment length is expressed as number basis. The sequence of these numbers identify the genetic profile. The electropherograms was processed by the GeneScan v 3.7 software (Applied Biosystems Inc.).

The alleles of each locus were represented on electropherograms with one or two peaks, depending on whether it is a locus homozygous or heterozygous. Furthermore, depending on the compound with which the primers were labeled, the peaks have been represented by different colors:

• blue, those marked with 6-FAM;

• green, those labeled with HEX;

• black, those marked with NED;

• red, those marked with PET.

The GeneScan software, for each peak, calculate the molecular weight expressed as number of base pairs comparing them with a reference standard (GeneScan 500 LIZ Size Standard). The sequence of these numbers identify the genetic profile. After obtaining the genetic profile of each sample, it was included in the *Database Viticolo Italiano* (DVI, Italian *Vitis* Database) and the standardization was performed, using the variety Sangiovese as a reference. The standardization allows to uniform the data obtained by different laboratories, thus reducing or eliminating the discrepancies due to the shift in the number of base pairs.

2.2.2.5. Genetic profile comparison

SSR profiles were compared with those detected by other scientific Institution and with those included in the national and international databases for cultivar identification:

- The European Vitis Database: http://www.eu-vitis.de.
- Italian Vitis Database: http://www.vitisdb.it.
- Vitis International Variety Catalogue (VIVC): http://www.vivc.de/.

2.2.3. Qualitative characteristics of the grapes

The grapes quality was assessed from a technological and phenolic point of view in order to evaluate the potential interest of these genotypes for the oenologic use. The measurements were performed in the year 2012 for the accessions of the Alto Tavoliere area, in the year 2013 for the accession of Monti Dauni area and in the year 2014 for those of Gargano area.

2.2.3.1. Technological parameters

The technological parameters (total soluble solids, titratable acidity, pH) were assessed whit common techniques. For each accession, about 300 berries were sampled from bunches, picking them from different parts of the plant. These berries were divided into three groups of about 100 berries and crushed manually. The resulting juice was poured through a strainer, centrifuged and separated from the solid residues, in order to obtain clear juice.

Soluble solid content was determined on the clear juice using digital refractometer (Atago WM7). The value, expressed in °Brix, is read directly on the display by placing a clear juice drop upon the prism-cell, after calibrating the instrument with deionized water.

Titratable acidity and pH were determined on the juice by automatic titration (Titralyser, Laboratoires Dujardin-Salleron, France). The titratable acidity was determined by the titration with NaOH (0.1 N) up to pH 7.0 of 10 ml of clear juice diluted 5 times with deionized water. It was expressed as tartaric acid multiplying the milliliters of used NaOH by 0.75 that is the equivalent weigh of tartaric acid divided by the juice milliliters used and by the NaOH normality.

The pH was determined immersing directly the electrode on clear juice and displaying the result on the display of the titration. Before of the measurement, the instrument was calibrated by a buffer at pH 4.0 and a buffer at pH 7.0.

2.2.3.2 Indices of phenol compound content

Grape phenol compounds will be analyzed, at grape maturity, by determining the total content in polyphenols, flavonoids, anthocyanins (red grapes), proanthocyanidins, flavans reacting with vanillin

of the skins and seeds, as well as hydroxycinnamic acid content of the pulp. For the different classes of phenol compounds, the content was determined by phenol extraction, using proper solutions and techniques and then by analyzing their total content at the proper wavelength range by using the spectrophotometric techniques. All the analysis can be conducted according to Di Stefano and Cravero (1991).

For each accession, about 100 berries were harvested from every part of the plant and divided randomly into groups of 10 berries. The extraction of phenol compounds was performed with two extraction solutions consisting of:

• ethyl alcohol, deionized water and concentrated hydrochloric acid in the proportion 70:30:1 (extraction solution based on hydrochloric ethanol);

• 5 g of tartaric acid, 22 ml of 1 N NaOH, 40 mg of potassium metabisulfite, 500 ml of deionized water, 120 ml of 95% ethanol and deionized water to 1 liter (tartaric buffer at pH 3.2).

For each extraction solution, three groups of 10 berries (repetitions) were formed, except the Gargano accessions for which it was possible to form a single group of 10 berries for each extraction solution because the heavy rain and hail occurred before harvest destroyed most of grapes.

The 10 berries of each repetition were weighed and prepared for analysis. We proceeded to separate the skins, pulp and seeds with the help of a scalpel. The skins were dried with absorbent paper, removing any residual pulp and it was weighed. The seeds were washed under running water and they were passed with deionized water, and finally they were counted and weighed. The pulp was poured in a beaker containing 50 mg of potassium metabisulfite ($K_2S_2O_5$), it was pressed to make them as homogeneous as possible, and then it was centrifuged and separated from the liquid (juice); finally, the latter was diluted 10 times with 10N sulfuric acid (H_2SO_4) to prevent tartaric precipitation.

The skins obtained by the first three groups of 10 berries were immersed in 25 ml of extraction solution based on hydrochloric ethanol for 24 hours at room temperature and in dark and the seeds were immersed in a same amount of extraction solution, however, left for 72 hours always at room temperature and in dark. The skins and seeds obtained from the other three groups were immersed in

50 ml of tartaric buffer at pH 3.2 and left under the same conditions of the first three groups. At the end, skins and seeds were separated from the extraction solution and the extracts were stored at -20 °C until analysis.

Acid hydroxicinnamiltartaric assay

The acids hydroxicinnamiltartaric content was determined by reading spectrophotometric at the wavelength of 320-325 nm on the juice obtained after centrifugation of the pulp and dilution with sulfuric acid. The absorption spectrum was recorded at the wavelength between 230 and 400 nm using quartz cells whit 1 centimeter optical path length against sulfuric acid considering how absorbance value the peak between 320 and 325 nm. The acids hydroxicinnamiltartaric content expressed as caffeic acid (mg/ L of the juice) was determined with the following relationship:

$$E \bullet (10/0.9) \bullet d$$

where E is the value of the absorbance; d is the dilution factor of the juice (10 in our case) and 0.9 is the absorbance of a caffeic acid solution to 10 mg/L.

Total polyphenol assay

The content of total polyphenols was determined by an oxidation reaction using the Folin-Ciocalteu reagent. A volume of 100 µl of extract was placed into a 20 ml flask. Then, 5 ml of distilled water, 1 ml of Folin-Ciocalteu reagent and after 3-5 minutes 4 ml of sodium carbonate solution (Na_2CO_3) to concentration 10% were added. Finally, the mixture was brought to volume with distilled water and left at room temperature and in the dark for 90 minutes. After, the absorbance reading was performed in the spectrophotometer UV-1700 (Shimadzu Europa GmbH, Germany) at the wavelength of 750 nm using quartz cells whit 1 centimeter optical path length against a blank prepared in the same way as samples, replacing only the extract with distilled water. The index of total polyphenols expressed as (+)catechin (mg/kg of grapes) was calculated with the following formula:

$$186.5 \bullet E_{750}/V \bullet X/P$$

where 186.5 is the correlation coefficient between absorbance and concentration obtained using the (+) catechin as standard; E_{750} is the value of the absorbance; V is the volume of extract used (in our case 100 µl); X is the volume of the extraction solution; P is the 10 berries weight.

Total anthocyanin and flavonoid assay

The content of anthocyanins and flavonoids was determined by dilution the extract from 10 to 100 times with the hydrochloric ethanol solution for grape skins extracts and distilled water for grape seeds extracts. Then, the absorbance was read using quartz cells whit 1 centimeter optical path length against a blank containing the solution used for the dilution.

For total flavonoids, that have an absorption peak at the wavelength of 280 nm, an absorption spectrum was registered at the wavelength ranged between 230 and 400 nm. The absorbance E'_{280} ranged between the maximum at the wavelength of 280 nm and the tangent plotting to the spectrum conduct for the minimum point in the ultraviolet (graphic method). The total flavonoids index expressed as (+) catechin (mg/kg of grapes) was calculated with the following formula:

$$E'_{280} \bullet 82.4 \bullet d \bullet X/P$$

where E'_{280} is the absorbance calculated using the graphical method; 82.4 is the molar extinction coefficient of (+)catechin; d is the dilution; X is the volume of the extraction solution; P is the weight 10 berries.

For total anthocyanins, that are present only in the red grape skins, the absorbance reading was performed at the wavelength of 540 nm and their content was expressed as malvidin-3-glucoside (mg/kg of grapes) using the following equation:

$$E_{540} \bullet 16.17 \bullet d \bullet X/P$$

where E_{540} is the value of absorbance; 16.17 is the molar extinction coefficient of malvidin-3-glucoside; d is the dilution; X is the volume of the extraction solution; P is the weight 10 berries.

Total proantocyanidin assay

The proanthocyanidin content was determined by Bate-Smith reaction based on heating in acidic conditions. In these conditions, the proanthocyanidins are transformed into cyanidins and catechins that have an absorption peak at the wavelength of 532 nm. An extract aliquot (200 µl), 12.3 ml of ethanol and 12.5 ml of concentrated hydrochloric acid containing 300 mg L^{-1} of ferrous sulfate ($FeSO_4 \bullet 7H_2O$) were placed in a 50 ml flask immersed in ice water and incased with aluminum film. The flasks were immersed in hot water bath equipped with a refrigerant for 50 minutes at 90 °C. For red grape skin extracts were recorded the absorption spectrum at the wavelength between 360 and 700 nm using quartz cells whit 1 centimeter optical path length against air, before that the flasks were immersed in hot water bath and the absorbance at wavelength of 532 nm ($E°_{532}$) was calculated by the method graphic described above. After 50 minutes, the flasks were cooled in ice and water and the absorption spectrum at the wavelength between 360 and 700 nm was recorded using quartz cells whit 1 centimeter optical path length against air. The absorbance at 532 nm (E'_{532}) wavelength was calculated by the graphic method. The content in proanthocyanidins expressed as cyanidin chloride (mg/kg of grapes) was determined with the following relationship:

$$1162.5 \bullet \Delta E/V \bullet X/P$$

where 1162.5 is a coefficient obtained with the following relation, considering the 25 ml of the reaction mixture, a yield of 20%, the molecular weight of cyanidin chloride (MW = 322.7 g mol^{-1}) and the molar extinction coefficient at a wavelength of 535 nm of cyanidin in 95% ethanol + 0.1 ml of HCl ($\varepsilon = 34700$): $1162.5 = 25 \bullet MW/\varepsilon \bullet (1/0.2) \bullet 1000$; $\Delta E = E'_{532} - E°_{532}$ in the case of red grapes and $\Delta E = E'_{532}$ in the case of white grapes; V is the volume of extract used (in our case 200 uL); X is the volume of the extracting solution; P is the weight of 10 berries.

Flavan reacting with vanillin assay

Vanillin is an aromatic aldehyde that it reacts in an acid condition with the positions 6 e 8 free of the proanthocyanidins giving red compounds: the intensity of red colorization lowers, as the

polymerization degree is high. A volume of 500 µl of extract diluted 10 times with methanol and 3 ml of 4% vanillin in methanol were placed in a dark glass tube; after 5 minutes 1.5 ml of concentrated hydrochloric acid was added, immersing the tube in water and ice. The reading of absorbance was performed after 15 minutes at the wavelength of 500 nm using quartz cuvettes with 1 cm optical path against a blank prepared in the same way as the sample, but substituting the vanillin with methanol. The content flavans reactive to vanillin expressed as (+)catechin mg/kg of grapes was calculated with the following formula:

$$290.8 \bullet E_{500} \bullet d \bullet X/P$$

where E_{500} is the value of the absorbance; d is the dilution; X is the volume of the extraction solution; P is the weight 10 berries.

The relationship between the content of flavans reactive to vanillin (FRV) and the content in proanthocyanidins (PA) was calculated to determine the polymerization degree of proanthocyanidins: the lower this index, the higher the polymerization degree.

Statistical analysis

Analysis of variance (ANOVA) and Duncan test were performed (significance level $p \leq 0.05$ or $p \leq 0.01$) using Assistat Software version 7.7 beta.

2.2.4. Entering data in the Italian Vitis Database

To enter accession data in the IVD, the first step is the registration of its microsatellite profile. The database provides for the inclusion of at least nine microsatellite loci (recommended by the EU-project GrapeGen06 (Table 3).

Table 3 - Microsatellite loci list.

Name	Allele Size – Min.	Allele Size – Max.
VVS2	123	165
VVMD5	215	270
VVMD7	180	270
VVMD27	165	216
VrZAG62	181	220
VrZAG79	230	270
VVMD25	229	275
VVMD28	210	283
VVMD32	228	280

In order to standardize the microsatellites profiles analyzed by different Institutions, the database has an internal process based on the comparison between the genetic profile of the reference accession Sangiovese as resulting from the lab analysis, and that one assumed as standard for the system (Table 4).

Table 4 – Microsatellite profiles of cv Sangiovese.

	SSR locus (number base pairs)													
	VVS2	**VVMD5**	**VVMD7**	**VVMD27**	**VrZAG62**	**VrZAG79**	**VVMD25**	**VVMD28**	**VVMD32**	**VMC1b11**	**VVMD17**	**VVMD21**	**VVMD24**	**VVMD6**
Sangiovese reference profile of UniFG-lab	133	225	240	180	196	243	240	237	253	172	212	242	209	190
	133	235	264	186	198	259	240	247	257	172	222	248	215	208
Sangiovese profile of DVI system	133	225	239	179	194	243	242	237	253	167	212	243	208	190
	133	235	263	185	196	259	242	247	257	167	222	249	214	208

The inclusion of each data in the IVD must be accompanied by representative pictures of the main grapevine organs (shoot, leaf and bunch) and the by the descriptive traits:

- ampelographic, ampelometric and phenological-productive traits reported in the second edition of the "Code des caractéres descriptifs des variétés et espèces de Vitis" (OIV, 2009) (Table 5);
- ampelometric traits generated by the SuperAmpelo software (Table 6);
- vegetative-productive traits (Table 7).

Table 5 - Ampelographic, ampelometric and phenological-productive traits.

Code OIV	Ampelographic traits
001	Young shoot: opening of the shoot tip
003	Young Shoot: intensity of anthocyanin coloration on prostrate hairs of tip
004	Young Shoot: density of prostrate hairs on tip
006	Shoot: attitude (before tying)
007	Shoot: color of dorsal side of internodes
008	Shoot: color of ventral side of internodes
016	Shoot: number of consecutive tendrils
051	Young leaf: color of the upper side of blade (4 th leaf)
053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)
067	Mature leaf: shape of blade
068	Mature leaf: number of lobes
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade
075	Mature leaf: blistering of upper side of blade
076	Mature leaf: shape of teeth
079	Mature leaf: degree of opening / overlapping of petiole sinus
080	Mature leaf: shape of base of petiole sinus
081-1	Mature leaf: teeth in the petiole sinus
081-2	Mature leaf: petiole sinus base limited by veins
083-2	Mature leaf: teeth in the upper lateral sinuses
084	Mature leaf: density of prostrate hairs between the main veins on lower side of blade
087	Mature leaf: density of erect hairs on main veins on lower side of blade
151	Flower: sexual organs
153	Inflorescence: number of inflorescences per shoot
155	Shoot: fertility of basal buds (buds 1-3)
202	Bunch: length (peduncle excluded)
204	Bunch: density
206	Bunch: length of peduncle of primary bunch
208	Bunch: shape
209	Bunch: number of wings of the primary bunch
220	Berry: length
221	Berry: width
223	Berry: shape
225	Berry: color of skin
231	Berry: intensity of flesh anthocyanin coloration
236	Berry: particularity of flavor
241	Berry: formation of seeds

Code OIV	Ampelometric traits
601	**Mature leaf: length of vein N1**
602	Mature leaf: length of vein N2
603	Mature leaf: length of vein N3
604	Mature leaf: length of vein N4
605	Mature leaf: length petiole sinus to upper lateral leaf sinus
606	Mature leaf: length petiole sinus to lower lateral leaf sinus
607	Mature leaf: angle between N1 and N2 measured at the first ramification
608	Mature leaf: angle between N2 and N3 measured at the first ramification
609	Mature leaf: angle between N3 and N41) measured at the first ramification
610	Mature leaf: angle between N3 and the tangent between petiole point
612	Mature leaf: length of tooth N2
613	Mature leaf: width of tooth N2
614	Mature leaf: length of tooth N4
615	Mature leaf: width of tooth N4
616	Mature leaf: number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 including the limits
Code OIV	**Phenological-productive traits**
301	Time of bud burst
303	Time of beginning of berry ripening (veraison)
351	Vigor of shoot growth
502	Bunch: weight of a single bunch
503	Berry: single berry weight
504	Yield per m2
505	Sugar content of must
506	Total acid content of must
508	must specific pH

Table 6 - Ampelometric traits generated by the SuperAmpelo software.

Distances	Base of the tooth located at the end of N2	Media height of the teeth of the right side
Leaf length	Height of the tooth on the end of N4'	Media of the base of the teeth of the right side
Leaf width	Height of the tooth on the end of N4	Media height of the teeth of the left side
Leaf length including the petiole	Height of the tooth on the end of N2	Media of the base of the teeth of the left side
Petiole length	Base of the tooth located at the end of N4	**Angles**
Length of vein N1	Base of the tooth located at the end of N2'	Angle between N1 and N2 measured at the first bifurcation
Distance between the ends of veins N2 and N2'	**Rations**	Angle between N1 and N2' measured at the first bifurcation
Distance between the ends of veins N3 and N3'	Multiplication between length and width of the leaf	Angle between N2 and N3 measured at the first bifurcation
Distance between the ends of veins N4 and N4'	Ratio between length and width of the leaf	Angle between N2 and N3' measured at the first bifurcation
Width of petiole sinus / Distance between points SP and SP'	Ratio between the length of the petiole OP and the length of the vein N1	Angle between N3 and N4 at the first fork of N3
Length of vein N2	Ratio between the distance from the sinus and the length of the vein N2	Angle between N3' and N4'
Length of vein N2'	Ratio between the distance from the sinus and the length of the vein N2'	Angle between N1 and N2 measured at the ends of the veins
Length of vein N3	Ratio between the distance from the petiole sinus to the lower right sinus OI and the length of vein N3	Angle between N1' and N2' measured at the ends of the veins
Length of vein N3'	Ratio between the distance from the petiole sinus to the lower left sinus OI' and the length of vein N3'	Angle between N2 and N3 measured at the ends of the veins
Distance between petiole point and end of vein N4	Ratio between the length of the vein N2 and the length of the vein N1	Angle between N2' and N3' measured at the ends of the veins
Distance between petiole point and end of vein N4'	Ratio between the length of the vein N2' and the length of the vein N1	Angle between N3 and N4 measured at the ends of the veins
Length of vein N4	Ratio between the length of the vein N3 and the length of the vein N1	Angle between N3' and N4' measured at the ends of the veins
Length of vein N4'	Ratio between the length of the vein N3' and the length of the vein N1	Angle of opening of the petiole sinus measured at SP and at SP'
Length of vein N5	Ratio between the length of the vein N4 and the length of the vein N1	Angle between D and D' with the center in N1
Length of vein N5'	Ratio between the length of the vein N4' and the length of the vein N1	Angle between S and S' with the center in N1
Vein N3, length from the petiole sinus to vein N4	Ratio between the length of the vein N5 and the length of the vein N1	Angle between I and I' with the center in N1
Vein N3', length from the petiole sinus to vein N4'	Ratio between the length of the vein N5' and the length of the vein N1	Angle between N2 and N3 measured at the petiole point and between N2 and N3 tooth tip
Distance from the petiole sinus to the upper right sinus	Ratio between the sum of the angles a + b and the sum of the distance between the petiole sinus and upper right sinus OS and the petiole sinus and lower right lower right sinus OI	Angle between N2 and N3 measured at the petiole point and between N2' and N3' tooth tip
Distance from the petiole sinus to the upper left sinus	Ratio between the sum of the angles a' + b' and the sum of the distance between the petiole sinus and upper right sinus OS' and the petiole sinus and lower right lower right sinus OI'	Sum of the angles alpha + beta
Distance from the petiole sinus to the lower right sinus	Ratio between the height and the base of the teeth of the right side	Sum of the angles alpha' + beta' + gamma'
Distance from the petiole sinus to the lower left sinus	Ratio between the height and the base of the teeth of the left side	Sum of the angles alpha + beta + gamma
Distance between the tooth tip of N2 and the tooth tip of the first ramification (secondary vein) of N2	Ratio between the height and the base of the tooth at the end of the vein N2	Sum of the angles alpha' + beta'
Distance between the tooth tip of N2' and the tooth tip of the first ramification (secondary vein) of N2'	Ratio between the height and the base of the tooth at the end of the vein N2'	
Altezza del dente posto all'estremità di N2'	Ratio between the height and the base of the tooth at the end of the vein N4	
Base of the tooth located at the end of N4'	Ratio between the height and the base of the tooth at the end of the vein N4'	

Table 7 - Vegetative-productive traits.

Plant spacing & training system	**Fertility**	Weight of 100 berries (g)
Training system	Number of bunch per shoots at flowering (number/shoot)	Berry diameter (average of 25 berries) - height (mm)
Pruning System	Fertility of basal buds (bunch/bud)	**Production's qualitative characteristics**
Distance between rows (m)	**Production's quantitative characteristics**	Titratable acidity of must (g/l)
Distance on the row (m)	Number of bunches per vine (number/vine)	Seeds total polyphenols (g/kg)
Vigor	Number of bunches per meter of row (number/m)	Skin total polyphenols (g/kg)
Number of shoots/canes per vine (number/vine)	Bunch's weight (g)	pH
Cane's weight (g)	Grape production per vine (kg/ceppo)	Sugar content of must (°Brix)
Number of shoots/canes per meter of row (number/m)	Grape production per hectare (t/ha)	
Pruning wood's weight per vine (kg)	Grape production per meter of row (kg/m)	
Pruning wood's weight per vine meter of row (kg)	Berry diameter (average of 25 berries) - width (mm)	

Furthermore, to them, have been associated a range of information including site of selection, the holding institution, collection vineyard, information of trueness-to-type, and the literature. Each accession is associated with a variety and the accessions with the same profile microsatellite the same variety. Among the accessions associated with a variety is given an accession as "accession main varieties" and the information entered for this accession describe the variety. Also in the variety are inserted other information:

- registration in the in the Italian Catalogue of Grapevine Varieties;
- synonyms;
- released clones;
- historical references;
- distribution and variation;
- technological use.

References 2° Chapter

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3. CHAPTER: RESULTS AND DISCUSSION

3.1 Analysis of Genetic profile

3.1.1. DNA extracted

The graph trend obtained by scanning the samples in the wavelength range between 220 and 330 nm showed the absence of proteins and contaminants for most of the accessions. Figure 1 shows the absorption spectra of some the analyzed accessions.

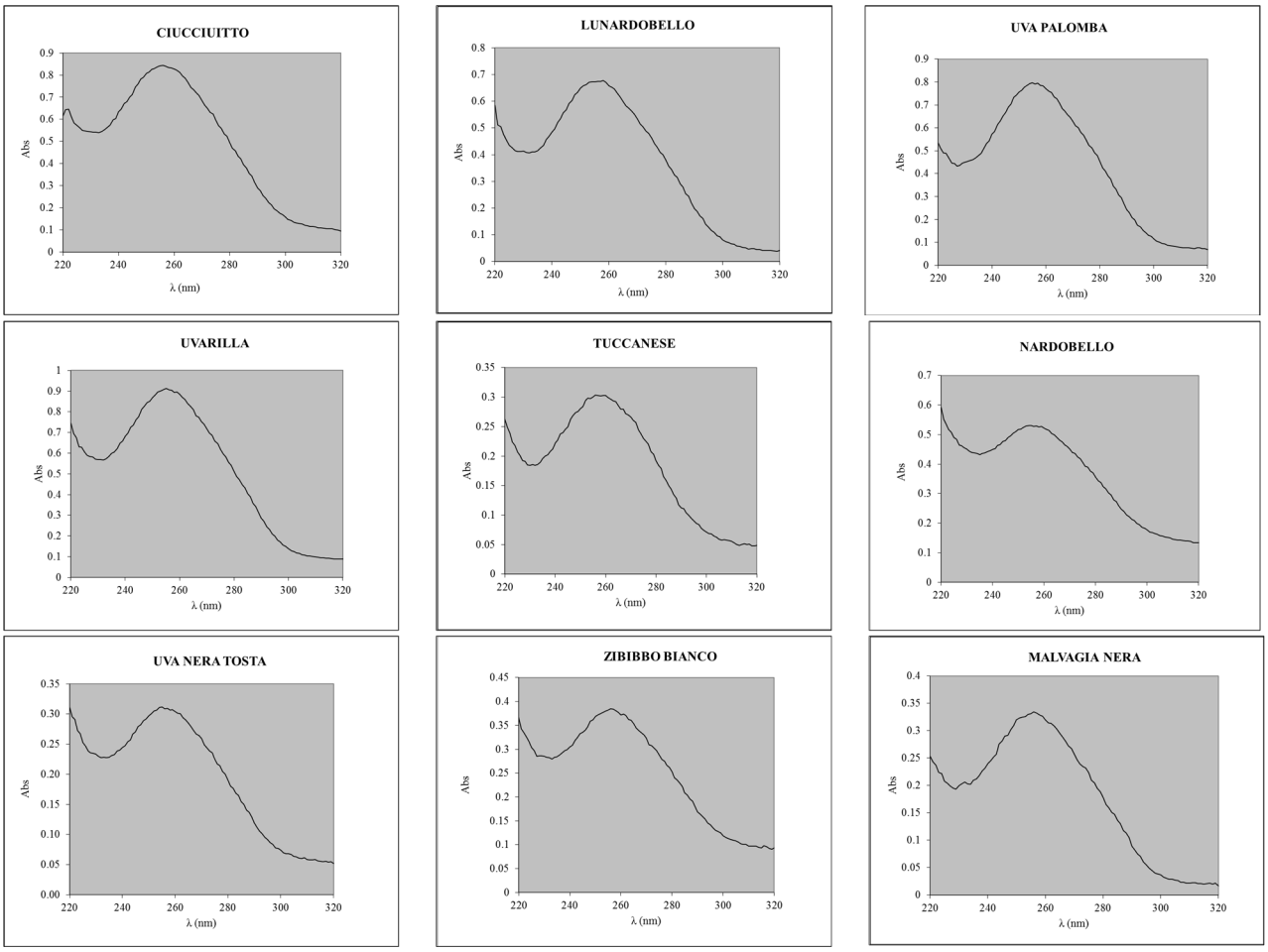


Figure 1 – Absorption spectra of some accessions.

The average yield obtained by DNA extractions using CTAB method was approximately 500 ng/µl, while, using the Qiagen kit, it was approximately 130 ng/µl. The average Abs_{260}/Abs_{280} ratio of all he accessions was about 1.6, irrespectively by the extraction method. For some samples, the absorbance

ratio was very high (1.9); this means that DNA with a high purity degree had been extracted (Table 8-9).

Table 8 - Quantity and quality of DNA extracted using CTAB method.

Accessions (Alto Tavoliere area)	Abs_{260}/Abs_{280}	[DNA] ng/µl	Accessions (Gargano area)	Abs_{260}/Abs_{280}	[DNA] ng/µl
Anonimo	1.5	267	Bell'Italia	1.8	398
Biancoreale	1.81	387	Lugliese	1.44	192
Bombino bianco (Az.Celeste)	1.66	614	Malvagia Nera	1.79	640
Bombino bianco (Az.Fortore)	1.77	385	Moscatello del Vasto	1.52	315
Ciucciuitto	1.67	414	Moscatello di Vico	1.46	855
Lunardobello	1.73	328	Moscatiddone Bianco	1.43	998
Malvasia Bastarda	1.87	440	Moscato Saraceno	1.4	795
Selvaggio	1.52	501	Moscato Tamburro	1.41	382
Squaccianosa (Az. Celeste)	1.43	653	Nardobello	1.47	647
Squaccianosa (Az.Fortore)	1.45	373	Sanguinella	1.39	480
Tuccanese moscio	1.66	597	Scannapecora	1.54	618
Uva Palomba	1.7	385	Somarello Rosso	1.48	467
Uvarilla	1.72	443	Tinturino	1.41	465
Average	**[DNA] ng/µl: 490**		Uva della Macchia	1.46	274
			Uva Nera Tosta	1.59	380
Average	**Abs_{260}/Abs_{280}: 1.57**		Uva Sagra	1.45	530
			Zibibbo	1.47	489

Table 9 - Quantity and quality of DNA extracted using Qiagen Kit.

Accessions	Abs_{260}/Abs_{280}	[DNA] ng/µl
Bombino Nero falso	1.42	155
Chiapparone	1.59	141
Moscatiddone Nero	1.71	143
Tuccanese	1.68	99
Uva Pane	1.66	135
Average	**1.61**	**134**

3.1.2. Analysis of the amplified products

In the first phase of the work, not all the 14 microsatellite loci gave amplified products. The loci that gave a negative response were subjected to a second and a third analysis, by changing the PCR conditions. In particular, the annealing temperatures, the cycles number, the magnesium chloride and primer concentration were varied to make the reaction more selective. The PCR has been one of the

most critical points for the success of the analysis; however, the conditions chosen for the amplification made possible to obtain highly reproducible results.

The accessions of the "Alto Tavoliere" area showed good amplification at the first analysis, since all the 14 loci of 6 accessions amplified. For the other 7 accessions the amplification of some loci failed, in particular for the accessions Tuccanese Moscio, Anonimo, Ciucciuitto, Lunardobello, Uva Palomma, Uvarilla; the amplification of these loci was repeated by increasing the concentration of the DNA in the samples.

The accessions of the Gargano area, which DNA was extracted using the CTAB method, showed good amplification at the first analysis for the VrZAG62, VVMD27, VVMD7, VVMD6, VVMD25 and VVMD24 loci, while for the other loci it was necessary to repeat the amplification, especially for some accessions. In particular, the amplification was repeated for the following loci:

- VVMD5, for most of the accessions, increasing the concentration of Mg^{++}, of the primers and of the extracted DNA;
- VMC1b11, for accessions Lugliese, Uva della Macchia Sanguinella, Moscato Saraceno, Moscatello del Vasto e Moscatello di Vico, increasing the concentration of Mg++, of the primers and of the extracted DNA;
- VVMD28, for all accessions except for Uva Sagra, Somarello Rosso, Moscato Tamburro and Moscatiddone Bianco, increasing the annealing temperature in order to improve the primer specificity, since bands with incorrect molecular weight due to non-specific annealing of one or both primers appeared in the electrophoretic gel.

The amplification problems had with these accessions may be due to a number of factors, such as the environment and the climate in which the plant grows, possible attacks by pathogens, the age of the plant and, in general, the growth conditions of the plant that contribute to the production of secondary metabolites, proteins, polysaccharides and polyphenols that are accumulated in the organs and affecting the quality and quantity of extracted DNA. In general, to get a good amount and quality of extracted DNA it is preferable to use young and healthy plant material.

The accessions of Gargano area, which DNA was extracted using the kit, presented the same problems of accessions which DNA was extracted using the method of CTAB for loci VVMD5 and VMC1b11, (the other loci were amplified at the first analysis).

Finally, for the accession Tuccanese of Monti Dauni area, which DNA was extracted using the kit, all the loci were amplified at the first analysis.

For the 14 analyzed loci, the electrophoretic bands demonstrated: the presence of polymorphism, good separation of the alleles, the absence of multiple bands and of bands with molecular weight greater than that of the studied loci. The pictures showing the amplified products of some accessions are reported in Fig. 2 and 3.

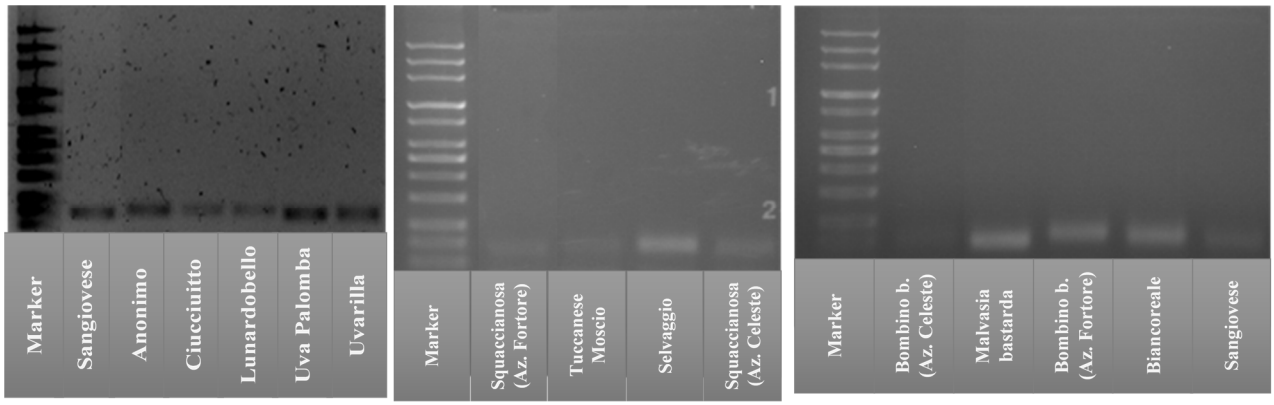


Figure 2 - Accessions of Alto Tavoliere Dauno area: VVS2 locus.

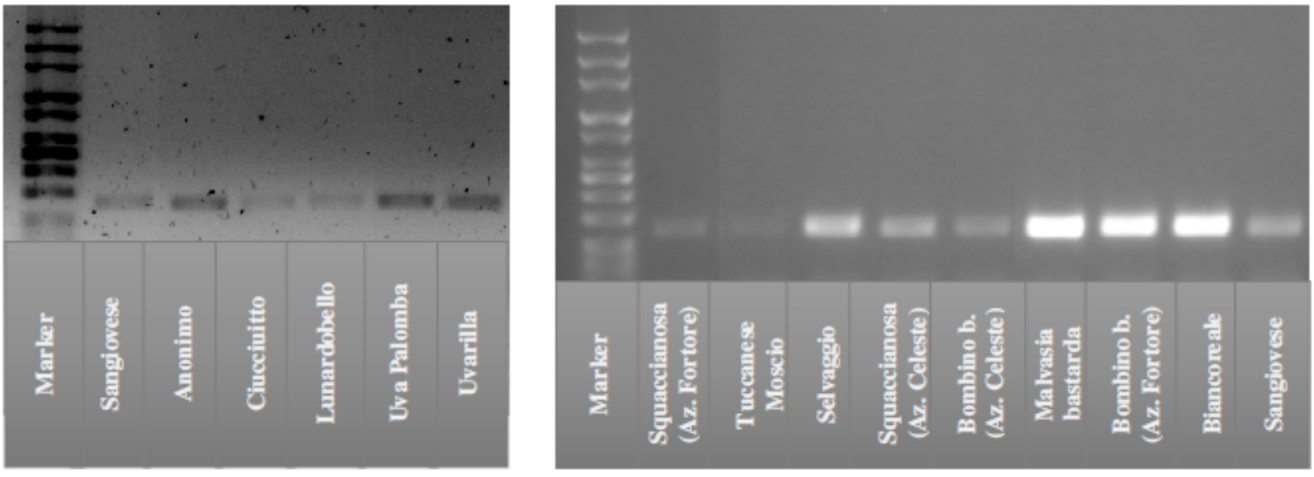


Figure 3 - Accessions of Alto Tavoliere Dauno area: ZAG79 locus.

The 14 microsatellite loci produced bands of size comparable with that of typical intervals for each locus. Therefore, it was possible to proceed to the sequencing of the fragments in order to obtain the SSRs allele size.

3.1.3. Analysis of microsatellite loci

Amplified alleles are visualized as bands on gels or are represented by peaks on electropherograms. Using an appropriate molecular weight marker, a specific software determines, for each peak, the fragment length expressed as number basis. An example of electropherogram processed by the GeneScan software v3.7 is reported in Fig. 4.

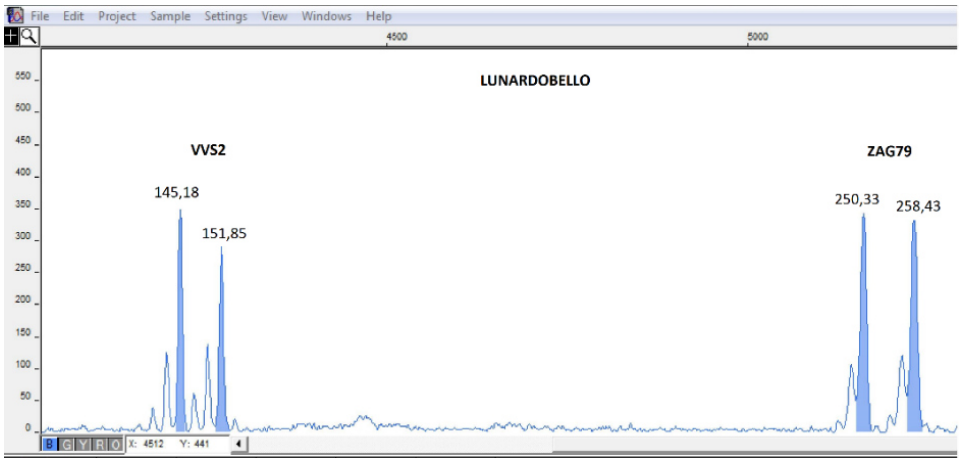


Figure 4 - Electropherogram of loci VVS2 and ZAG79 (accession Lunardobello).

The DNA profiles of the 35 accessions are shown in Tables 10 and 11.

Table 10 - Genetic profile of white-berry accessions of the Alto Tavoliere area.

Accessions	VVS2	VVMD5	VVMD7	VVMD27	ssrVrZAG62	ssrVrZAG79	VVMD25	VVMD28	VVMD32	VVMD6	VVMD17	VVMD21	VVMD24	VMC1b11
Anonimo	143	227	248	190	188	239	240	239	259	208	222	248	209	184
	145	245	250	192	196	247	254	257	263	208	222	264	217	188
Biancoreale	133	225	244	190	190	251	240	237	263	190	222	248	209	172
	145	245	248	190	198	257	240	261	273	208	222	254	209	190
Bombino Bianco (Az. Celeste)	145	227	250	182	192	251	240	247	259	200	222	242	209	180
	151	231	254	194	202	259	262	251	273	208	222	248	209	190
Bombino Bianco (Az. Fortore)	145	227	250	182	192	251	240	247	259	200	222	242	209	180
	151	231	254	194	202	259	262	251	273	208	222	248	209	190
Ciucciuitto	145	233	240	182	190	247	238	247	251	208	222	248	209	172
	151	233	254	194	190	259	248	251	251	210	222	254	217	194
Malvasia Bastarda	133	225	240	180	196	245	240	239	259	200	222	242	209	176
	133	225	254	184	202	251	254	239	273	210	222	254	209	188
Lunardobello	145	227	250	182	192	251	240	247	259	200	222	242	209	180
	151	231	254	194	202	259	262	251	273	208	222	248	209	190
Selvaggio	143	231	240	182	190	243	240	239	259	190	222	242	209	172
	145	231	254	186	202	259	254	249	273	206	222	248	213	190
Squaccianosa (Az. Celeste)	133	225	240	190	196	249	238	239	263	190	220	248	209	172
	143	231	250	194	198	251	254	247	275	200	222	248	217	190
Squaccianosa (Az. Fortore)	133	225	240	190	196	249	238	239	263	190	220	248	209	172
	143	231	250	194	198	251	254	247	273	200	222	248	217	190
Tuccanese Moscio	133	225	240	182	190	243	240	239	259	208	212	248	209	172
	133	227	250	186	204	251	262	261	263	208	220	264	213	190
Uva Palomma	133	225	250	180	192	257	238	231	273	200	220	242	209	190
	145	227	254	182	202	259	240	251	273	208	222	256	209	194
Uvarilla	133	225	240	186	196	247	238	251	253	190	212	248	209	188
	153	235	264	192	198	251	248	261	273	206	220	248	215	190

Table 11 - Genetic profile of accessions of the Monti Dauni area and Gargano area.

Black-berry accessions	VVS2	VVMD5	VVMD7	VVMD27	ssrVrZAG62	ssrVrZAG79	VVMD25	VVMD28	VVMD32	VVMD6	VVMD17	VVMD21	VVMD24	VMC1b11
Bombino Nero falso	135	233	244	186	190	243	254	237	253	208	222	248	209	188
	151	235	250	186	192	251	262	261	273	208	222	248	217	194
Malvagia Nera	137	239	248	182	202	247	238	237	263	208	222	248	209	188
	153	245	250	192	204	259	254	261	273	208	238	258	213	194
Moscatiddone Nero	135	233	248	182	190	247	254	237	253	208	222	248	209	188
	153	235	250	186	202	251	262	261	273	208	238	258	209	194
Moscato Tamburro	133	225	248	180	194	251	240	271	263	190	220	254	213	190
	133	227	252	186	206	255	248	271	265	210	220	264	217	190
Sanguinella	133	225	248	180	202	237	238	251	257	208	220	242	209	180
	143	235	250	182	206	259	238	261	265	210	220	248	209	188
Somarello Rosso	133	225	252	180	202	247	240	247	251	200	222	248	209	176
	143	239	254	184	202	251	254	251	251	210	222	256	209	194
Tinturino	133	233	240	182	190	243	240	239	251	190	222	248	209	178
	151	237	244	190	198	245	248	263	273	206	222	248	213	188
Tuccanese	133	225	240	180	196	243	240	237	253	190	212	242	209	172
	133	235	264	186	198	259	240	247	257	208	220	248	215	172
Uva della Macchia	133	225	248	180	192	251	238	251	253	208	222	242	209	172
	145	227	250	192	192	259	240	261	259	208	222	242	209	180
Uva Nera Tosta	137	227	250	180	192	251	238	261	251	208	222	242	209	184
	145	239	254	182	202	259	240	261	273	208	222	258	213	194

White-berry accessions	VVS2	VVMD5	VVMD7	VVMD27	ssrVrZAG62	ssrVrZAG79	VVMD25	VVMD28	VVMD32	VVMD6	VVMD17	VVMD21	VVMD24	VMC1b11
Bell'Italia	143	227	240	180	188	251	240	251	251	208	222	248	211	190
	145	239	244	194	190	257	254	261	273	208	236	254	217	190
Chiapparone	133	225	250	180	188	251	238	251	259	200	222	242	213	188
	135	227	250	194	202	255	240	261	273	210	222	264	217	190
Lugliese	145	227	248	186	194	239	240	237	253	210	222	248	209	176
	155	235	248	186	196	251	248	249	263	210	224	256	209	190
Moscatello del Vasto	133	227	234	180	188	251	240	249	265	208	220	248	213	190
	133	235	250	194	198	255	248	271	273	210	222	266	217	194
Moscatello di Vico	133	227	240	180	188	249	240	239	259	208	212	254	213	190
	143	239	250	180	190	255	254	249	273	208	222	264	217	194
Moscatiddone Bianco	133	227	250	180	188	247	248	247	265	190	212	254	213	172
	149	231	252	194	206	255	248	271	273	210	220	264	213	190
Moscato Saraceno	133	225	250	180	188	249	238	251	259	200	222	242	213	188
	135	227	250	194	202	255	240	261	273	210	222	266	217	190
Nardobello	133	225	250	180	202	251	238	239	253	210	222	248	209	172
	145	227	250	194	204	259	240	261	259	210	222	266	213	186
Scannapecora	145	225	240	180	198	243	238	251	253	190	222	248	207	188
	145	239	254	180	202	251	240	257	257	210	222	248	209	188
Uva Pane	135	233	248	180	198	251	248	247	271	190	220	256	209	172
	153	235	254	194	206	257	254	247	273	210	222	266	209	176
Uva Sagra	133	225	250	180	192	257	238	231	273	200	220	242	209	190
	145	227	254	182	202	259	240	251	273	208	222	256	209	194
Zibibbo	133	225	240	186	188	243	248	237	259	208	222	254	209	190
	135	231	250	186	190	251	254	261	273	208	222	254	209	190

The results obtained from the analysis of the sequences of the 14 microsatellite loci amplified for all the accessions showed that all samples possess one or two alleles per locus. This indicates that the locus may be in the homozygous condition (only one allele) or in a state of heterozygotes (two alleles). Comparing the genetic profile of the 35 accessions within themselves, 30 genotypes were found. The two accessions of Bombino Bianco (Alto Tavoliere) had the same genetic profile, as well as the two accessions of Squaccianosa found in the same area. In addition, three overlaps of the genetic profile resulted for other three groups of accessions: Uva Palomma (Alto Tavoliere) and Uva Sagra (Gargano), Chiapparone and Moscato Saraceno both of the Gargano area, Bombino Bianco and Lunardobello both of the Alto Tavoliere area.

Comparing the genetic profile of the 35 accessions with those of genotypes enrolled in the genetic databases of other scientific Institutions, 23 identifications were obtained.

Among the 23 genotypes identified, 20 genotypes resulted synonymous of varieties mentioned in the National Catalogue of Grapevine Varieties (RNVV) (Table 12), 3 genotypes resulted not registered in the RNVV (Table 13).

The genetic profile of 7 accessions were not found in any database, hence, at the moment, they may be considered as "unique genotypes" (Table 14).

Table 12 - Genotypes registered in the RNVV.

White-berry accessions of Alto Tavoliere area	**Official name**
Biancoreale	Biancame
Bombino bianco (Az. Celeste) / Bombino bianco (Az. Fortore) / Lunardobello	Bombino Bianco, Passerina
Ciucciuitto	Sultanina Bianca
Malvasia Bastarda	Malvasia Bianca
Selvaggio	Bianco d'Alessano
Squaccianosa (Az. Celeste) / Squaccianosa (Az. Fortore)	Minutolo
White-berry accessions of Gargano area	**Official name**
Bell'Italia	Damaschino
Chiapparone / Moscato saraceno	Moscato di Terracina
Lugliese	S. Anna di Lipsia
Moscatello del Vasto	Moscato Bianco
Moscatello di Vico	Moscato giallo
Moscatiddone Bianco	Moscato d'Alessandria
Scannapecora	Malvasia Bianca Lunga
Uva Pane	Baresana
Zibibbo	Regina
Black-berry accessions of "Gargano" area	**Official name**
Moscatiddone Nero	Moscato d'Amburgo
Moscato Tamburro	Moscato d'Adda
Sanguinella	Primitivo
Somarello Rosso	Somarello Rosso
Black-berry accession of "Monti Dauni" area	**Official name**
Tuccanese	Sangiovese

Table 13 - Genotypes not registered in the RNVV.

Accessions	**Corresponding name**	**Refernces**
Bombino Nero falso	Uva Nera Antica	de Palma *et al.*, 2013
Tinturino	Petit Bouschet	D'Onofrio, 2015
Uva Palomma/Uva Sagra	Palumbo/Uva Carrieri	de Palma *et al.*, 2012, Schneider *et al.*, 2014

Table 14 - Genotypes with unique genetic profile.

Anonimo
Malvagia Nera
Nardobello
Tuccanese Moscio
Uva della Macchia
Uva Nera Tosta
Uvarilla

3.2. Qualitative characteristics of the grapes

3.2.1. Technological parameters

3.2.1.1. Technological parameters evaluated for the black-berry accessions of Monti Dauni and Gargano area

The results of the analyses performed at grape harvest (mid September) are shown in Graph 1.

The accession **Tuccanese** found in the Monti Dauni area, which genetic profile corresponded to that of Sangiovese, showed technological traits (SST 22 °Brix, TA 6.7 g/L, pH 3.40), comparable to the averager of some Sangiovese accessions grown in Tuscany (Scalabrelli *et al*., 2015).

Among the black-berry accessions found in the Gargano area (Tab. 12), **Moscatiddone Nero**, identified as Moscato d'Amburgo that is a dual-purpose variety (Fregoni and Zamboni, 2005), was able to reach a high TSS content (22.8 °Brix) as it is typical of this genotype when grown in Apulia (Novello *et al*., 1995). The same tendency was pointed out in Muscatidrunni nero, another Moscato d'Amburgo biotype described in the IVD and grown in the Trapani province (Sicily) (Ansaldi *et al.*, 2015a). The pH of Moscatiddone Nero was interesting, since it was limited to 3.3, but also the must acidity was very low (3.41 g/L). Moscato d'Amburgo is known to give poor wine, especially as concerns the color intensity (Fregoni and Zamboni, 2005).

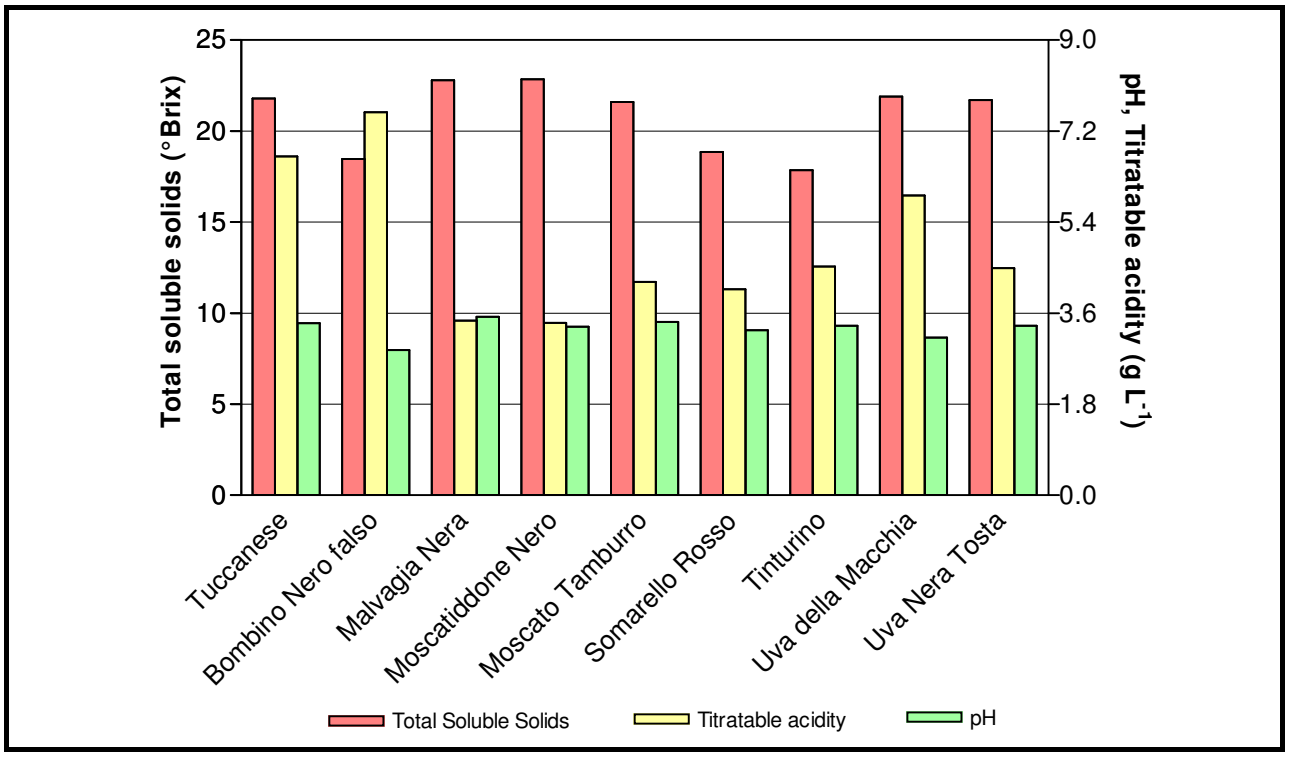
Moscato Tamburro was also identified as a table grape variety, that is, Moscato d'Adda (Tab. 12) a variety selected in 1987, by Pirovano, starting from a Moscato d'Amburgo seedling (Fregoni and Zamboni, 2005). Technological traits were not so far from those of Moscatiddone Nero.

Somarello Rosso, corresponding to the homonymous varieties (Tab. 12), showed medium TSS content (18.8 °Brix) and medium-low TA (4.1 g/L) and pH (3.26), according to the average values of other two accessions of the same variety grown in the Bari province (Apulia) (de Palma *et al.*, 2014).

Tinturino, that corresponds to the variety Petit Bouschet grown in France (Tab. 12), showed a not satisfying TSS content (17.85 ° Brix), low TA (4.52 g/L) and medium pH (3.35), similarly to an accession of same variety included in the IVD and cultivated in Tuscany with the name called Colorino di Lucca. This genotype has pigmented pulp and thus gives deeply colored wines, but poor in structure (D'Onofrio *et al.*, 2015a).

Bombino Nero falso, which genetic profile correspond to that of Uva Nera Antica found in Abruzzo (not to Bombino nero typical of the Apulia region), seemed to be just sufficient in SST content (18.5 °Brix), but very interesting for TA level (7.6 g/L) and for its very low pH (2.87). These two latter traits are unusual for Apulian grapes.

Finally, accessions **Malvagia Nera**, **Uva della Macchia** and **Uva Nera Tosta**, that by genetic profiles did not correspond to other genotypes already known, proved to be characterized by high TSS content and low levels of titratable acidity, as it is common in warm-arid environments. In particular, Malvagia Nera reached the highest TSS content (22.8 °Brix) and the lowest TA (3.45 g/L). Uva della Macchia showed a low pH value (3.12) not common for grapes growing in the same environment.



Graphic 1-Technological parameters evaluated for black-berry accessions of Gargano and Monti Dauni areas.

3.2.1.2. Technological parameters evaluated for the white-berry accessions of Gargano area

The results of the analyses performed at grape harvest (mid September) are shown in Graph 2.

Among white-berry accessions, the accessions **Uva Pane** and **Zibibbo**, that were respectively identified as Baresana and Regina of RNVV (Tab. 12), are both table grape genotypes typically grown in the Bari province. At grape harvest, total soluble solids content (TSS) was lower in Uva Pane (14.5 °Brix) than in Zibibbo (about 20 °Brix, very high for table graps), while titratable acidity (TA) showed opposite tendency (5.74 g/L and 3.90 g/L, respectively). Thus, the TSS/TA ratio was 25 for the former and 51 for the latter variety. Typical values of maturity indices for Baresana harvested in the second half of September are 16-18 °Brix and 5-6 g/L, with TSS/TA 26-36 (AA.VV., 1989), and for Regina harvested between 10th and 20th of September for 17.6 °Brix and 5 g/L, with TSS/TA 35 (Colapietra, 2004).

Chiapparone and **Moscato Saraceno**, although were the same genotype (registered in the RNVV as Moscato di Terracina, Tab. 12), differed in TSS and TA content: Moscato Saraceno showing higher

sugar accumulation and lower titratable acidity (21.59 °Brix; 3.44 g/L) than Chiapparone (19.72 °Brix, 5.28 g/L). This latter showed very similar values when compared to an other accession of Moscato di Terracina described in IVD (D'Onofrio *et al*., 2015b), that is Moscato Bianco di Lucca.

Moscatiddone Bianco, that has been identified as Zibibbo or Moscato d'Alessandria enrolled in the RNVV, reached about 21 °Brix similarly to two Zibibbo accessions described in IVD and grown in Sicily (Ansaldi, *et al*., 2015b; Ansaldi *et al*., 2015c), while TA (4.85 g/L) and pH (3.25) were lower than the average values of those accessions.

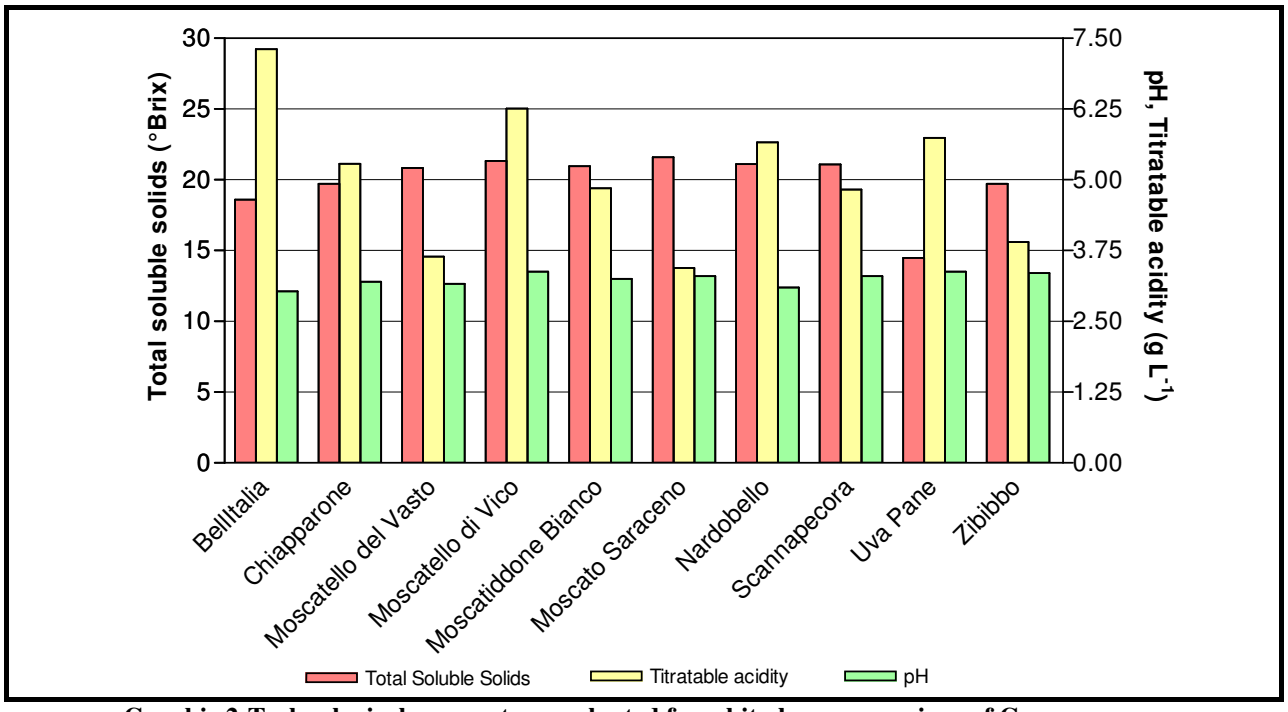
Moscatello del Vasto, identified as Moscato Bianco (Tab. 12), showed sugar (20,84 °Brix) and acid levels (3,64) similar to those of a Moscato bianco biotype grown in Sicily, Trapani province, included in IVD (Ansaldi *et al*., 2015d); the TSS content was also similar to that of two Moscato bianco accessions respectively grown in Tuscany, Pisa province (D'Onofrio *et al*., 2015c), and Piedmont, Cuneo province (Schneider *et al.*, 2013), while the acidity (3.64 g/L) was lower by 2.76 g/L respect to the former and by about 2 g/L respect to the latter; it is known that the high air temperatures reached in warm environments increases cellular respiration consuming privileged substrates, including malic acid, and, as consequence, the acid level is typically quite low.

Moscatello di Vico, identified as Moscato giallo (Tab. 12), showed TSS (21.34 °Brix) and a pH value (3.38) similar to those found in Moscato Saraceno; the TA value (6.26 g/L) was the highest among the "Moscato" accessions described in the present research. A satisfying TA level is important for wine freshness, savoriness and longevity, and is particularly useful in white wine to avoid a flat taste.

Bell'Italia, which genetic profile was the same of Damaschino (Tab. 12), a typical dual-purpose variety grown in the Trapani province (Sicily) (Ansaldi *et al*., 2015e), showed technological traits (18.60 °Brix, 7.31 g/L, pH 3.3) useful for winemaking.

Scannapecora, corresponding to Malvasia Bianca Lunga (Tab. 12), showed technological traits (21.9 °Brix, 4.83 g/L, pH 3.3) very similar to those of Malvasia Bianca lunga accessions grown in Tuscany (D'Onofrio and Scalabrelli, 2015).

Finally, **Nardobello**, that seemed to have a "unique" genetic profile, proved to have interesting technological attitude since their grape reached satisfying sugar accumulation of 21.10 °Brix, medium acidity level 5.66 g/L, and very low pH 3.10, unusual for Apulian grapes having medium-to-low acidity. Low must pH is important for a good wine color and stability.

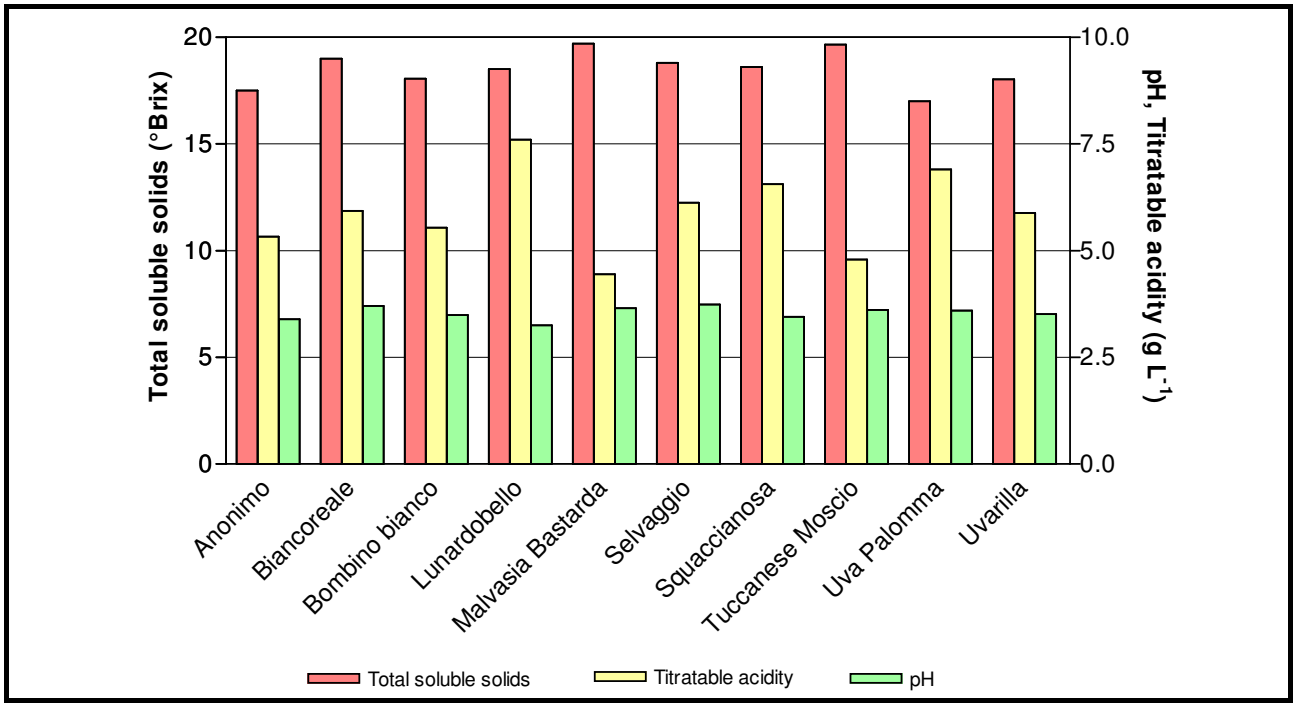


Graphic 2-Technological parameters evaluated for white-berry accessions of Gargano area.

3.2.1.3. Technological parameters evaluated for the accessions of Alto Tavoliere area

The accessions found in AltoTavoliere area showed a total soluble solids (TSS) content ranging between about 17.20 °Brix in **Uva Palomma** and **Anonimo** and 20 °Brix in **Tuccanese Moscio** and **Malvasia Bastarda** (Graph 3)**.** All the other accessions showed a tendency for an intermediate TSS concentration (18-19 °Brix). The pH, that is important for the chemical and microbiological wine stability, was low for **Lunardobello** (3.25), while **Biancoreale** (3.70) and **Selvaggio** (3.74) showed an opposite tendency. Among the other genotypes, **Squaccianosa** (3.45) and **Uvarilla** (3.51) had pH very close to that of the **Bombino Bianco** (3.49). High pH are quite common in warm environments and as consequence of the low acidity. **Lunardobello**, that had the lowest pH, showed the highest

titratable acidity (7.60 g/L). As concerns the capacity to maintain a quite high titratable acidity, also the genotype **Uva Palomma** (6.90 mg/L) showed an interesting tendency.

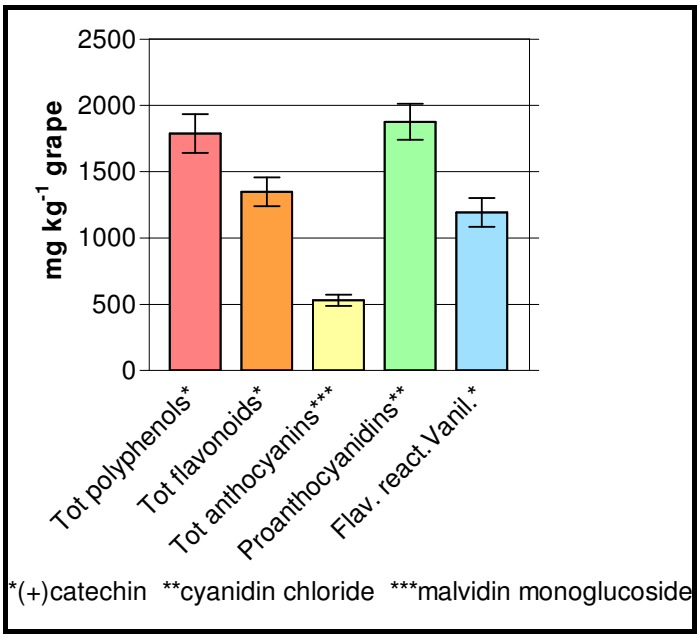


Graphic 3 - Technological parameters evaluated for accessions of Alto Tavoliere area.

3.2.2. Indices of the total content of the main phenol compounds

3.2.2.1. Indices of the total content of the main skin phenol compounds of Monti Dauni area

The accession called Tuccanese, identified as Sangiovese, showed a great content of phenolic compounds (Graph. 4). The total polyphenols and anthocyanins amounts of Tuccanese were very close to that reported in the DVI for the variety Sangiovese. However, the ratio FRV/PA was 0.63, that is a high values. The ratio between flavans reactive to vanillin and proanthocyanidin is related to the average degree of polymerization of the flavan compounds and, thus, to their reactivity and astringency: the higher the ratio, the lower the polimerization and the astringency (Souquet *et al.*, 1996; Cagnasso *et al.*, 2005-2006).



Graphic 4 - Indices of the total content of the main phenolic compounds of accessions of Monti Dauni area.

3.2.2.2. Indices of the total content of the main skin phenol compounds of Gargano area

The results phenol analyses on grapevine accessions of the Gargano area should be considered not plenty representative, because of the heavy rain and hail occurred before harvest; these events caused metabolite dilution and loss of grapes. Nevertheless, the grapes appeared quite well endowed from the phenolic point of view (Graph. 5 e Graph. 7).

Black-berry accessions

The PF content ranged between about 700 mg/kg grape in **Uva della Macchia** and **Somarello Rosso** and about 1400 mg/kg grape in **Uva Nera Tosta, Malvagia Nera** and **Tinturino. Moscato Tamburro** and **Moscatiddone Nero** showed a medium content in PT (about 1000 mg/kg grape).

The FL content ranged between about 650 mg/kg grape in **Somarello Rosso** and about 2500 mg/kg grape in **Malvagia Nera**. **Tinturino** (2000 mg/kg grape), **Uva Nera Tosta** (1650 mg/kg grape) and **Moscato Tamburro** (1700 mg/kg grape) showed a content very close to that of Malvagia Nera, while **Uva della Macchia** (830 mg/kg grape) and **Bombino Nero falso** (1000 mg/kg grape) showed a FL content very close to that of Somarello Rosso.

The total anthocyanins (TA) index had the lowest value in **Somarello Rosso** (about 40 mg/kg grapes) and the highest value in **Malvagia Nera** (1060 mg/kg grape). **Moscato Tamburro, Tinturino** and **Uva Nera Tosta** showed a TA content similar each to other (about 650 mg/kg grape).

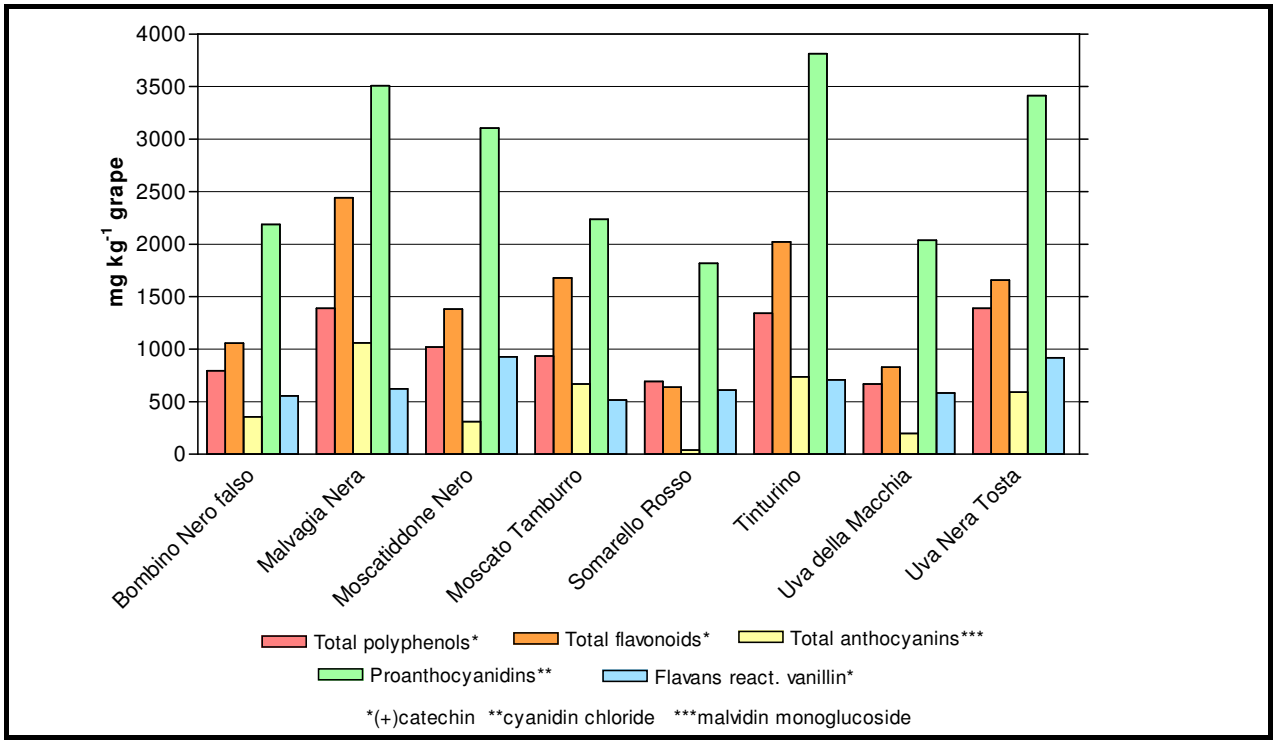
The PA content, quite high in all accessions, ranged between about 1800 mg/kg grape in **Somarello Rosso** and 3800 mg/kg in **Tinturino**. **Malvagia Nera** (3500 mg/kg grape) and **Uva Nera Tosta** (3400 mg/kg grape) showed a PA content similar between them; **Moscato Tamburro** (2240 mg/kg grape) and **Bombino Nero falso** (2190 mg/kg grape) did the same.

The FRV content ranged between 520 mg/kg grape in **Moscato Tamburro** and about 925 mg/kg grape in **Moscatiddone Nero** and **Uva Nera Tosta**. The other accessions showed an intermediate value.

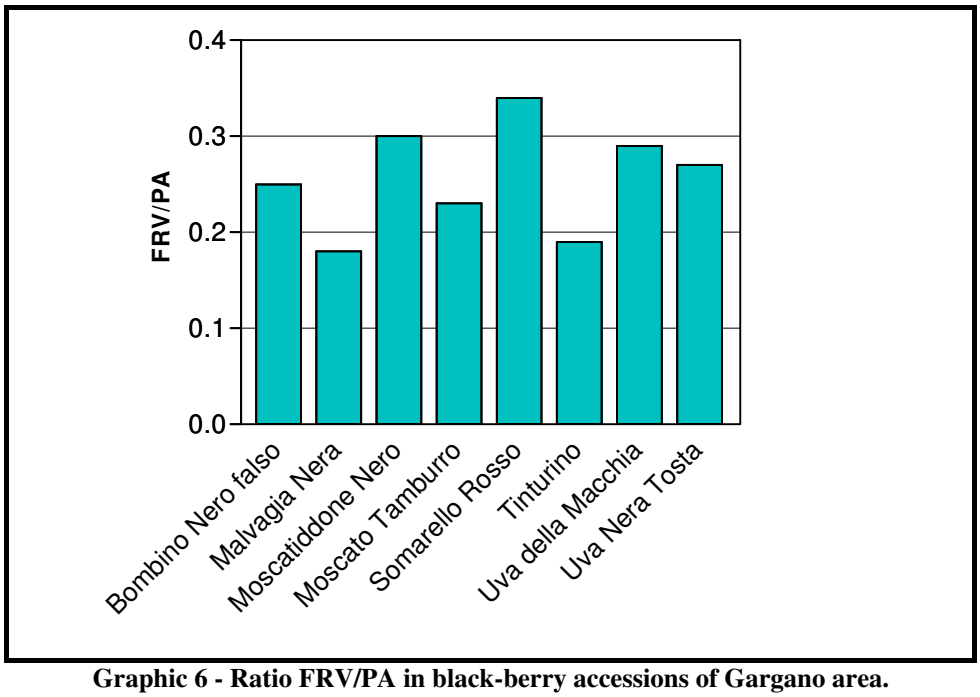
The phenolic content of these accessions was compared with that of black-berry accession of Monti Dauni area "Tuccanese" (Graph.4), that was identified as Sangiovese, and that is locally used to produce an appreciated appreciated. All black-berry Gargano accessions showed a lower content in PT (-30% for Uva Nera Tosta and Malvagia Nera) and FRV (-30% for Uva Nera Tosta and Moscatiddone Nero) than the Tuccanese, while they showed an opposite tendency as for the PA content (+50% for Tinturino). As for the content in FL and TA, Tuccanese showed intermediate values than those of the Gargano accessions.

Malvagia Nera seemed a very interesting accession because of its richness in phenol compounds, especially as concerns the anthocyanin and the proanthocyanidin content: it could be suitable for the production of well-structured and very-well colored wines with stable color, thanks to the possible co-pigmentation reactions between anthocyanin and proantocyanidin compound. Moreover, the FRV/PA ratio was very low (0.18) (Graph. 6): this means that only about 20% of the skin tannins of this accession is constituted by low molecular weight oligomers.

Uva Nera Tosta, as well as **Uva della Macchia,** were well equipped with phenolic compounds, and moreover, the FRV/PA ratio was about 0.30 (Graph 6).



Graphic 5 - Indices of the total content of the main phenolic compounds of the skins of black-berry accessions of Gargano area.



Graphic 6 - Ratio FRV/PA in black-berry accessions of Gargano area.

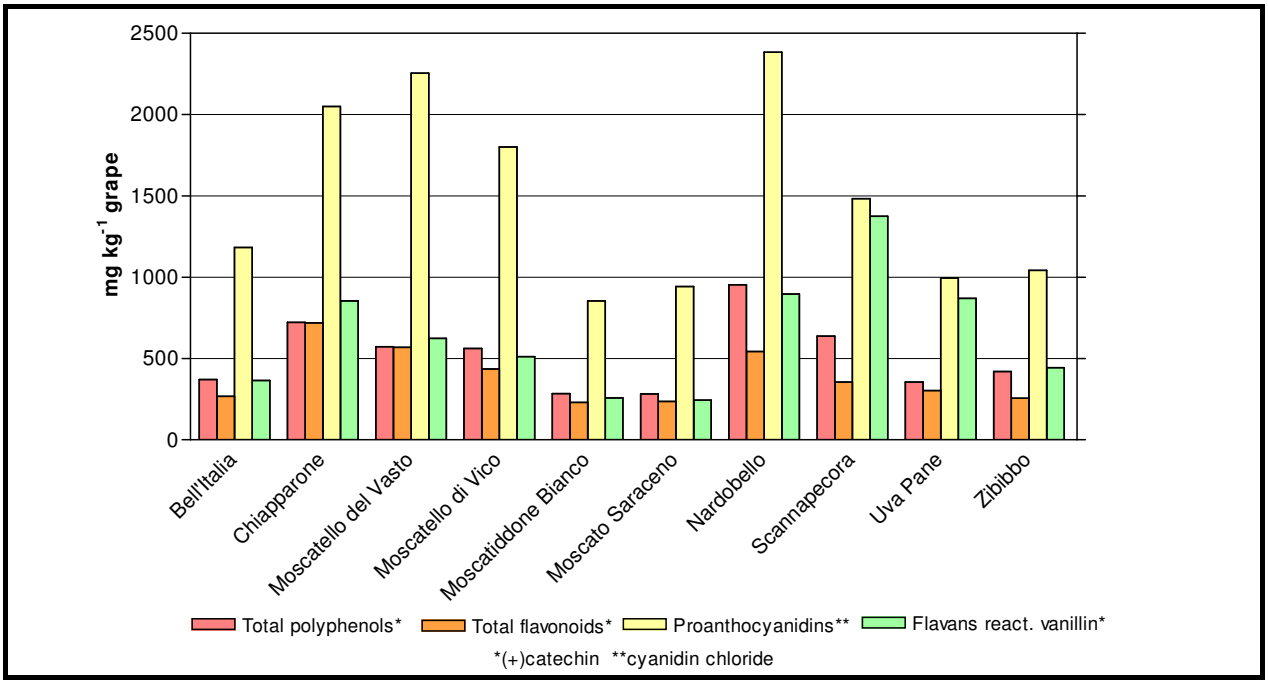
White-berry accessions

The skin total polyphenol content (PF) ranged between about 280 mg/kg grape in **Moscato Saraceno** and **Moscatiddone Bianco** to 954.11 mg/kg grape in **Nardobello. Uva Pane** and **Zibibbo** (both table grape varieties) as well as **Bell'Italia** (dual-purpose variety) showed quite low values and very close to each other (about 380 mg/kg grape). Also, **Moscatello di Vico** and **Moscatello del Vasto** showed PF content very close to each other, approximately 567 mg/kg grape, that is a value of intermediate level.

The skin total flavonoid content (FL) ranged was between 230 in **Moscatiddone Bianco** and 720 mg/kg grape in **Chiapparone**. **Moscato Saraceno** (about 240 mg/kg grape) had FL content close to the lowest value. **Zibibbo, Bell'Italia** and **Uva Pane** had FL content (about 270 mg/kg grape) very close to each other, as it was noticed for the PF content.

The index of total skin proanthocyanidins (PA) was low in **Moscatiddone Bianco** (855 mg/kg grape) and **Moscato Saraceno** (942 mg/kg grape) (the former, moreover, is a dual-purpose variety), while it reached high values in **Nardobello** (2383 mg/kg grape), **Moscatello del Vasto** (2254 mg/kg grape) and **Chiapparone** (2050 mg/kg grape). **Uva Pane** and **Zibibbo**, ones more, showed a similar phenol content (about 1020 mg/kg grape), while **Bell'Italia** overcame the other two accessions by 13%. **Scannapecora** (1483 mg/kg grape) and **Moscatello di Vico** (1800 mg/kg grape) showed a medium-high total proanthocyanidin content.

The index of favans reactive to vanillin (FRV) in berry skin ranged between 250 mg/kg of grape in **Moscato Saraceno** and **Moscatiddone Bianco** and 1375 mg/kg of grape in **Scannapecora**. **Uva Pane** showed a FRV content (870 mg/kg grape) almost twice than those of **Bell'Italia** and **Zibibbo,** and very close to that of **Chiapparone** (855 mg/kg grape) and **Nardobello** (about 900 mg/kg grape).

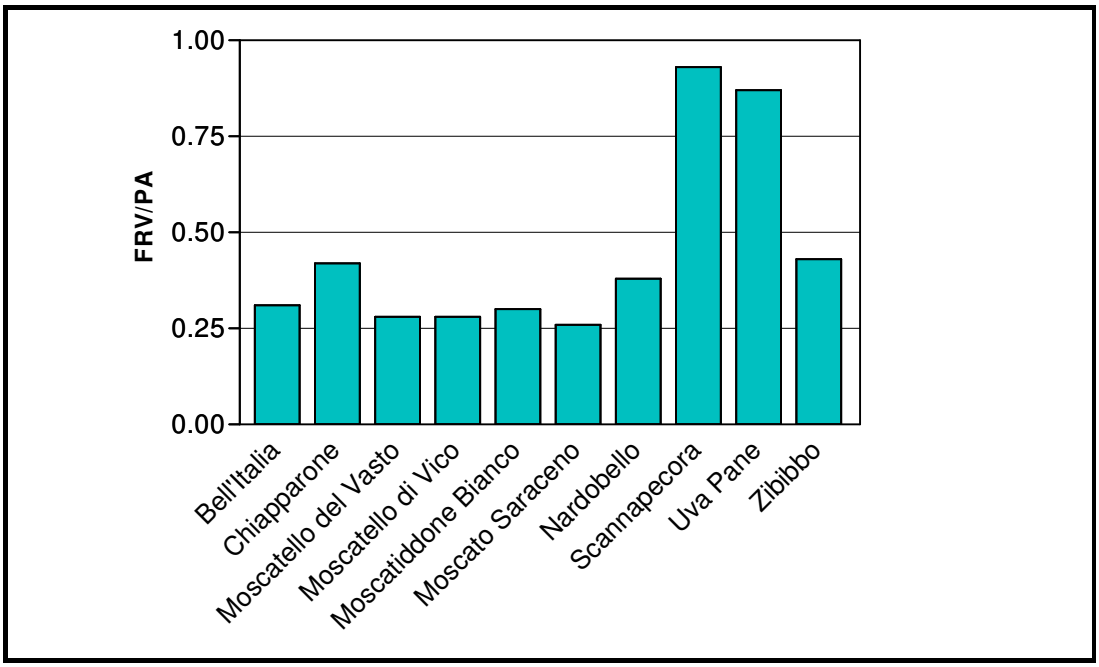


Graphic 7 - Indices of the total content of the main phenolic compounds of the skins of white-berry accessions of Gargano area.

The values found of the indices of the total polyphenol, flavonoid and proanthocynidin content found in the skin of **Moscatello del Vasto** were compared with those of the other wine-grape genotypes (excluding the genotypes identified as table grape varieties), since Moscatello del Vasto corresponds to Moscato Bianco, that is a very famous wine-grape variety. Some accessions showed phenol contents higher or close to those of the reference genotype.

Nardobello revealed the most interesting phenolic profile, especially as for its high content in proanthocyanidins, and could be suitable for the obtaining of a well-structured mono-variety wine, providing a special care during the winemaking (i.e. temperature and oxygen-exposure control) in order to protect the must from the oxidations that cause browning reactions. **Chiapparone**, that was identified as Moscato di Terracina, followed Nardobello as for phenolic richness; however, PA content was 10% lower than that of Moscatello del Vasto. **Moscatello di Vico**, that was identified as Moscato Giallo, had same polyphenols of the reference variety, but lower flavonoids and proanthocyanidins (about -20%). **Scannapecora**, that was identified as Malvasia Bianca Lunga, showed slightly higher polyphenol content (+11%), but markedly lower flavonoid (-37%) and proanthocynidin content (34%) than Moscatello del Vasto.

The FRV/PA ratio of the wine-grape accessions (Graph. 8), Moscatello del Vasto, Moscatello di Vico, Moscatiddone Bianco and Moscato Saraceno were very interesting since all they showed a value of about 0.25.



Graphic 8 – Ratio FRV/PA in white-berry accessions of Gargano area.

3.2.2.2. Indices of the total content of the main skin phenol compounds of Alto Tavoliere area

The accession selected in the Alto Tavoliere area, that were all white-berry genotypes, showed a satisfying content in total polyphenols. Among them, it was included as a reference variety Bombino Bianco, as main variety typical of this area. As summarized in Tab. 15, **Lunardobello,** that was identified as Bombino Bianco, showed similar skin phenol contents for PF (1090 mg/kg grape), PA (1370 mg/kg grape) and FRV (890 mg/kg grape), but higher FL content (1180 mg/kg grapes). **Tuccanese Moscio** showed a lower content of PT (880 mg/kg grape), FL (790 mg/kg grape) e FRV (715 mg/kg grape) than Bombino Bianco, but a higher content of PA (1260 mg/kg grape).

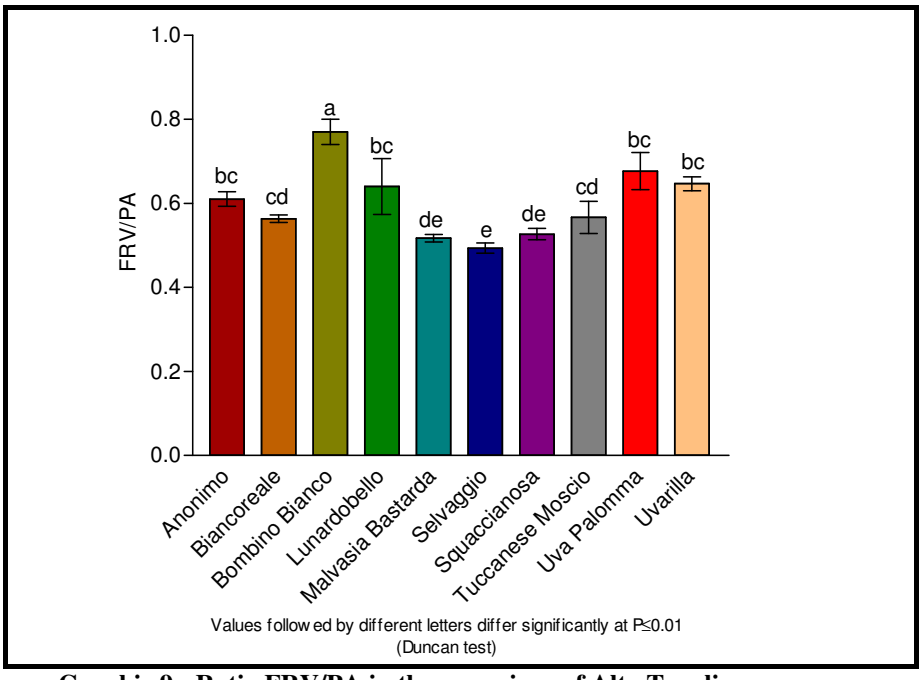
All the other accessions showed phenol contents lower than those of Bombino Bianco; however, among them, **Uva Palomma** seemed the most rich in skin phenol compounds, while **Biancoreale**

was at the opposite. **Anonimo, Selvaggio** and **Squaccianosa** had PF, PA and FRV contents statistically similar between them and very close to those of Uva Palomma; **Malvasia Bastarda** and **Uvarilla** showed phenol content similar between them and similar to those of Biancoreale.

Table 15 - Phenolic compounds of accessions of Alto Tavoliere area

Parameters	Anonimo	Biancoreale	Bombino Bianco	Lunardobello	Malvasia Bastarda	Selvaggio	Squaccianosa	Tuccanese Moscio	Uva Palomma	Uvarilla	Significance
Total polyphenols (+)catechin (mg kg^{-1} grape)	633.69 ±11.55 cd	319 ±55.76 e	1204.94 ±4.17 a	1087.1 ±151.48 a	493.23 ±42.89 de	609.71 ±54.41 cd	600.38 ±20.39 cd	881.25 ±69.34 b	747.54 ±36.54 bc	469.12 ±82.40 de	**
Total flavonoids (+)catechin (mg kg^{-1} grape)	303.61 ±6.93 ef	226.98 ±61.19 f	977.95 ±70.70 b	1177.59 ±107.13 a	378.19 ±25.58 ef	469.01 ±46.21 de	356.26 ±37.20 ef	788.94 ±65.83 c	601.78 ±42.13 d	256.77 ±54.01 f	**
Proanthocyanidins cyanidin chloride (mg kg^{-1} grape)	797.68 ±32.22 cd	437.65 ±93.09 e	1124.04 ±22.63 ab	1369.38 ±188.12 a	633.2 ±53.48 de	791.47 ±31.49 cd	726.59 ±38.67 d	1260.19 ±58.46 a	988.93 ±18.85 bc	570.64 ±110.25 de	**
Flav. react.Vanil. (+)catechin (mg kg^{-1} grape)	483.64 ±23.07 bc	246.36 ±52.65 c	864.68 ±47.50 a	897.59 ±219.30 a	327.08 ±22.44 c	392.07 ±25.08 c	383.31 ±29.15 c	715.54 ±67.29 ab	667.12 ±44.11 ab	367.84 ±65.19 c	**
Values followed by different letters differ significantly at p≤0.05 (*) or p ≤ 0.01 (**) (Duncan test)											

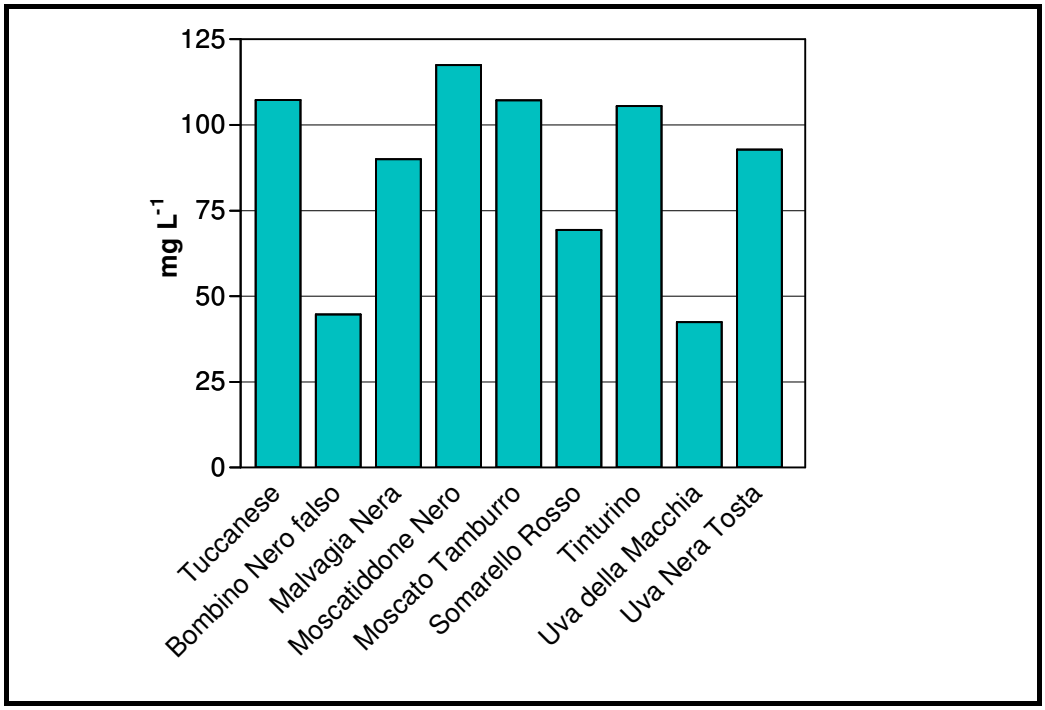
The rate FRV/PA (Graph. 9), related to the ability of proanthocyanidins polymerization, was medium-high for most of the genotypes (≈ 0,50-0.60). Nevertheless, FRV/PA of **Bombino Bianco** was even higher (0.77).



Graphic 9 - Ratio FRV/PA in the accessions of Alto Tavoliere area.

3.2.2.3. Hydroxycinnamyl tartaric acids content in the pulp of black-berry and withe-berry accessions of Monti Dauni and Gargano area

Tuccanese, the black-berry accession of Monti Dauni area, showed a high hydroxycinnamyl tartaric acids (HCTA) content (107.33 mg/L) (Graph. 10). Some black-berry accessions of Gargano area showed very similar values; this is the case of two genotypes of the Muscat family, that is, **Moscato Tamburro** and **Moscatiddone Nero** (107.22 and 117.44 mg/L, respectively). **Tinturino** showed HCTA content (105.56 mg/L) close to that of the Muscat group of varieties, while **Malvagia Nera** and **Uva Nera Tosta** reached values of about 91 mg/L. The lowest content was found in Uva della Macchia (42.44 mg/L), close to Bombino Nero falso (44.67 mg/L).

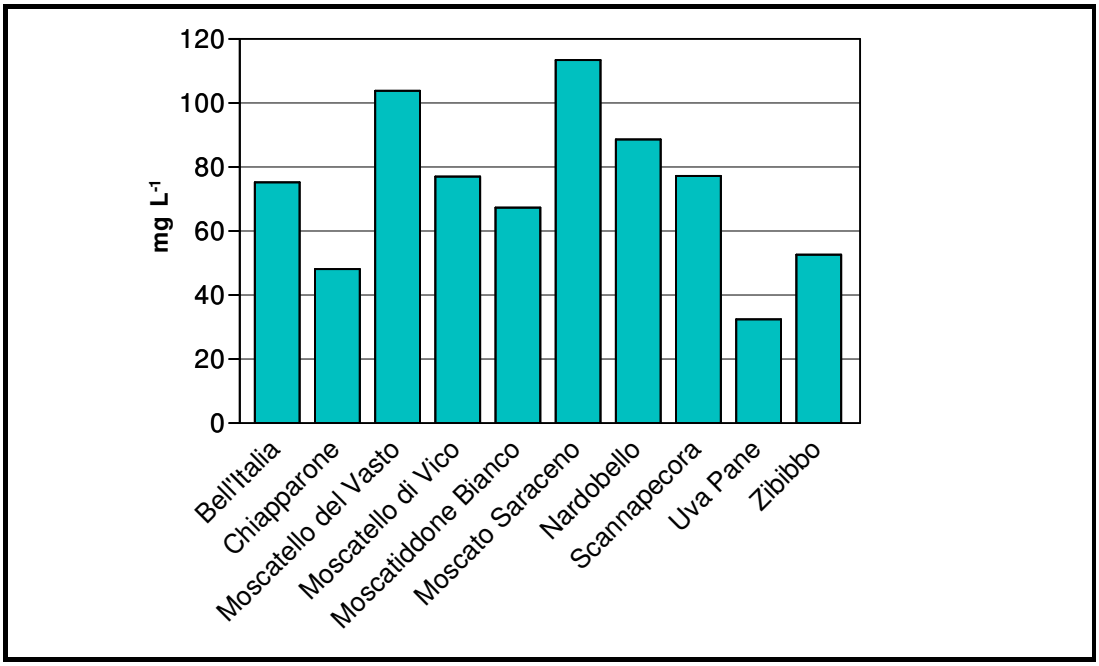


Graphic 10 - HCTA content in the pulp of the black-berry accessions of Gargano and Monti Dauni areas (millignams of caffeic acid per liter of pulp juice).

The white-berry accessions had HCTA content lower than that of the black-berry genotypes (-12% on average) (Graph. 11). The HCTA content ranged from 32 mg/L in **Uva Pane** to 113 mg/L in **Moscato Saraceno**. **Chiapparone** (same genetic profile of Moscato Saraceno) and **Zibibbo** (50 mg/L) showed values quite close to the lowest threshold, while **Moscatello del Vasto** (104 mg/L) was quite close to the highest one. All the other accessions had intermediate values. It is to notice that

also among the white-berry accessions, the several Muscat genotypes showed the higher HCTA concentration (Moscato Saraceno. Chiapparone and Moscatelo del Vasto).

Hydroxycinnamic acids are precursors of volatile phenols, but also the main substrates responsible for the oxidative browning of musts: a low HCTA content enhances the winemaking aptitude of grapes, even though the browning phenomenon depends not only by their concentration, but also by the relationship between HCTA and the antioxidant compound concentration in the musts.



Graphic 11 - HCTA content in the pulp of the white-berry accessions of Gargano area.

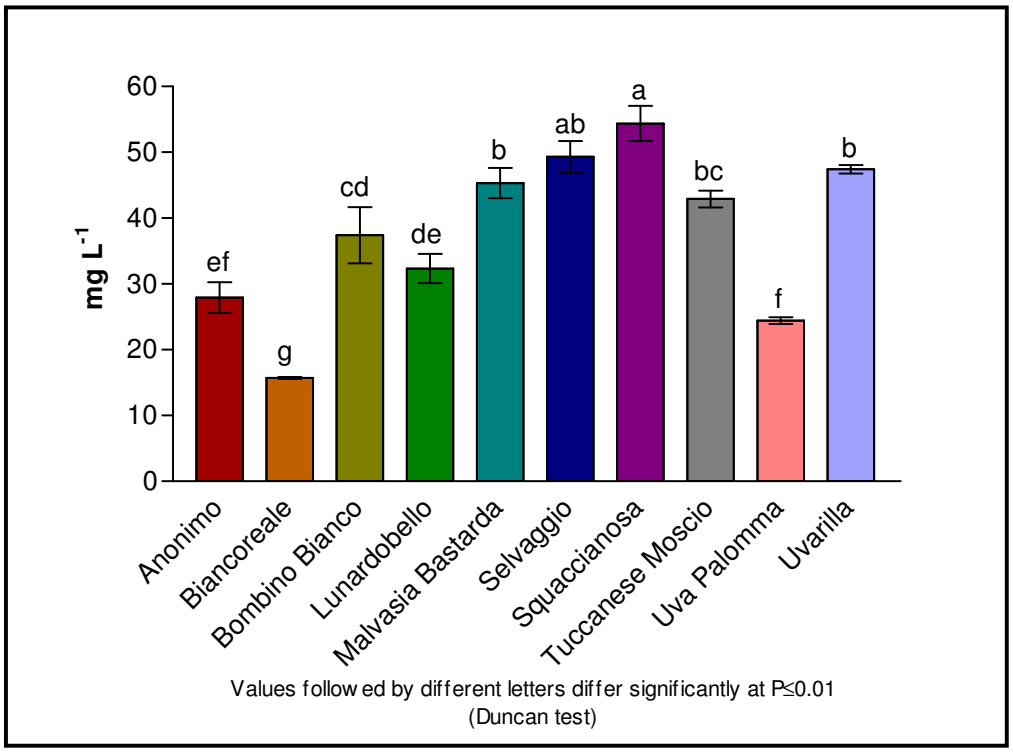
3.2.2.4. Hydroxycinnamyl tartaric acids content in the pulp of with-berry accessions of Alto Tavoliere area

The HCTA content (Graph. 12) ranged between about 16 mg/L in **Biancoreale** (very low value) and about 55 mg/L in **Squaccianosa**.

Uva Palomma (24.5 mg/L) and **Anonimo** (28.0 mg/L) showed values similar each to other, as well as **Malvasia Bastarda** (46.0 mg/L) and **Uvarilla** (47.5 mg/L). The HCTA average content of **Lunardobello** (32.30 mg/L) and **Tuccanese Moscio** (43 mg/L) was similar to that of **Bombino**

Bianco (37.40 mg/L). Finally, **Selvaggio** (49.30 mg/L) showed a HCTA content statistically similar to that of Squaccianosa.

Overall, the HCTA content was lower than that of all he white-berry accessions of the Gargano area.



Graphic 12 - HCTA content in the pulp of the accessions of Alto Tavoliere area (millignams of caffeic acid per liter of pulp juice).

3.3. Italian Vitis Database description

Microsatellite profiles of all the investigated accessions, together with other descriptive information, were entered in the IVD.

Since it is not possible to report all data, one of the investigated genotypes is illustrated below, as example. It has been chosen Selvaggio, a white-berry grapevine found in the Ato Tavoliere Dauno area and identified as Bianco d'Alessano.

Bianco d'Alessano

release 13/05/2015, **updated** at 2015-07-14 12:29:06
url http://www.vitisdb.it/varieties/show/1101

Information managed by

Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente (SAFE)-Università degli Studi di Foggia

How to cite this source

de Palma L., Tarricone L., De Michele M., Sacco L., Limosani P., Carparelli P., 2014. Bianco d'Alessano. In Italian Vitis database http://it.grapedb.org ISSN 2282-006X

Botanical information

Name: **Bianco d'Alessano**
Genera: **Vitis**
Type of origin: **spontanea**
Subspecie: **sativa**
Specie: **Vitis vinifera**
Variety: **for wine**
Variety group: **Neutre**
Trueness to type: **confirmed by ampelography and SSR-markers**
Variety.code: **IVD-var_30**

Registration

Registered in the National Catalogue: **yes**
Code: **028**
Official name: **Bianco d'Alessano**

Synonyms

Documented synonyms (2)
synonyms documented by the Istitution that appear with the eventual support of the literature

Verdurino **Acchiappapalmento**

Released clones (2)

I - CRSA - Regione Puglia C2 **I - CRSA - Regione Puglia C5**

Historical references

Variety of undefined origin and of ancient cultivation in the Puglia region; in the "Bullettino Ampelografico" Frojo (1875) mentions the grape variety Butta palmento (Lecce) with its synonyms Bianco di lassame (Trani, Andria, Barletta), Bianco di palmento (Taranto province), Acchianca palamento (Ostuni, in Brindisi province) and Bianco d'Alessano (Carovigno, in Brindisi province).
Bianco d'Alessano, possibly starting from the Lecce province (where Alessano town is located), wasspread aroundother viticulturalareas likelybecause of its highproductivity, not for other features. In facts, Frojo described this variety asvery productive, but sensitive to rot and poor of flavors and other quality traits.
According to Carparelli and collaborators (2006), in the '40sBianco d'Alessano growing lost part of its importance in favor of more profitable crops, while,in the '50s, it arouse new interest since the neutral wines had a good remuneration.

Distribution & variation

Del Gaudio and Nico (1960) date back to 1870 the Bianco d'Alessano growingin the Martina Franca area (Taranto province), mention the same town and Crispiano as the most important for the cultivation of thisvariety and report its constant association with cv. Verdeca, considered by growers as even more productive and by wine-makersas suitable to confera light green colorto the "Martina Franca wine".

Presently, Bianco d'Alessanois grown almost exclusively in Puglia, especially in "Valle di Itria". It is included amongthe varieties suitable for cultivation into all the Apulian viticultural areas. According to data of the Italian Census of Agriculture 2010, Bianco d'Alessano is grown in Apulia on411 hectares, 67% of which (equally distributed amongthe provinces of Brindisi, Bari and Taranto) are destined to the production of DOC wines. At the begenning of '70s the surface interested by this variety was about 3830 hectars.

Technological use

Bianco d'Alessano wines are neutral, dry, with pale yellow color, suitable as base for vermouth wines, not suitable for aging.

The adoption of proper viticultural and oenological techniques allowed to obtain by this variety interesting wines, with golden hues, preservable from oxidation.

Grapes show average sugar accumulation about 20 °Brix, titratable acidity 5-6 g/L, pH 3.4-3.6.

This cultivar is included in the variety base of Apulian DOC wines Lizzano, Locorotondo, Martina Franca and Gravina and IGT Puglia, Daunia, Murgia, Valle d'Itria, Tarantino, Salento.

Bibliographies (3)

Carparelli P., Moretti G., Tarricone L., Gardiman M., 2006. Rivalutazione enologica del Bianco di Alessano. Atti informatici Convegno Nazionale "I vitigni autoctoni minori: aspetti tecnici, normativi e commerciali". Torino (Villa Gualino), 30 novembre-1 dicembre 2006.

Del Gaudio S., Nico G., 1960 Bianco d'Alessano. Principali vitigni da vino coltivati in Italia - Volume I, Ministero dell'Agricoltura e delle Foreste.

Frojo G., 1875. Relazione sugli studi ampelografici eseguiti nelle Puglie. Bullettino Ampelografico, Ministero d'Agricoltura Industria e Commercio, Anno 1875 Fascicolo I. Tipografia Eredi Botta, Roma, 1876.

Selvaggio

release 08/06/2015, **updated at** 2016-01-04 11:31:35
url http://www.vitisdb.it/accessions/show/15646

Information managed by

Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente (SAFE) - Università degli Studi di Foggia

How to cite this source

de Palma L., Poli G., De Michele M., Limosani P., 2013. Selvaggio. In Italian Vitis database http://it.grapedb.org ISSN 2282-006X

General information

Name: **Selvaggio**

Code: **ITA428-TM4/11**

Country of selection: **Italia**

Region of selection: **Puglia**

Provincial of selection: **Foggia**

Locality of selection: **Torremaggiore (FG)**

Holding institution: **Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente (SAFE) Università degli Studi di Foggia**

collection vineyard: **Az. Fortore, Torremaggiore (FG)**

Variety & clone

Type of origin: **spontanea**

Genera: **Vitis**

Specie: **Vitis vinifera**

Subspecie: **sativa**

Variety: **Bianco d'Alessano**

Clone: **n.a.**

Trueness to type: **confirmed by ampelography and SSR-markers**

Microsatellite profile

loci:	**predefined loci (9)**																	
SSR locus:	**VVS2**		**VVMD5**		**VVMD7**		**VVMD27**		**VrZAG62**		**VrZAG79**		**VVMD25**		**VVMD28**		**VVMD32**	
allele:	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
size:	143	145	231	231	240	254	182	186	190	202	243	259	240	254	239	249	259	273

Standardized microsatellite profile

loci:	predefined loci (9)																	
SSR locus:	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79		VVMD25		VVMD28		VVMD32	
allele:	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
size:	143	145	231	231	239	253	181	185	188	200	243	259	242	256	239	249	259	273

Other locus info available online

Images

Shoot

leaf

bunch

berry

Related bibliography (2)

de PALMA L., LOPRIORE G., TARANTINO A., TARRICONE L., SOLETI F., POLI G., 2010. Caratteristiche di genotipi viticoli a bacca bianca reperiti nell'area della D.O.C. "San Severo". Ital. Hort. vol. 17 (suppl. al n.3), 334-338. Atti II Convegno Nazionale di Viticoltura, Marsala (T), 14 -19 luglio 2008.

de PALMA L., LIMOSANI P., DE MICHELE M., TAMBORRA P., TARRICONE L., NOVELLO V., 2011. Evaluation of experimental wines obtained from "new old" white-berry genotypes of the Northern province of the Apulia region. Le Progrès Agricole et Viticole vol. 128, 477-484.

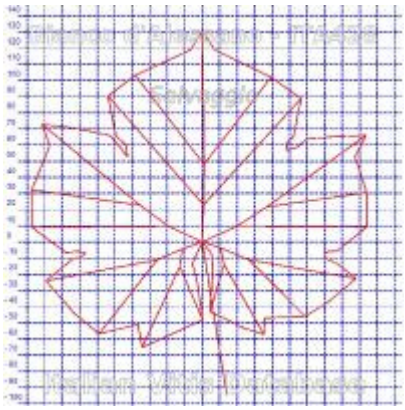
Accessions of the same variety (2)

- **Bianco d'Alessano** - Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente (SAFE) - Università degli Studi di Foggia
- **Selvaggio** - Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente (SAFE) - Università degli Studi di Foggia

Ampelography

OIV	Description	Value	
001	Young shoot: opening of the shoot tip	1	closed
003	Young Shoot: intensity of anthocyanin coloration on prostrate hairs of tip	5	medium
004	Young Shoot: density of prostrate hairs on tip	5	medium
006	Shoot: attitude (before tying)	5	horizontal
007	Shoot: color of dorsal side of internodes	2	green and red
008	Shoot: color of ventral side of internodes	1	green
016	Shoot: number of consecutive tendrils	1	2 or less
051	Young leaf: color of the upper side of blade (4 th leaf)	2 / 3	yellow / bronze
053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)	7 / 9	high / very high
067	Mature leaf: shape of blade	2 / 4	wedge-shaped / circular
068	Mature leaf: number of lobes	2 / 3	three / five
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
075	Mature leaf: blistering of upper side of blade	3 / 5	weak / medium
076	Mature leaf: shape of teeth	3 / 5	both sides convex / mixture between both sides straight (note 2) and both sides convex (note 3)
079	Mature leaf: degree of opening / overlapping of petiole sinus	3 / 5	open / closed
080	Mature leaf: shape of base of petiole sinus	1 / 3	U-shaped / V-shaped
081-1	Mature leaf: teeth in the petiole sinus	1	none
081-2	Mature leaf: petiole sinus base limited by veins	1	not limited
083-2	Mature leaf: teeth in the upper lateral sinuses	1	none
084	Mature leaf: density of prostrate hairs between the main veins on lower side of blade	5	medium
087	Mature leaf: density of erect hairs on main veins on lower side of blade	5	medium
151	Flower: sexual organs	3	fully developed stamens and fully developed gynoecium
153	Inflorescence: number of inflorescences per shoot	2	1,1 to 2 inflorescences
155	Shoot: fertility of basal buds (buds 1-3)	5 / 9	medium (1,1-1,3) / very high (>1,9)
202	Bunch: length (peduncle excluded)	5 / 7	medium / long
204	Bunch: density	5 / 7	medium / dense
206	Bunch: length of peduncle of primary bunch	3	short
208	Bunch: shape	1	cylindrical
209	Bunch: number of wings of the primary bunch	2 / 3	1 - 2 wings / 3 - 4 wings
220	Berry: length	3	short
221	Berry: width	3	narrow
223	Berry: shape	2	globose
225	Berry: color of skin	1	green yellow
231	Berry: intensity of flesh anthocyanin coloration	1	none or very weak
236	Berry: particularity of flavor	1	none
241	Berry: formation of seeds	3	complete

Ampelometry



ampelometric leaf

OIV	Description	Value	
601	Mature leaf: length of vein N1	5	medium (135 mm)
602	Mature leaf: length of vein N2	7	long (125 mm)
603	Mature leaf: length of vein N3	7	long (95 mm)
604	Mature leaf: length of vein N4	9	very long (55 mm and over)
605	Mature leaf: length petiole sinus to upper lateral leaf sinus	5	medium (70 mm)
606	Mature leaf: length petiole sinus to lower lateral leaf sinus	7	long (75 mm)
607	Mature leaf: angle between N1 and N2 measured at the first ramification	7	large (56°-70°)
608	Mature leaf: angle between N2 and N3 measured at the first ramification	5	medium (46°-55°)
609	Mature leaf: angle between N3 and N41) measured at the first ramification	7	large (56°-70°)
610	Mature leaf: angle between N3 and the tangent between petiole point	9	very large (> 70°)
612	Mature leaf: length of tooth N2	5	medium (14 mm)
613	Mature leaf: width of tooth N2	7	wide (18 mm)
614	Mature leaf: length of tooth N4	3	short (10 mm)
615	Mature leaf: width of tooth N4	5	medium (14 mm)
617	Mature leaf: length between the tooth tip of N2 and the tooth tip of the first secondary vein of N2	7	long (56-70 mm)

Superampelo		
Distances		
Descriptor	**Value**	**Standard deviation**
Base of the tooth located at the end of N2'	17.250	5.471
Distance between petiole point and end of vein N4'	74.433	4.625
Leaf length Including the petiole	226.450	10.345
Base of the tooth located at the end of N4	13.750	2.982
Distance between the tooth tip of N2 and the tooth tip of the first ramification (secondary vein) of N2	65.133	12.375
Height of the tooth on the end of N4	8.633	2.115
Vein N3, length from the petiole sinus to vein N4	11.150	2.430
Distance between petiole point and end of vein N4	67.583	4.445
Length of vein N3	91.183	7.584
Petiole length	96.000	10.773
Distance between the tooth tip of N2' and the tooth tip of the first ramification (secondary vein) of N2'	61.583	15.725
Height of the tooth on the end of N2	13.783	2.032
Length of vein N5	30.383	5.896
Height of the tooth on the end of N4'	11.450	1.601
Length of vein N1	130.467	6.924

Distance between the ends of veins N2 and N2'	185.617	17.394
Distance from the petiole sinus to the lower right sinus	69.950	7.711
Vein N3', length from the petiole sinus to vein N4'	12.367	2.968
Distance between the ends of veins N4 and N4'	70.833	9.701
Base of the tooth located at the end of N2	17.483	1.686
Base of the tooth located at the end of N4'	17.067	1.595
Width of petiole sinus / Distance between points SP and SP'	-14.350	6.021
Length of vein N4	59.317	5.638
Length of vein N2	114.750	8.347
Distance from the petiole sinus to the upper right sinus	74.550	10.872
Length of vein N5'	35.967	5.316
Length of vein N2'	117.837	6.702
Leaf width	189.917	12.691
Altezza del dente posto all'estremità di N2'	13.250	4.486
Distance between the ends of veins N3 and N3'	177.733	8.917
Leaf length	197.117	8.685
Length of vein N4'	65.767	4.139
Distance from the petiole sinus to the lower left sinus	67.533	9.824
Length of vein N3'	93.250	5.745
Distance from the petiole sinus to the upper left sinus	67.883	11.350

Angles

Descriptor	Value	Standard deviation
Angle between N2' and N3' measured at the ends of the veins	53.450	5.131
Angle between S and S' with the center in N1	62.533	13.672
Angle between N3 and N4 measured at the ends of the veins	43.617	5.248
Angle between N2 and N3' measured at the first bifurcation	46.367	7.735
Angle between D and D' with the center in N1	106.867	5.724
Sum of the angles alpha' + beta' + gamma'	169.350	15.676
Angle between N3 and N4 at the first fork of N3	55.083	7.839
Angle between N1' and N2' measured at the ends of the veins	51.433	7.853
Sum of the angles alpha' + beta'	111.250	10.317
Angle between N1 and N2 measured at the ends of the veins	54.750	3.624
Sum of the angles alpha + beta	106.883	4.711
Angle between N2 and N3 measured at the first bifurcation	44.717	6.197
Angle between N2 and N3 measured at the petiole point and between N2' and N3' tooth tip	74.967	5.000
Angle between N3' and N4' measured at the ends of the veins	47.200	2.694
Sum of the angles alpha + beta + gamma	161.933	11.338
Angle between N3' and N4'	58.117	6.209
Angle between N1 and N2 measured at the first bifurcation	62.117	4.309
Angle between N2 and N3 measured at the ends of the veins	49.800	7.758
Angle between N1 and N2' measured at the first bifurcation	64.850	4.992
Angle of opening of the petiole sinus measured at SP and at SP'	28.583	17.721
Angle between N2 and N3 measured at the petiole point and between N2 and N3 tooth tip	69.150	7.959
Angle between I and I' with the center in N1	52.550	5.139

Rations

Descriptor	Value	Standard deviation
Ratio between the distance from the petiole sinus to the lower left sinus OI' and the length of vein N3'	0.726	0.108
Ratio between the length of the vein N2' and the length of the vein N1	0.904	0.041
Ratio between the length of the vein N5' and the length of the vein N1	0.277	0.046
Ratio between the length of the vein N3 and the length of the vein N1	0.699	0.051
Ratio between the length of the petiole OP and the length of the vein N1	0.739	0.102
Ratio between the height and the base of the tooth at the end of the vein N4	0.669	0.285
Ratio between the length of the vein N2 and the length of the vein N1	0.879	0.035
Ratio between the length of the vein N5 and the length of the vein N1	0.233	0.043
Ratio between the length of the vein N3' and the length of the vein N1	0.718	0.072
Ratio between length and width of the leaf	1.039	0.026

Ratio between the distance from the petiole sinus to the lower right sinus OI and the length of vein N3	0.770	0.088
Ratio between the height and the base of the tooth at the end of the vein N4'	0.671	0.083
Ratio between the height and the base of the tooth at the end of the vein N2'	0.764	0.082
Ratio between the length of the vein N4 and the length of the vein N1	0.455	0.043
Ratio between the sum of the angles a' + b' and the sum of the distance between the petiole sinus and upper right sinus OS' and the petiole sinus and lower right lower right sinus OI'	0.015	0.003
Ratio between the distance from the sinus and the length of the vein N2'	0.580	0.118
Ratio between the height and the base of the tooth at the end of the vein N2	0.789	0.104
Ratio between the distance from the sinus and the length of the vein N2	0.650	0.083
Multiplication between length and width of the leaf	37522.770	4244.780
Ratio between the sum of the angles a + b and the sum of the distance between the petiole sinus and upper right sinus OS and the petiole sinus and lower right lower right sinus OI	0.013	0.002
Ratio between the length of the vein N4' and the length of the vein N1	0.506	0.049

Phenology & Production

OIV	Description	Value	
301	Time of bud burst	5	medium
303	Time of beginning of berry ripening (veraison)	5	medium
351	Vigor of shoot growth	5 / 9	medium (70-80 g) / very strong (>110 g)
502	Bunch: weight of a single bunch	3	low (250-350 g)
503	Berry: single berry weight	3	low (2,5-3,5 g)
504	Yield per m2	5 / 7	medium (1,4-1,6 kg) / high (1,8-2,0 kg)
505	Sugar content of must	5	medium (17,2-18,8)
506	Total acid content of must	3	low (5,2-6,8)
508	Must specific pH	7	high (3,4-3,5)

Vegetative productive

Plant spacing & training system	Value	Standard deviation	Number of years
Training system	Counter-espalier		
Pruning System	Guyot		
Distance between rows (m)	2.500		
Distance on the row (m)	1.000		
Vigor	**Value**	**Standard deviation**	**Number of years**
Number of shoots/canes per vine (number/vine)	12.000		2
Number of shoots/canes per meter of row (number/m)	12.000		2
Cane’s weight (g)	75.000		2
Pruning wood’s weight per vine (kg)	0.900		2
Pruning wood’s weight per vine meter of row (kg)	0.900		2
Production's quantitative characteristics	**Value**	**Standard deviation**	**Number of years**
Bunch’s weight (g)	247.670	21.079	3
Grape production per vine (kg/ceppo)	2.700		2
Number of bunches per meter of row (number/m)	10.240		2
Grape production per meter of row (kg/m)	2.700		2
Grape production per hectare (t/ha)	10.800		2
Weight of 100 berries (g)	203.000		2
Berry diameter (average of 25 berries) - width (mm)	14.600		2
Berry diameter (average of 25 berries) - height (mm)	14.200		2
Number of bunches per vine (number/vine)	10.240	0.817	3

Production's qualitative characteristics	Value	Standard deviation	Number of years
Skin total flavonoids (g/kg)	0.469		2
Seeds total flavonoids (g/kg)	1.042		2

Skin total polyphenols (g/kg)	0.609		2
Seeds total polyphenols (g/kg)	0.988		2
pH (pH)	3.740	0.231	4
Titratable acidity of must (g/l)	6.120	0.963	4
Sugar content of must (°Brix)	18.800	0.844	4

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4. CHAPTER: FINAL REMAKS

The 35 accessions found in three different areas of the Foggia province, most of which are white-berry genotypes, showed 30 different genetic profiles; the remaining profiles overlapped someone of the others.

Comparing the 30 genetic profiles with those included in international databases or with those detected by other scientific Institution, it has been possible to identify 23 different genotypes. Most of them (87%) were found to match cultivars enrolled in National Catalogue of Grapevine Varieties (RNVV); hence, the names by which they are known in the Foggia province are synonyms of official varieties. The remaining genotypes (13%) are not enrolled in RNVV. The genetic profile of the other 7 genotypes was not found in any database; thus, by now, each of these accessions can be considered as being a "unique genotype".

Among the accessions that have been found to be synonyms of varieties enrolled in RNVV, 30% are table grape varieties, 10% are the dual-purpose varieties, while the majority (60%) are the wine grape varieties. Some of these latter, such as Selvaggio, Lunardobello, Squaccianosa and Sanguinella, are typical of the Apulia region and correspond to the official varieties named Bianco d'Alessano, Bombino Bianco, Minutolo, Primitivo. Other wine grape accessions, such as Moscatello del Vasto and Tuccanese, correspond two of the main Italian varieties, that is, Moscato Bianco and Sangiovese, widely grown all the national viticultural areas. One accession, i.e. Bell'Italia, is typical of Sicily region and officially called Damaschino. Finally, some other accessions such as Scannapecora, are widespread in central part of Italy with the official name of Malvasia Bianca Lunga, while Malvasia Bastarda is grown in several Southern regions with the official name of Malvasia Bianca.

The other accessions identified, but not enrolled in the RNVV, correspond to genotype grown in France (Tinturino identified as Petit Bouschet), in Abruzzo, Chieti Province (Bombino Nero falso

identified as Uva Nera Antica), or also in Puglia, Bari province (Uva Palomma and Uva Sagra, both identified as Palumbo or Uva Carrieri).

As concerns the oenological potential of the accessions, all of them, excluding the table grape genotypes, showed interesting traits. The accessions identified as synonymous of varieties enrolled in the RNVV produced grapes with positive attribute from both the point the technological and the phenolic of view: in many cases, their analytical values were found very similar to those typical of the variety to which they correspond, despite the different agro-environmental conditions in which they were grown. This means that these genotypes are adapted to several environments and that, generally speaking, their grapes have quite stable qualitative features.

Among the accessions considered by now as "unique genotypes", **Nardobello** (white berry), thanks to its performing technological and phenolic traits, should be suitable for the production of mono-varietal wines with a good level of alcohol, stability, structure, color and flavor. **Tuccanese Moscio** (white berry) showed a good attitude for sugar accumulation in berry juice and proanthocyanidins in berry skin: it could be suitable used for the production blended wines but perhaps also for mono-varietal wines. **Malvagia Nera** (black berry), thanks to the high sugar content, could be used to produce high alcoholic wines; moreover, the excellent content in anthocyanins and proanthocyanidins are expected to have a positive effect on color, structure and stability. However, it showed a tendency for low must acidity that could be contrasted, in the vineyard, by growing a leafier canopy to protect grapes direct insolation, or by applying supplementary irrigation, in order to contain the increase of temperature and, thus, the acid oxidation. Finally, **Uva Nera Tosta** (black berry), showed an overall satisfactory potential for the oenological use of its grape, very close to that of Malvagia Nera; also in his case the grape could be used to produce blended wines but, may be, also for mono-varietal wines. Obviously, the propagation of these latter four varieties and the and utilization of their grapes is conditioned to their previous enrolment in the RNVV, that is, to the demonstration of their validity and suitability

for cultivation and for wine-making and to the acceptance of these demonstration by a national board of scientists and specialists.

In conclusion, the first part of this study has highlighted the richness of old grapevine genotypes grown in the Foggia province. However, most of these genotypes have resulted to be typical not only of this area, but also of other areas of the Apulia, or of areas of other Italian regions, or sometime of foreign regions. Evidently, these genotypes have found climate and soil conditions suitable for their growth and production, becoming naturalized in the environment of the Daunia area.

The second part of this study has highlighted the oenological skills of the grape produced by these genotypes, analyzing the technological and the phenolic traits that may be useful to support the making of mono-varietal wines or that of wines obtained by blending more local varieties.

The third part of this research activity has been the entering of all the data in the Italian Vitis Database; this fact that makes all the information available for farmer, technicians and scientists interested in the grapevine growing.