

Enhancement of the Tuscan grapevine germplasm: Nocchianello Bianco and Nocchianello Nero cultivars

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Abstract. As part of a decades-long project to safeguard the viticultural biodiversity in the wine-growing district of Pitigliano (Tuscany, Italy), the research activity carried out made it possible to rediscover two valuable autochthonous grapevine varieties, otherwise doomed to extinction: Nocchianello Bianco (white-berried) and Nocchianello Nero (black-berried). Both the cultivars were thoroughly characterized at the genetic, phenological, and ampelographic levels, as well as the yield and grape quality were evaluated. Thanks to the results obtained, Nocchianello Bianco and Nocchianello Nero were officially reintroduced in the local and regional winemaking scenario, following the registration in the Italian Catalogue of Grapevine Varieties and the addition to the list of grapevine varieties suitable for cultivation in Tuscany.

1. Introduction

Nocchianello Bianco and Nocchianello Nero are two old autochthonous grapevine varieties recovered in the area around Pitigliano (Tuscany, Italy), where an important project to preserve the viticultural genetic heritage was carried out in the last decades of the past century [1]. The vine-growing area included in the renowned “Maremma Toscana” territory has a very remote tradition that dates to the Etruscan period [2] and has not undergone substantial changes until the phylloxera invasion at the end of 1800. Subsequently, in the 1960s, the renewing process of the vineyards with allochthonous international cultivars and the transition to specialized cultivations led to a further reduction of the viticultural biodiversity. Even the production regulation of the Bianco di Pitigliano denomination of controlled origin wine, widely used in this wine district, has further helped reduce the cultivated varieties, limiting them mainly to Trebbiano Toscano, Verdello, and Malvasia Bianca Lunga [3].

The information about Nocchianello Bianco and Nocchianello Nero in the literature is very scarce; according to the opinion of elderly local winegrowers, the etymology of both cultivar names could derive from the Italian word “nocca” or “noccola” (meaning knuckle), probably referring to the bunch shape, as it resembles a hand closed in a fist.

Exploiting the existing grapevine germplasm collection created as part of the previous recovery project [1], we recently pursued the research activity to confirm the genetic fingerprinting of Nocchianello Bianco and Nocchianello Nero grape cultivars and to evaluate their ampelographic, phenological, productive, and qualitative characteristics ahead of the safeguard from extinction and a relaunch on the local winemaking scenario.

2. Materials and methods

2.1 Experimental vineyard characteristics

Nocchianello Bianco and Nocchianello Nero vines were planted in 1995 in a vineyard collection set up in the municipality of Pitigliano (Grosseto, Italy).

The vineyard had a medium dough of volcanic origin soil (tuff and pumice) with a sandy texture and no active limestone. The vines were grafted on SO4 rootstock and were grown with a single high wire trellis system.

In this area, the climate is characterized by low minimum temperatures during winter and intense heat with low relative humidity in summer. Pest management was normally scheduled with calendar sprays at 10-day intervals.

2.2 Genetic analyses

Thirteen SSR markers were used for genotyping: the nine proposed as common grape markers for international use within the framework of the GrapeGen06 European project (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79) [4], plus VMC6E1, VMC6F1, VNC6G1 [5], and VMCNG4b9 [6]. The SSR analyses were performed following the protocol of [7]. True-to-type identifications were validated by comparing the obtained SSR profiles with those stored in the CREA-VE molecular database.

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2.3 Phenology, ampelography, and ampelometry

The main phenological growth stages (bud break – BBCH 09; flowering – BBCH 65; veraison – BBCH 83; maturity – BBCH 89) [8] of Nocchianello Bianco and Nocchianello Nero vines were observed recording the dates as the day of the year (DOY) for five consecutive vegetative seasons, in the time frame 2013-2017.

The ampelographic characteristics of the main vine organs were described according to the recommended OIV - International Organisation of Vine and Wine methodology [9], and the ampelometric measurements on adult leaves were carried out using the software SuperAmpelo [10].

2.4 Yield and grape quality

Yields and grape qualitative data were evaluated for three consecutive years, corresponding to 2015-2017 vintages. At the harvest, the main production traits were determined: yield *per vine* (kg), clusters number *per vine*, cluster weight (g), and berry weight (g). Then, the technological maturity of grapes (sugar content - °Brix; total acidity – g/L tartaric acid; pH) was assessed. The parameters observed were compared with Trebbiano Toscano and Sangiovese, two Tuscan (white-berried and red-berried, respectively) grape varieties present as reference cultivars in the same experimental vineyard.

Lastly, Nocchianello Nero berry skins extracts were used to analyse the anthocyanin profile by HPLC following the method of [11]. Also in this case, a comparison with Sangiovese was carried out to check the typical differences of the variety under observation.

3. Results and Discussion

3.1 Genetic identification

According to the molecular results, Nocchianello Bianco and Nocchianello Nero were confirmed as two independent genotypes with unique microsatellite profiles (Table 1), perfectly matching the reference SSR profiles already present in the CREA-VE molecular database.

Despite the homonymy, Nocchianello Bianco and Nocchianello Nero turned out to be genetically distant from each other; except for the identical allele lengths in the locus VVS2, they share only one allele in few other SSR loci (highlighted in bold in Table 1).

From further observations (data not shown), it was seen that Nocchianello Bianco has a higher number of alleles overlapping Trebbiano Toscano SSR profile, and the same happens between Nocchianello Nero and Sangiovese, suggesting a rather close relationship between the respective pairs of grapevine varieties.

Table 1. Microsatellite profiles at 13 SSR loci of Nocchianello Bianco and Nocchianello Nero cultivars (allele lengths in bp). The alleles matching between the cultivars are shown in bold.

Locus SSR	Nocchianello Bianco		Nocchianello Nero	
VVS2	133	133	133	133
VVMD5	228	246	226	226
VVMD7	239	239	239	247
VVMD25	245	245	243	243
VVMD27	191	194	179	194
VVMD28	247	251	231	237
VVMD32	253	257	253	253
VrZAG62	187	193	193	203
VrZAG79	244	248	248	248
VMC6E1	141	161	161	165
VMC6F1	133	139	131	145
VMC6G1	177	197	169	187
VMCNG4b9	158	158	152	162

3.2 Vine phenology

In both the cultivars considered, bud break (DOY range 91-100) and flowering (DOY range 145-161) dates occurred on average in overlapping periods (Fig. 1). On the contrary, veraison took place earlier for Nocchianello Bianco (DOY range 206-217) than for Nocchianello Nero (DOY 211-222). The grapes reached maturity with an average difference of 15 days between the two varieties (DOY range 248-258 for Nocchianello Bianco; DOY range 263-274 for Nocchianello Nero).

In general, the trend of the phenological growth stages of Nocchianello Nero is similar to Sangiovese but with a delay of about ten days in reaching full ripeness. Nocchianello Bianco has phenological growth stages comparable to Trebbiano Toscano, but it completes grape maturation about ten days before (data not shown).

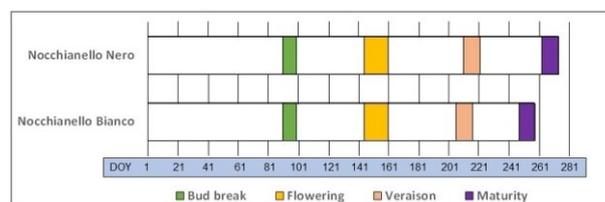


Fig. 1. Main phenological growth stages of vine development. The colored boxes represent the ranges recorded in five different vegetative seasons (2013-2017). DOY = day of the year.

3.3 Ampelography

Nocchianello Bianco has a medium-sized, pentagonal, five-lobed leaf blade with strong blistering on the upper side and a twisted profile. The bunch is medium-long and narrow, with medium density and conical shape with 1-2 wings. The berry is globose, medium-short in length and width, and has yellow-green color. The pulp is juicy,

colorless, and contains an average of 2 seeds. The skin has medium thickness and is slightly waxy (Fig. 2 A-C). Nocchianello Nero has a medium-large, pentagonal, five-lobed leaf blade with a wavy edge, strong blistering on the upper side, and twisted profile. The bunch has medium length, is narrow, dense, conical or funnel-shaped with 1-2 wings. The berry is globose, with medium size and blue-black color. The pulp is colorless, juicy, and contains an average of 2 seeds. The skin is thick and very waxy (Fig. 2 D-F).

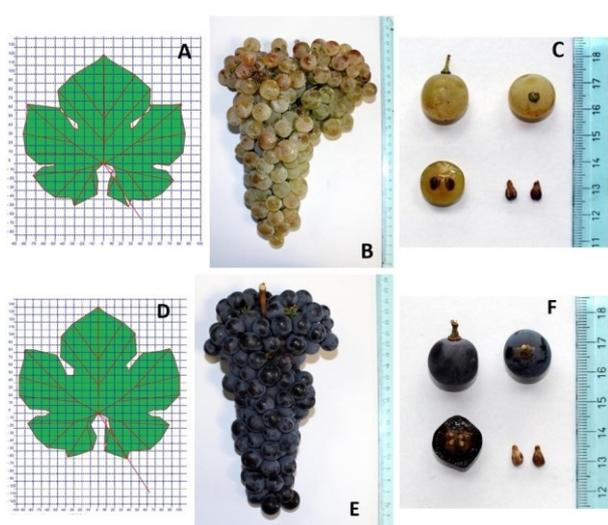


Fig. 2. Ampelometric profile of the adult leaf (A-D), photos of the mature bunch (B-E), berry and seed details (C-F) of Nocchianello Bianco and Nocchianello Nero, respectively.

3.4 Yield and grape quality

Nocchianello Bianco and Nocchianello Nero have shown good fertility, with medium vigor and regular grape productions in the different vintages considered (Table 2). Nocchianello Bianco was less productive than Trebbiano Toscano, with a lower yield *per vine* mainly due to a lower cluster weight (Table 2a). Nocchianello Nero also revealed a significantly lower grape production with respect to Sangiovese, which on average has a higher number of clusters *per vine* (Table 2b).

Both the cultivars have always reached appropriate sugar contents, maintaining good values of total acidity and pH, within an optimal range for winemaking. In detail, Nocchianello Bianco displayed must technological maturity characteristics similar to Trebbiano Toscano (Table 2a), while Nocchianello Nero had higher values of total acidity (with corresponding lower pH) than Sangiovese (Table 2b), an optimal feature given the growing environment, where the climate is abundantly warm during the ripening period.

Nocchianello Bianco has proven to be a variety exploitable for blending with local cultivars (for example, Trebbiano Toscano), perfectly suitable for the wines traditionally produced in the Pitigliano wine district.

On the contrary, Nocchianello Nero, given its interesting anthocyanin profile (Table 3) very different from Sangiovese and characterized by a prevalence of

malvidin (64%) and a high content of trisubstituted anthocyanins (86%), turns out to be proper for monovarietal winemaking. These features enable predicting high color stability in wines and, hence, great aptitude for medium-long aging.

Table 2. Production and technological maturity parameters of Nocchianello Bianco and Trebbiano Toscano (a), and Nocchianello Nero and Sangiovese grapes (b); 2015-2017 average values, \pm standard deviation.

(a)

Parameter	Nocchianello Bianco	Trebbiano Toscano
Yield <i>per vine</i> (kg)	3,59 \pm 0,36	4,66 \pm 0,52
Clusters <i>per vine</i> (n)	10 \pm 1,8	10 \pm 2,2
Cluster weight (g)	411 \pm 130	498 \pm 124
Berry weight (g)	2,54 \pm 0,95	2,22 \pm 0,55
Sugar content ($^{\circ}$ Brix)	21,7 \pm 1,3	22,0 \pm 1,6
Total acidity (g/L tartaric acid)	5,68 \pm 2,03	5,71 \pm 1,87
pH	3,16 \pm 0,18	3,27 \pm 0,09

(b)

Parameter	Nocchianello Nero	Sangiovese
Yield <i>per vine</i> (kg)	3,53 \pm 0,61	5,20 \pm 0,90
Clusters <i>per vine</i> (n)	15 \pm 3,3	18,5 \pm 4,2
Cluster weight (g)	229 \pm 56	231 \pm 37,4
Berry weight (g)	1,55 \pm 0,24	1,73 \pm 0,19
Sugar content ($^{\circ}$ Brix)	21,8 \pm 1,5	21,4 \pm 2,2
Total acidity (g/L tartaric acid)	7,15 \pm 1,06	5,98 \pm 1,55
pH	3,17 \pm 0,06	3,24 \pm 0,14

Table 3. Anthocyanin profile (%) obtained by HPLC of Nocchianello Nero and Sangiovese berry skins extracts.

Anthocyanin	Nocchianello Nero	Sangiovese
Cyanidin	1,75 %	21,80 %
Delphinidin	4,13 %	13,11 %
Peonidin	10,50 %	16,83 %
Petunidin	9,17 %	14,17 %
Malvidin	63,78 %	32,88 %
Σ acylated anthocyanins	10,67 %	1,21 %

4. Conclusions

The research undertaken has allowed completing the recovery activity, reaching the formal recognition of two valuable grapevine cultivars belonging to the Tuscan viticultural heritage, otherwise doomed to extinction. As a result, in 2017 Nocchianello Bianco and Nocchianello Nero were officially added to the Italian Catalogue of Grapevine Varieties [12] with the codes 858 and 859. Then, since 2018, both the cultivars were included in the list of grapevine varieties suitable for cultivation in the Tuscany region.

The reintroduction of these two ancient autochthonous cultivars can be considered an important achievement, not only for the protection of the local grapevine biodiversity but also for the enhancement of the viticultural tradition linked to the territory of origin.

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