

# NEW WHITE GRAPE VARIETY OBTAINED FROM SELF-POLLINATION OF THE "SAGRANTINO" RED GRAPE VARIETY FROM UMBRIA (ITALY)

## ABSTRACT

The high presence of heterozygosity in the *Vitis vinifera* species makes it useful in self-pollination as an instrument for genetic improvement (Mullins et al., 1992). The genetic recombination that is achieved with self-pollination may cause the appearance of new morphological characteristics (Todorov, 1981, 1987, 1992, 1995), allowing the use of the filial generation for the selection and creation of new varieties.

With the aim of increasing variability in the population of Sagrantino di Montefalco (a variety with a polyphenol content higher than those of all Italian varieties) (Mattivi et al., 2002b) and of selecting some genotypes with superior characteristics (best value anthocyanin/polyphenol), self-pollination of this cultivar was conducted in 1999. The recombinant genetic profiles of the progenies are useful for identifying the parents and the unknown origins of this ancient variety.

The study was carried out at the Azienda Agricola Arnaldo Caprai and provided, in 2006 and 2007, an examination of 250 genotypes selected from the 1,800 obtained by self-pollination. Thirty-seven of these showed white berry, in relation to the appearance of the recessive gene for color (Bonner and Oliveira, 1990).

From the analysis of the data collected in 2006 to 2007, it was observed that, starting with a red berry cultivar, genotypes were obtained with white berries that were very different from each other, with high variability in the considered parameters (production, phenology, acid levels, pH, and polyphenol content). To select individuals with white berry with different peculiarities both for phenological, qualitative and quantitative parameters were chosen six genotypes of the original 37 that were representative of the various groups identified by cluster analysis.

For these individuals, additional data were collected in 2010 that have revealed that, due to the type of selection, a good representation of the variability of the starting group was maintained, also allowing the identification of some genotypes that are particularly interesting for future enrollment in the national register of varieties.

**Keywords: self-pollination; segregation; genetic improvement; Sagrantino**

## INTRODUCTION

The concept of genetic erosion must be introduced to describe the Italian situation in terms of genetic resources with regard to the *Vitis vinifera* species. This erosion is represented by the constant loss of genetic variation associated with the abandonment of native varieties.

The invasion of Phyloxera, which entered European viticulture at the beginning of the last century, marked the beginning of this phenomenon (De Astis, 1937). An example of this phenomenon is well represented by the Tuscan situation, in which over a hundred varieties were planted in the early years of the twentieth century, while, as of today, fewer than 10 varieties occupy more than 95% of this viticultural area (Storchi et al., 2007).

The loss of variability is also emphasized in the face of the needs of modern viticulture, which requires grape varieties with specific characteristics, generally regulated by the demands of an increasingly globalized market (Mastromauro et al., 1992), which seems to favor wines produced with international grapes.

It is necessary to underline that biodiversity originates mainly from breeding, while anthropogenic selection, particularly for the *Vitis vinifera* species, has chosen and multiplied only biotypes of greater interest, causing the loss of the remaining germoplasm (Intrieri et al. 2007). Several studies show, however, that weak clonal selection allows growers to obtain an improved population while maintaining the initial variability (Bogoni et al., 1993 Valenti et al., 1994).

In this context, another tool to increase and improve the autochthonous germoplasm and to address the phenomenon of genetic erosion is self-pollination (Valenti et al., 1990). The *Vitis vinifera* species includes a series of highly heterozygous individuals, characterised by different alleles at many genetic loci (Filippetti et al., 1999); for this reason, this species is one of those, among the arboreal plants, that is best suited as a self-pollination tool for genetic improvement (Mullins et al., 1992).

The inbreeding that occurs as a result of self-pollination, particularly in the first generation, can result in the appearance of new characteristics (e.g. compactness of the cluster), making it useful to use filial generation for the establishment of new varieties (Todorov, 1981 1987, 1992, 1995).

The use of self-pollination is, therefore, interesting, with the aim of increasing the degree of genetic variability as well as developing some characteristics such as resistance to pests, the color, and the characteristics of the bunch and the berry (Bonner and Oliveira, 1990). Though the progeny often present signs of inbreeding depression, usually resulting in lower productivity (Poni, 1989), it is possible to achieve varietal improvement (seedlessness or muscat taste) through lines of selfing (Todorov et al., 2002).

## **MATERIALS AND METHODS**

In the present trial, the Sagrantino grape variety was studied. It is a native Umbria cultivar with red berries, late ripening, and the highest polyphenolic content among all the Italian cultivars (Mattivi et al., 2002b), with mean values of 4,000-5,000 mg/kg but often even higher. The test took place at the Arnaldo Caprai Montefalco (PG) farm.

With the aim of increasing the variability in the Sagrantino population and selecting some genotypes with improved features, such as the best value for the relation between anthocyanin and polyphenol, in 1999, a self-pollination trial was conducted with this cultivar. The genetic recombination that occurs with this technique can also help to identify the parents as well as the origins of this ancient vine, which are still unknown.

After the self-pollination, followed by the germination of the obtained seeds in protected cultivation, a field of mother plants was created. Using the wood of these vines for propagation, in 2003, it was decided to plant a vineyard in which there were 250 accessions. Of these 250, 37 were of the white grapes, resulting from the appearance of the recessive gene for color (Bonner and Oliveira, 1990). Twenty-one of these white grape genotypes have been identified as interesting for characterisation and possible selection.

The absence of contamination and the actual descendants of seedlings from the self-pollination of Sagrantino have been verified thanks to the characterisation of each individual new genotype for 10 microsatellite loci (VVS2, VVMD5, VVMD7, VVMD27, ZAG62, ZAG79, VVMD25, VVMD28, VVMD31, and VVMD32). These loci have been used previously for the characterisation of the germoplasm of company varietal collection.

At the same time as the selection and study of the red grape genotypes, the characterisation of the white grape genotypes was then carried to assess their features and characteristics and to identify any new varieties that may be interesting in increasing the heritage of grapes of the region. From

2006 to 2007, vegetative-productive values (production, average weight of the bunch, fertility, and phenology) and the analytical data of musts (sugars, pH, and acidity) were collected for the 21 white grape genotypes. The contents of extractable polyphenols in the skins and seeds were also determined using the method proposed by Mattivi et al. (2002a).

Through cluster analysis, an evaluation of the parameters considered to be most interesting to the characterisation (production, sugar content, acidity, pH and extractable polyphenols of the skins) was carried out, and the 21 genotypes with white berries were divided into three groups with similar characteristics for the aforementioned parameters. For each group, one or more genotypes were then chosen, with six genotypes representing the variability between clusters. The parameters most considered for the selection of the best genotypes of each group (obtained through the cluster analysis) were the production, the balance between the sugar component and the acidity, and the total extractable polyphenolic content of the skins. Additional data were then collected in 2010 for six of these genotypes, with the aim of definitively characterising them and evaluating the inscription of the most interesting for the National Register of Varieties.

Unfortunately, it was not possible to realize the microvinifications for each genotype identified because of the few available vinestocks for each of them. In 2010, however, three microvinifications were made by combining grapes from more of the selected genotypes. The groupings were established on the basis of the divisions that emerged through the cluster analysis used for the selection described above. The three resulting wines were then subjected to tasting by a panel expert to characterise the sensory profile of these genotypes derived from white Sagrantino grapes.

Statistical analysis was performed using the SPSS statistical software (SPSS, Inc., Chicago, IL), through which the cluster analysis was conducted; this statistical method consists of a multivariate analysis that brings together individuals with common characteristics associated with each one and only one group. For the sensory profile data, the applied method combined univariate ANOVA and the Duncan post hoc test.

## **RESULTS AND DISCUSSION**

### **Characterisation of the population (2006-2007)**

The white grape genotypes born from the self-pollination of Sagrantino show a large variability for the parameters considered, both in terms of phenology and for the vegetative-productive and qualitative parameters. The phenological data (not shown) reflect a wide heterogeneity in the development and maturation at different phenological stages, each of which delineates at least three groups: early, medium, and late. It is, however, difficult to generalize since genotypes with early budding do not necessarily show precocity in other phases, reaching in some cases even late maturation.

Data recorded for 2006 to 2007 show that self-pollination leads to depression in the seedlings (Poni, 1989); the yield (Graph 1) is, in fact, lower than 1 kg per plant for almost all of the genotypes: the production values are between 0.32 kg/vine (A2) and 1.83 kg/vine (B3), while the average value of the genotypes is 0.83 kg/vine. Moreover, there is a strong correlation between productivity and the

average weight of the bunch, which have two overlapping trends (Figure 1). Considering the average weight of the cluster (Figure 1), two genotypes (B6 and B3) show the highest values of the population, respectively, 176 and 145 g, while nine genotypes record an average weight of the bunch between 94 g (B4) and 124 g (A6), above the average of the population (91 g). Ten genotypes are identified as having weights that are lower than average and ranging between 41 g (A2) and 91 g (B5).

In analyzing the data of the grapes and musts from 2006 and 2007, while representing each genotype as a variety in its own right, the influence of the parent Sagrantino can be observed; the majority of white grape genotypes share a high accumulation capacity for both sugars (Figure 2) (average 23.9 ° Brix) and polyphenols (Graph 3) (mean extractable polyphenols 3,948 mg/kg).

A large group of genotypes (13) reaches sugar levels between 21.9 and 24 ° Brix (Graph 2); between them, six genotypes retain an appreciable acidity (between 6.9 and 7.8 g/L) (Graph 2), with the remaining seven recording acidic values between 4.7 and 6.3 g/L. Seven genotypes reach high sugar concentrations ranging between 24.9 and 26.4 ° Brix, and in this case, some (B7, A1, and C2) keep good acidity (between 7 and 7.3g/L); the remaining four show instead acidic values between 4.9 and 6.2 g/L. The behavior of the genotype B6 is interesting to note because of its sugar accumulations that are appreciably lower than the average (20.3 ° Brix) and an acidity of 5.5 g/L (Graph 2).

The total extractable polyphenolic content, though these genotypes present white grapes, is generally high (Graph 3), but with wide variability: four genotypes are characterised by high polyphenolic content, between 4,936 (E1) and 5,594 mg/kg (B7), fourteen present an average content in relation to the population, ranging from 3,096 (B3) to 4,705 mg/kg (C2), and three genotypes show values well below the average, with values of 2,598 (B6), 2,725 (A6), and 2,793 mg/kg (B2).

It is also interesting to put in evidence the relationship between the polyphenols of the skins and those of the seeds: for a white grape variety, excluding the maceration of the solid parts, the extraction of polyphenols from grape seeds is negligible. In this context, it is necessary to note that some genotypes with very high values of total extractable polyphenols actually present a medium to low concentration of polyphenols in the skin; in this case, high cumulative data (skins + seeds) is related only to a high content of polyphenols in grape seeds. An example of this feature is evident for genotype B7, for which the concentration of total extractable polyphenols (5,594 mg/kg) is the sum of 1,765 mg/kg contained in the skins and 3,829 mg/kg extractable from grape seeds (Graph 3).

### **Selection of the best genotypes and their characterisation (2006-2007 and 2010)**

As mentioned above, a selection of six genotypes was carried out among the 21 white grapes studied, based on the above data. The selection was, however, made by identifying six individuals with good characteristics that were representative of the variability of the population. For this reason, a cluster analysis was carried out (Graph 5) for the parameters of interest (production, sugar content, acidity, pH, and extractable polyphenols of the skins), which identified three groups with similar characteristics; only two genotypes (D1 and E1) do not fall within the above groups but

form two separate groups, resulting also in unattractively low acidity (5.6 to 6.3 g/L), high pH (3:31 to 3:49) and a polyphenolic content that exceeds 4,900 mg/kg.

The selection was therefore made by identifying the genotypes with the desired characteristics but making sure to choose at least one individual for each of the three groups defined by the cluster analysis.

Among the six selected genotypes, one belongs to group A (A1), three are included in group B (B2, B3, and B6), and two are part of group C (C2 and C4) (Graph 4). For these six genotypes, additional data were collected in 2010, which, when combined with the data for 2006 to 2007, allowed the definition of their characteristics (Table 1).

### **Microvinification and taste (2010)**

As mentioned before, although it was not possible to conduct separate microvinification of individual genotypes due to the low productivity combined with the small number of plants, three wines were produced in 2010. These wines were obtained by unifying the grapes of various individuals on the basis of the groups identified through the cluster analysis (Graph 4) utilized for the genotype selection (2006-2007), obtaining three wines (A, B, and C), each of which was produced from genotypes with homogeneous characteristics. This type of assemblage can be considered comparable to a varietal assemblage that could characterise future commercial wines.

A statistical analysis of the data obtained through the tasting does not show significant differences between the three wines but, rather, outlines a similar sensory profile (Graph 5). The average sensory profile indicates a balanced wine with high olfactive intensity; the descriptors most widely perceived by tasters are floral, honey, and fruity aromas and, in particular, notes of citrus and spices. Other descriptors perceived with less intensity are a hint of vegetable (both dry and fresh), the aroma of dried fruit, red fruit, and balsam (Graph 5). The gustative taste outlines a structured wine with an intense perception of alcohol, a low sensation of bitterness, with a strong acidity that adds up to a sapid sensation (Graphic 5).

In looking at the single profiles of the three wines (Graph 5), the wine of group A shows an evident perception of mature and dried fruit and notes of honey; that of group B has a less intense smell and is less acidic and less structured. The wine of group C shows a clear note of linalool, while it is little identified with the notes of spice and dried fruit found in the other two wines. In general, the wine made from the white grape genotypes seems interesting both for the complexity of the aromatic profile and for the balanced taste determined by the contrast among the structure, the alcohol content, and the acidity-sapidity.

### **CONCLUSIONS**

It is interesting to note that self-pollination seems to be a valuable tool to increase the variability and to determine the appearance of new features in generated seedlings that often differentiates them from the original variety. These peculiarities are related both to phenology and to morphological, vegetative-productive, and qualitative parameters.

The characterisation of the 21 white grape genotypes showed a wide variability in the population for all major parameters. The methods used for the selection (cluster analysis followed by the identification of the best individuals within the individual cluster) has allowed, at least in part, the maintaining of the variability that characterises the entire initial population. From the analysis of the characteristics found in the six genotypes derived from the selection, it appears clear that each of these potential new varieties show interesting aspects on several fronts: some genotypes (B2, C2, C4, and A1) for sugar content associated with a medium or high preservation of acidity and low pH; a genotype (B6) with good production that presents particularly low sugar concentrations, with values and acidic pH in the average range for the population. Another interesting aspect shared by the entire population is the sensory profile that originated from tastings: all three wines produced have a complex olfactive profile and an important structure but are supported by the acidity and sapidity. As of today, the multiplication of these six genotypes is still in place; in the next few years, the planting of a new field for comparison will be provided to make a definitive characterisation as well as to allow the separate vinification of each genotype. The future perspective is to consider possible inclusion in the National Register of Varieties.

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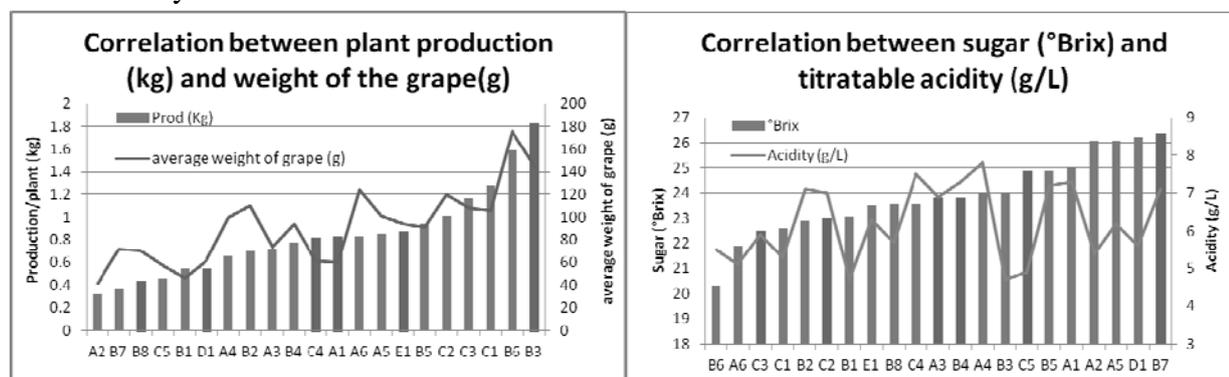
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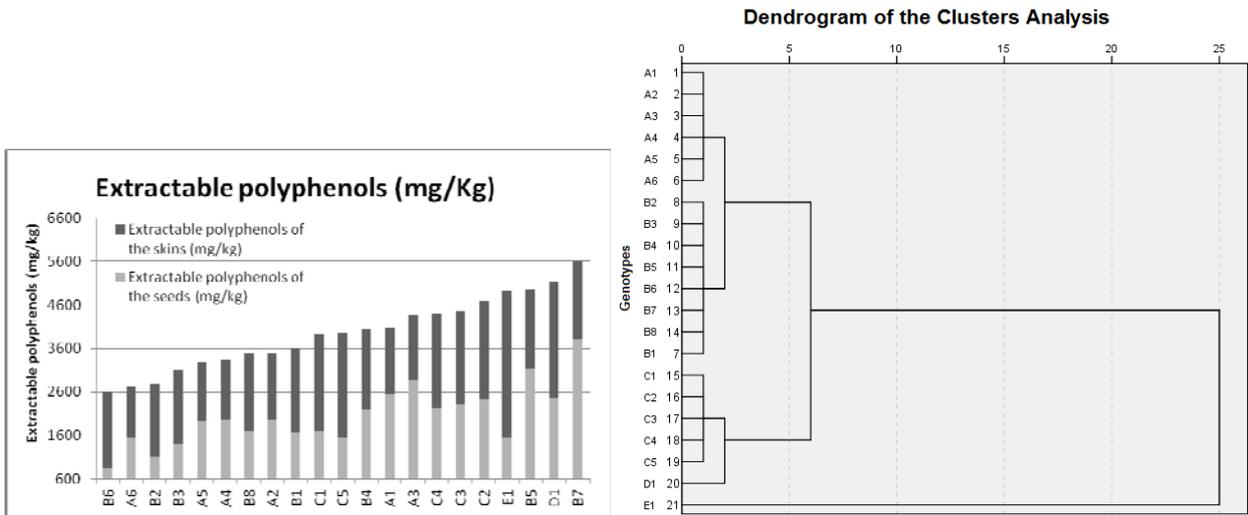
## ATTACHMENTS

Average 2006-2007-2010							
Genotype	Production (Kg/plant)	Sugar (°Brix)	Tritatable acidity (g/L)	pH	Extractable polyphenols of the skins (mg/kg)	Extractable polyphenols of the seeds (mg/kg)	Total extractable polyphenols (mg/Kg)
B6	1.59	20.2	5.87	3.21	1758.7	691.6	2450.3
B2	0.70	22.8	8.08	3.09	1772.7	1272.0	3044.7
C2	1.01	23.3	8.65	3.04	2872.3	2410.8	5283.1
C4	0.82	23.7	8.18	3.00	2301.4	1821.3	4122.7
B3	1.83	22.9	5.56	3.26	1795.5	1618.8	3414.3
A1	0.83	24.7	7.77	3.00	1389.2	2249.7	3638.9

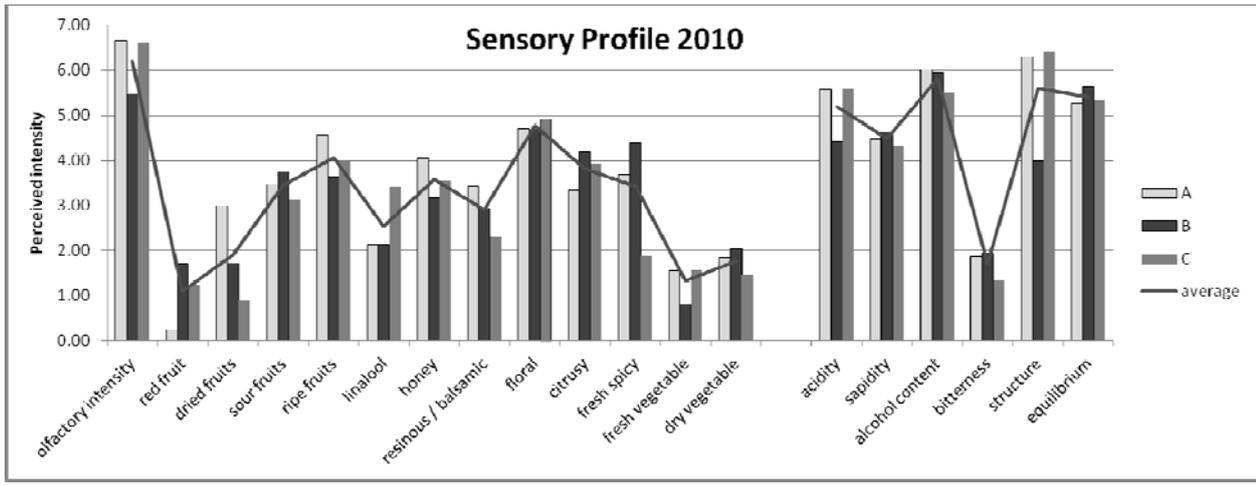
Table 1. Characterization table (average data 2006-2007-2010) of the 6 genotypes selected by Cluster Analysis



Graph 1. Correlation between the plant production and the average weight of the grape (average 2006-2007). - Graph 2. Correlation between the concentration of sugar and titratable acidity (average 2006-2007).



Graph 3. Relationship between extractable polyphenols from the skins and extractable polyphenols from the seeds compared to the total extractable polyphenols (skins + seeds). - Graph 4. Dendrogram obtained from the cluster analysis using the average data 2006-2007.



Graph 5. Sensory profile of the 3 wines tasted and average profile (2010 data)