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COMPARISON OF TOURIGA NACIONAL WITH THREE PORTUGUESE MINOR GRAPEVINE VARIETIES ON CANOPY, PRODUCTION AND QUALITY IN THE DOURO REGION

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Abstract: Some Portuguese minor grapevine varieties may reveal a good oenological potential for diversifying the wine market. Therefore, it is important to characterize them. This work has been carried out in the Douro Region and compared the Touriga Nacional variety with three Portuguese minor grapevine varieties (Preto Martinho, Malvasia Preta and Cornifesto). During 2016, 2017 and 2018 several parameters related to canopy, production and grape quality were determined. Radial visualization revealed eight relevant parameters for characterizing the differences among these varieties, showing that pruning weight was the main feature for Touriga Nacional, clusters weight for Malvasia Preta, must total soluble solids for Cornifesto and must tannins for Preto Martinho. According to the parameters analysed, Malvasia Preta showed the highest number of shoots and yield, Cornifesto stood out by the total soluble solids content. Preto Martinho exhibited the greatest richness in phenolic compounds (total polyphenols, tannins and total anthocyanins). In this study, Preto Martinho exhibited higher concentrations in total soluble solids, tannins, anthocyanins and polyphenols than Touriga Nacional, it might be the minor grapevine variety with the best profile for diversifying the wine market in the Douro Region.
Keywords: Oenological potential; diversifying; yield; phenolic compounds.

INTRODUCTION

Several parameters are essential to evaluate differences among grapevine varieties, including canopy, production and grape composition. Martínez de Toda (2011) indicated that the influence of canopy on grape quality can be conditioned by variety, vegetative growth, vigour, Canopy Surface Area, and crop load. The canopy density can be evaluated by the *Point Quadrat* method

(Poni *et al.,* 1996; Smart & Robinson, 1991). According to Smart & Robinson (1991), the ideal canopy must have between 1 and 1.5 leaf layers in order to avoid excessive shadow inside the canopy, where photosynthetically active radiation is lower (Lopes, 1994). In warmer climates, other authors considered Leaf Layers Number (LLN) between 3 and 4 to be more adequate (Terry & Kurtural, 2011; Reynolds & Vanden Heuvel, 2009; Main & Morris, 2004). Canopy Surface Area intercepts directly solar radiation and its ideal value is around 21 000 m2/ha (Smart & Robinson, 1991). The number of shoots per linear meter is also a well-studied parameter in the relation between canopy and solar radiation interception. Smart & Robinson (1991) recommended 15 shoots/m to obtain a good production and quality for most grapevine varieties, although it depends on the number of clusters (Schneider, 1989). The vigour of a balanced vineyard ranges between 80 and 120 g of weight per shoot in Portuguese grapevine varieties (Oliveira, 2003; Queiroz, 2002; Lopes, 1994), while Kliewer & Dokoozlian (2005) referred values from 0.5 to 1 kg of pruning weight per linear meter.

According to Murisier & Zufferey (1997), a good indicator to evaluate a balanced development of grapevines and the potential of grape maturation is the relation between Canopy Surface Area and yield (denominated Carbohydrate Balance), with ideal values in the range from 1 to 1.2 m2/kg (Schneider, 1989). The relation between yield and pruning weight (Ravaz index) is also a parameter to define grapevine balance. According to Reynolds (2010), Ravaz index for a balanced grapevine of the Aramon variety must be near 4. Lopes (1994) reported similar values for many other varieties. However, Martínez de Toda (2011), Kliewer & Dokoozlian (2005) and Smart & Robinson (1991) proposed Ravaz index between 5 and 10.

The exposure of clusters to solar radiation can lead to higher phenolic compounds concentration (Guidoni *et al*., 2008; Poni *et al*., 2006). However, in warm areas, an excessive direct solar exposure can lead to extreme temperatures on the clusters (Cook *et al*., 2015; Spayd *et al*., 2002) with severe consequences for berry composition. These problems include sunburn, inhibition of sugar accumulation, accelerated degradation of organic acids, degradation and/or limitation of phenolic compound biosynthesis and consequent decrease in the anthocyanins concentration in berry skin (Yu *et al*., 2016; Martínez-Lüscher *et al*., 2014; Bergqvist *et al*., 2001). The optimal temperature for anthocyanins synthesis ranges between 17 and 26 °C (Martínez de Toda, 2011; Sadras *et al*., 2007).This temperature in the cluster zone can be achieved if LLN is maintained to a certain level in warm area (Terry & Kurtural, 2011; Reynolds & Vanden Heuvel, 2009; Main & Morris, 2004).

Total soluble solids, pH and phenolic compounds concentration are related to berry size, cultural practices, soil properties, water status and weather conditions, as well as variety (Mirás-Avalos *et al.,* 2019; Echeverría *et al.,* 2017; Triolo *et al.,* 2017; Bouzas-Cid *et al.,* 2015; Gil *et al.,* 2015). Total soluble solids usually decrease as the vegetative vigour increases (Bonilla *et al.*, 2015). Moreover, there is a trend to lower total soluble solids with increasing production (Wessner & Kurtural, 2013; Keller *et al.*, 2004). Some studies revealed that grapevines with lower canopy densities can reach 22 °Brix faster than grapevines with higher canopy densities (Bernizzoni *et al*., 2011; Terry & Kurtural, 2011; Morris, 2007). Other authors showed that a higher vigour led to a lower anthocyanins concentration in grapes (Filippetti *et al*., 2013; Cortell *et al*., 2007). However, these relations can be altered by climate conditions, as it is proven that in warmer climates low vigour can lead to unripen berries.

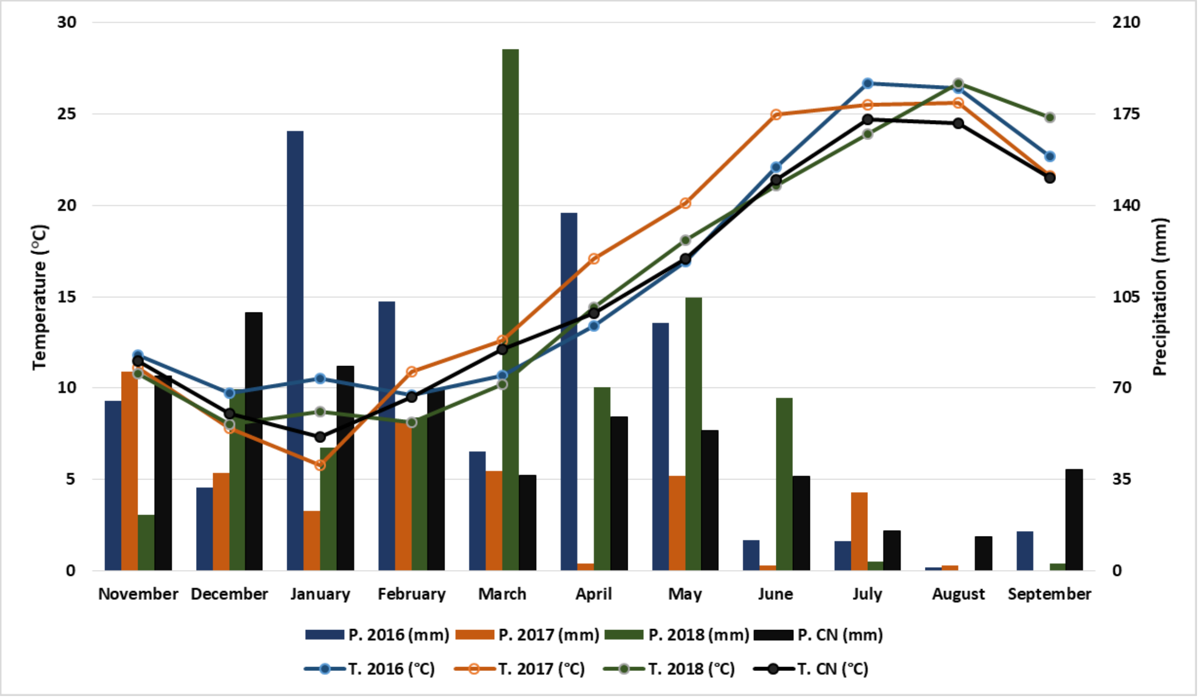
Touriga Nacional is a grapevine variety widely grown in Portugal. It is characterized by a small to medium leaf with sensitivity to sunburn, a small cluster, medium compact with thick skin berries and soft pulp. It shows a high vigour with tendency for coulure, secondary shoots emission and the yield is considered medium (Bohm *et al.,* 2011; Magalhães, 2008; Pereira & Sousa, 1990). Touriga Nacional is adapted to drought, maintaining high stomatal conductance and water use efficiency at low of leaf water potential values (Palliotti *et al.,* 2014). According to the Instituto da Vinha e do Vinho ranking of varieties planted in Portugal (IVV, 2018), Preto Martinho, Malvasia Preta and Cornifesto are considered minor grapevine varieties. Preto Martinho is characterized by a medium leaf, medium and compact cluster with medium thick skin berries and rigid pulp. It shows a low to medium vigour and medium yield (Eiras-Dias *et al.,* 1986). Malvasia Preta is characterized by having a small to medium leaf, a medium and compact cluster with medium thick skin berries and rigid pulp. It shows a medium to high vigour and medium yield (Sousa *et al.*, 2007; Pereira & Sousa, 1990). Cornifesto is characterized by having a small to medium leaf, a small and compact cluster with thin skin berries and rigid pulp. It shows a medium vigour and medium yield (Sousa *et al.,* 2007; Pereira & Sousa, 1990). Several countries are currently recovering and characterizing autochthonous varieties, nearly extinct in some cases, as potential producers of quality wines (Loureiro *et al.*, 2017; Milella *et al.*, 2016; Dobrei *et al.*, 2015; Merkouropoulos et al., 2015) and for preserving the genetic resources of a region. Some Portuguese minor grapevine varieties may reveal a good oenological potential for wine market diversification, leading to a distinct and exclusive product. Therefore, it is important to characterize the potential of these minor varieties regarding vegetative vigour, production and grape composition. In this context, the present work aimed to evaluate the differences of Touriga Nacional and three Portuguese minor grapevine varieties (Preto Martinho, Malvasia Preta and Cornifesto) present in the Douro Region on parameters related with canopy, production and grape quality.

MATERIALS AND METHODS

STUDY SITE AND PLANT MATERIAL

Data were collected on a vineyard located in Pinhão (41°10'44.04"N, 7°32'02.02"W; 230 m) at Cima-Corgo, Douro Region (Northern Portugal), during three consecutive years (206, 2017 and 2018). The vineyard is oriented to the north (northeast direction). The soil is silty-loam in texture, with low organic matter content (below 1%) and with acid pH. Temperature and precipitation data wererecorded by a weather station located near the vineyard (ADVID 2018; ADVID 2017; ADVID 2016) and they are presented in Figure 1.

The vineyard contains four grapevine varieties, Touriga Nacional, Preto Martinho, Malvasia Preta and Cornifesto. They were planted in 2002 on 1103P rootstock and systematized by terraces with one row. The grapevines were trained to a vertical shoot positioning with a pair of movable wires and were spur-pruned on a unilateral Royat Cordon system with 10 to 12 buds per grapevine. The grapevines were spaced 1 m within and 2.5 m between rows (around 4000 grapevines/ha). The height and width of the canopy were, respectively, 1.40 m and 0.55 m (average values for the four grapevine varieties).



CN: Climatological Normal (1971-2000).

Figure 1 - Monthly mean temperature and monthly precipitation for 2016, 2017 and 2018.

EXPERIMENTAL DESIGN

The experiment was laid out in a randomized block design with three repetitions per variety (twenty grapevines per repetition). The parameters were evaluated for all grapevines and data were organized by repetition. The samples to analyse the grape quality had an average of 60 berries per repetition (3 random berries per grapevine). All grapevine varieties were subjected to the same weather and soil conditions. Grapevines were managed without irrigation. Cultural practices and phytosanitary treatments were the same for the four varieties.

CANOPY, PRODUCTION AND QUALITY PARAMETERS

The methods to determine the parameters were those described in Martínez de Toda (2011), Murisier & Zufferey (1997), Poni *et al.* (1996) and Smart & Robinson (1991). In this study, we assessed budding rate, shoots per meter, vegetative vigour, pruning weight, Canopy Surface Area, Leaf Layers Number, porosity, interior leaves, carbohydrate balance, number of clusters, clusters weight, average weight per cluster, yield, interior clusters, dry clusters, coulure and Ravaz index. Grape composition was evaluated following the International Organisation of Vine and Wine methods (OIV, 2006) determining total soluble solids and pH in the pulp homogenate, while total polyphenols, tannins, and total anthocyanins were measured in the seeds and skins. Total polyphenols and tannins values are expressed in mg of epicatechin per gram of berry and total anthocyanins values are expressed in mg of malvidine-3-glucoside per gram of berry.

STATISTICAL ANALYSIS

The statistical analysis was performed using IBM SPSS Statistic 25. Data were subjected to a Levene's test to assess the homogeneity of variances, which complied the requirements of ANOVA in all cases. In order to determine significant differences among years and grapevine varieties, an analysis of variance and comparison of means (two-way ANOVA) was carried out. Differences between means were assessed using the Tukey test ($p < 0.05$). Results are expressed as mean ± standard deviation.

The parameters considered most relevant were vigour, pruning weight, clusters weight, Ravaz index, total soluble solids, total polyphenols, tannins and total anthocyanins. They were compared for each grapevine variety by applying a linear projection represented using the radial visualization (RadViz) (Matias, 2017; Rubio-Sánchez *et al.*, 2015). The neighbourhood between a data point and a parameter highlights the influence of this parameter in variety.

RESULTS AND DISCUSSION

According to Figure 1, the mean temperatures were higher in summer (July, August and September) during the studied period than the climatological normal "1971-2000", except for July 2018. When comparing with the mean temperatures between 2007 and 2014 in Pinhão referred by Real *et al.* (2018), there was an increase for 2016, 2017 and 2018. These warmer conditions can have influenced the occurrence of veraison and maturation with a tendency to accelerate the growing cycle in the studied years. In 2017 a drought was detected. When comparing all studied years, 2017 was the year with the earliest growing season, where the highest temperature until June and drought might have influenced this faster evolution of growing season.

According to Table 1, it was not observed an interaction ($p > 0.05$) between year and grapevine variety in vigour, Canopy Surface Area, pruning weight and Ravaz index. Simultaneously, no significant effects ($p > 0.05$) of the year were found for vigour and coulure. Since the statistical analysis revealed no significant effects ($p > 0.05$) of the grapevine variety on porosity and interior clusters, these parameters were excluded from the further analysis.

Table 2 shows the means of all parameters for the three studied years and the statistical differences among grapevine varieties and years. As an example, budding rate, Canopy Surface Area and total anthocyanins showed higher values in 2018, 2016 and 2017, respectively. This fact could be attributed to the occurrence of higher precipitation from November to May in 2016 and 2018 (646 mm in 2016, 572 mm in 2018, while only 271 mm in 2017), which fostered vegetative growth. Temperatures from June to September were similar in the three years (24.5 °C, 24.4 °C and 24.1 °C, in 2016, 2017 and 2018 respectively), exhibiting optimal values for anthocyanins synthesis (Echeverría *et al.,* 2017; Martínez de Toda, 2011; Sadras *et al.,* 2007).

Considering the fertility parameters, Malvasia Preta variety stood out with the highest budding rate and shoots/m due to its higher emission of secondary shoots, being greater than the standard values (15 shoots/m) indicated by Smart & Robinson (1991). For the vigour, there were significant differences between Touriga Nacional and the other grapevine varieties, as expected (Bohm *et al.,* 2011; Magalhães, 2008; Pereira & Sousa, 1990). According to the values referred by Oliveira (2003), Queiroz (2002) and Lopes (1994) regarding Portuguese grapevine varieties (from 80 g to 120 g/shoot), only Touriga Nacional had a vigour to support a balanced production. However, the other varieties presented similar values to those indicated by Martínez de Toda (2011), from 30 g to 60 g. Touriga Nacional presented the highest Canopy Surface Area, LLN and interior leaves. The highest budding

	Grapevine	Year	Grapevine* Year		Grapevine	Year	Grapevine* Year
Budding rate	***	**	*	Number of clusters	**	***	**
Shoots per meter	***	*	*	Clusters weight	***	***	**
Vigour	***	n.s	n.s	Average weight per cluster	***	***	**
Canopy Surface Area	**	***	n.s	Yield	***	***	**
Carbohydrate Balance	*	***	*	Pruning weight	***	***	n.s
Leaf Layers Number	**	***	**	Ravaz index	***	**	n.s
Porosity	n.s	*	*	Total soluble solids	***	**	***
Interior leaves	***	***	**	pH	***	***	**
Interior clusters	n.s	*	*	Tannins	***	***	***
Dry clusters	***	***	**	Total polyphenols	***	***	***
Coulure	***	n.s	**	Total anthocyanins	***	***	***

* Significant differences at p < 0.05; ** Significant differences at p < 0.01; *** Significant differences at p < 0.001; n.s -not significant

Table 1. Effects exerted by grapevine variety, year and their interaction on the vegetative growth, yield components and berry composition traits analysed.

	Touriga Nacional	**Preto Martinho**	**MalvasiaPreta**	**Cornifesto**	**2016**	**2017**	**2018**
Budding rate (%)	$108.0^{ab} \pm 4.3$	$111.9^{b} \pm 5.5$	$132.7^{c} \pm 11.5$	$104.8^{a} \pm 3.5$	a	a	b
Shoots/m	$16.3^{b} \pm 1.0$	$13.1^{a} \pm 1.3$	$18.3^{c} \pm 2.1$	$14.8^{b} \pm 2.2$	ab	a	b
Vigour (g/shoot)	$112.5^{b} \pm 14.6$	$57.4^{a} \pm 9.5$	$64.0^{a} \pm 11.0$	$53.6^{a} \pm 12.3$		n.s	
Prunning weight (g/grapevine)	$1482.1^{c} \pm 318.0$	$638.6^{a} \pm 186.0$	$955.9^{b} \pm 208.0$	$683.7^{a} \pm 267.0$	b	a	b

Canopy Surface Area (m^2/ha)	13427.9^{c} ±1517.5	12382.3^{a} ±1436.5	13311.0^{bc} ±975.6	12474.6^{ab} ±978.4	b	a	a
Leaf Layers Number	3.1^{b} ±0.8	2.9^{ab} ±0.9	2.7^{a} ±1.0	2.6^{a} ±1.1	b	a	a
Interior leaves (%)	38.9^{b} ±9.5	34.2^{a} ±13.6	32.3^{a} ±14.8	31.7^{a} ±12.6	c	a	b
C. balance (m^2/kg)	1.1^{ab} ±0.3	1.0^{a} ±0.2	1.0^{a} ±0.3	1.2^{b} ±0.3	a	b	b
Clusters weight (g/grapevine)	3352.2^{ab}±1087.0	3298.7^{ab}±1037.0	3910.0^{b}±1694.0	2688.0^{a}±689.0	b	a	a
Yield (kg/ha)	13408.9^{ab} ±4345.6	13194.8^{ab} ±4150.4	15639.6^{b} ±6775.5	10751.9^{a} ±2753.7	b	a	a
Dry clusters (%)	7.0^{a}±2.5	24.6^{b}±9.4	5.7^{a}±3.8	10.3^{a}±6.8	c	a	b
Coulure (%)	5.5^{b}±0.8	0.8^{a}±0.5	0.2^{a}±0.2	0.1^{a}±0.1		n.s	
Ravaz index	2.0^{a}±0.7	5.3^{c}±1.4	4.1^{b}±1.3	4.2^{b}±0.7	b	b	a
pH	4.0^{c}±0.3	3.6^{a}±0.1	3.8^{b}±0.1	3.8^{b}±0.1	a	b	a
Total soluble solids (°Brix)	20.3^{a}±0.6	22.1^{b}±1.5	19.8^{a}±1.9	22.1^{b}±1.0	a	b	b
Total polyphenols (mg/g)	16.9^{c}±10.2	25.0^{d}±21.6	14.0^{b}±3.6	8.4^{a}±1.2	a	b	a
Tannins (mg/g)	6.8^{b}±2.5	11.5^{c}±5.7	6.7^{b}±1.0	4.1^{a}±0.6	b	c	a
Total anthocyanins (mg/g)	2.0^{c}±1.5	4.2^{d}±4.5	1.4^{b}±0.7	0.8^{a}±0.1	a	b	a

Means ± standard deviations of parameters for the three studied years. Different letters within the same row for grapevinevarieties and years indicate significant differences according to the Tukey test ($p < 0.05$). n.s - not significant.

Table 2. Canopy, production and quality parameters analysed for the four varieties studied in three years.

rate and shoots/m presented by Malvasia Preta did not originate highest LLN and interior leaves. Since the LLN of the four varieties were within the ideal values for warm climates (Terry & Kurtural, 2011; Reynolds & Vanden Heuvel, 2009; Main & Morris, 2004), they might be able to avoid possible damage caused by excessive direct solar exposure on the clusters (Yu *et al.*, 2016; Cook *et al.*, 2015; Martínez-Lüscher *et al.*, 2014). For all grapevine varieties, the relation between the Canopy Surface Area and yield was around 1 to 1.2 m^2/kg, indicating of enough canopy for quality wine production (Murisier & Zufferey, 1997; Schneider, 1989). Preto Martinho exhibited the highest percentage of dry clusters, which demonstrated some sensitivity to sunburn. Touriga Nacional showed significant coulure values, usual for this variety (Bohm *et al.,* 2011; Magalhães, 2008; Pereira & Sousa, 1990). Malvasia Preta had the highest cluster weight (consequently the highest yield), while Cornifesto had the lowest. Comparing these grapevine varieties, the differences found may be related to budding rate and shoots/m. Malvasia Preta had the biggest clusters per grapevine while Cornifesto showed less and the smallest clusters per grapevine, which verifies the differences pointed out by Sousa *et al.* (2007) and Pereira & Sousa (1990). Cornifesto and Preto Martinho showed smaller berries when compared with Touriga Nacional and Malvasia Preta. Concerning pruning weight, the highest value was shown by the variety with highest vigour (Touriga Nacional), followed by the variety with the highest budding rate and shoots/m (Malvasia Preta). Preto Martinho, Malvasia Preta and Cornifesto exhibited pruning weight values within those proposed by Kliewer & Dokoozlian (2005). Preto Martinho exhibited the highest value of Ravaz index, similar to those indicated by Martínez de Toda (2011), Kliewer & Dokoozlian (2005) and Smart & Robinson (1991). Touriga Nacional showed the lowest value, within those proposed by Oliveira (2003) and Queiroz (2002) for this variety.

Regarding the quality parameters, there were significant differences between grapevine varieties, as expected (Bouzas-Cid *et al.*, 2015). Total soluble solids decreased when clusters weight was higher, as observed by Wessner & Kurtural (2013) and Keller *et al.* (2004). The grapevine variety exhibiting the highest vigour (Touriga Nacional) presented lower total soluble solids, as verified by Bonilla *et al.* (2015). In contrast, the varieties with lower vigour and shoots/m (Preto Martinho and Cornifesto) reached 22 °Brix more easily than varieties with higher canopy density (Touriga Nacional and Malvasia Preta), as previously pointed out (Bernizzoni *et al.,* 2011; Terry & Kurtural, 2011; Morris, 2007). Cornifesto and Preto Martinho had the lowest and the highest phenolic compounds concentrations, respectively. The lower vigour of Preto Martinho, when compared with Touriga Nacional, may have contributed to the higher phenolic compounds concentration (Filippetti *et al.,* 2013; Cortell *et al.,* 2007). Other reason for the higher concentration of those compounds can be related to the small size berries of Preto Martinho and its relation between skin and pulp as pointed out by some authors (Bonilla *et al,.* 2015; Gil *et al.*, 2015). Since that all varieties stayed under the same weather conditions, no irrigation and the same cultural practices, its influence on the grape composition is not applied as indicated by Mirás-Avalos *et al.* (2019) and Triolo *et al.* (2017). Preto Martinho had a reduced production caused by the highest percentage of dry clusters, however, the accumulation of phenolic compounds in the remaining clusters might have been influenced by the photo-assimilates distribution. Preto Martinho showed the lowest pH in contrast with Touriga Nacional.

Despite of lower vegetative growth and production capacity and a higher sensitivity to sunburn among the minor grapevine varieties studied, Preto Martinho seems to be the variety more similar, regarding must composition, to Touriga Nacional with high quality and recommended in the Douro Region (IVDP, 2019; Abade & Guerra, 2008). Due to its good oenological potential, Preto Martinho is a minor variety that can be valuable in the differentiation of wines from the Douro Region.

In Figure 2, The RadViz shows eight relevant parameters in varieties characterization, such as vigour, pruning weight, clusters weight, Ravaz index, total soluble solids, total polyphenols, total anthocyanins and tannins.

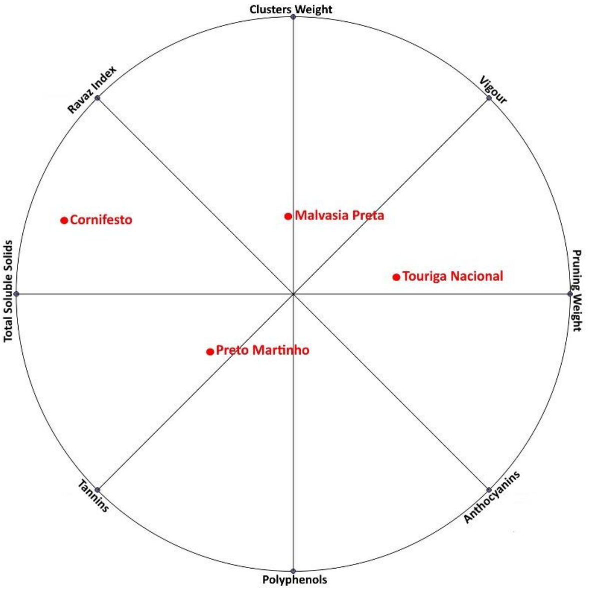


Figure 2 - Radial visualization applied to vigour, pruning weight, clusters weight, Ravaz index, total soluble solids, total polyphenols, total anthocyanins and tannins.

RadViz showed that: i) the pruning weight was the most relevant parameter for Touriga Nacional; ii) the clusters weight for Malvasia Preta; iii) total soluble solids for Cornifesto; iv) tannins for Preto Martinho.

CONCLUSIONS

This study reveals differences on several parameters in three minor Portuguese grapevine varieties (Preto Martinho, Malvasia Preta and Cornifesto) when compared with other widely grown in Portugal (Touriga Nacional), in particular in the Douro Region.

Malvasia Preta showed the highest number of shoots and yield, Cornifesto stood out by the total soluble solids content. Preto Martinho exhibited the greatest richness in phenolic compounds (total polyphenols, tannins and total anthocyanins), followed by Touriga Nacional.

Touriga Nacional is a variety known for its high quality and is recommended in the Douro Region. Since, in this study, Preto Martinho exhibited higher concentrations in total soluble solids, tannins, anthocyanins and polyphenols than Touriga Nacional, it might be the minor grapevine variety with the best profile for diversifying the wine market in the Douro Region.

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