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Breeding strategies for wine grapes: From genetic analysis of agronomic traits to wine sensory evaluation
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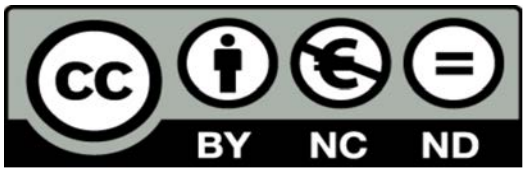

Breeding strategies for wine grapes:

"From genetic analysis of agronomic traits to wine sensory evaluation"

Cristina Manso Martínez

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UNIVERSIDAD DE LA RIOJA

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Breeding strategies for wine grapes:

"From genetic analysis of agronomic traits to wine sensory evaluation"

Memoria presentada por:

Cristina Manso Martínez

Para optar al grado de Doctor de la Universidad de La Rioja con mención de Doctorado Internacional

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Bajo la dirección de: Dra. Cristina Menéndez Menéndez

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Departamento de Agricultura y Alimentación

Las doctoras Cristina Menéndez Menéndez y María del Mar Hernández Álamos, investigadoras del Instituto de Ciencias de la Vid y del Vino (Universidad de La Rioja, CSIC, Gobierno de la Rioja,) y profesoras de la Universidad de La Rioja

INFORMAN:

Que la presente memoria titulada "Breeding strategies for wine grapes: from genetic analysis of agronomic traits to wine sensory evaluation" ha sido realizada por Cristina Manso Martínez en el Departamento de Agricultura y Alimentación de la Universidad de La Rioja bajo nuestra dirección, y reúne las condiciones exigidas para optar al grado de Doctor.

Lo que hacen constar en Logroño, a 19 de junio de 2020

Dra. Cristina Menéndez Menéndez

Dra. María del Mar Hernández Álamos

List of publications obtained from the PhD research

This PhD thesis has resulted so far in the following scientific papers:

1. Manso-Martínez, C., Sáenz-Navajas, M. P., Hernández, M. M., & Menéndez, C. M. (2020). Sensory profiling and quality assessment of wines derived from Graciano × Tempranillo selections. *LWT - Food Science and Technology.* https://doi.org/10.1016/j.lwt.2020.109394.

2. Manso-Martínez, C., Sáenz-Navajas, M. P., Hernández, M. M., & Menéndez, C. M. (2020). Wine quality and berry size: a case study with Tempranillo progenies. *LWT - Food Science and Technology* (under revision).

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Summary

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated and highest value horticultural crops in the world due to the economic value of wine, being one of the most valuable agricultural products in Europe. Current wine grape breeding programmes are focusing on wine quality, disease resistance and climate change adaptation which should also bring about new varieties that match new consumer preferences in the market. Therefore, relevant traits in wine grape breeding include berry weight and composition, high phenolic content, high acidity, early or late phenology, and moderate productivity with low alcohol content. Small berry size is a key factor that influences grape composition and presumably improves wine quality due to the higher concentration of aromatic and phenolic compounds in the grape skin. On the other hand, final berry shape is thought to be predictable at the ovary stage being that final berry size is correlated with flower morphology which could be also influenced by flower sex.

The main goal of this research was to develop strategies for wine grape breeding. Two specific objectives were considered: 1) analysis the genetic basis of target traits such as berry size and morphology, flower sex, production and phenological periods 2) assessment of the influence of berry size in the oenological composition of Tempranillo segregating progenies and Pinot Noir clones and the sensory evaluation of the wines derived from Tempranillo selections.

Two segregating progenies derived from Grenache × Tempranillo (130 genotypes) and Graciano × Tempranillo (151 genotypes) were evaluated for 26 traits, including berry, flower, seed, phenology and productivity for up to four years and the influence of sex was assessed at the phenotypic level. When compared with hermaphrodites in both genetic backgrounds, female plants showed rounder flower shape, larger flower diameter, lower number of seeds, and a delay in flowering and veraison onset dates.

The Grenache × Tempranillo progeny was genotyped using GBS methodology with 4452 polymorphic SNP (Single Nucleotide Polymorphism) markers. A consensus genetic map was constructed with 1296 SNP and 4 SSR markers covering 1540 cM distributed in 19 linkage groups, with an average interval length of 1.2 cM between markers. The Grenache map consisted of 1011 markers spanning 1364.5 cM while the Tempranillo map covered 1237.7 cM with 826 markers and an average distance of (1.4) and (1.5) cM respectively.

Quantitative Trait Loci (QTL) analyses of 26 traits including berry and flower morphology, must composition, productivity and phenological parameters were conducted in the Grenache × Tempranillo progeny in two to four seasons from 2014 to 2017; the influence of *Sex* locus was also assessed. In addition, QTL analyses were conducted for berry, flower, and seed traits in the Graciano progeny for two seasons. A QTL region in LG17 was found in Grenache × Tempranillo progeny significantly associated to berry size, productivity traits and phenology stages; whereas in LG7 and LG13 QTL for flower morphology and flowering date suggest close linkage or pleiotropic effects. In Graciano × Tempranillo population, regions in LG3 and LG5 were associated mainly to berry size and seed traits. QTL on LG5 for berry, seed and flower traits in Graciano × Tempranillo progeny covered the region of *FERONIA* locus, and a QTL on LG18 found for seed traits resulted associated to locus *SDI*. In relation to flower morphology, the QTL region on LG11 had the strongest and most stable effect over the two years in both genetic backgrounds and a candidate gene VIT_11s0016g03650 with a function associated to pollen morphology was proposed. Highly significant QTL were found for total acidity on LG4, LG12, LG13, LG14 and LG17 in Grenache × Tempranillo progeny. Concerning phenological traits, in Grenache × Tempranillo progeny, veraison dates showed significant associations with genomic

regions in LG11 and LG17; ripening dates resulted to be significantly associated to LG8, LG11 and LG13. In Graciano × Tempranillo progeny, co-localizations of QTL for flower morphology, seed traits and phenology events were detected in LG3 and LG11. Moreover, a QTL region in LG2 was detected for flower-morphology, seed, productivity traits, and phenological stages (flowering date, veraison), confirming the influence of flower sex in the genetic determinism of these characters.

Influence of berry size on wine composition was studied in two Tempranillo segregating progenies and in Pinot noir clones. Consistently, wines obtained from small berry genotypes presented higher proportions of phenolic compounds and deeper colour. Higher quality scores were obtained for small berry size wines regardless of genetic background and vintage; the wines were described as sweeter, fruitier and with greater astringency. Pinot Noir clones presented differences in berry morphology and nitrogen compounds independently of the subregion studied. Environmental conditions and rootstock were found to influence parameters such as berry size, nitrogen compound accumulation, and phenolic composition in Pinot Noir clones.

All traits except berry shape showed transgressive segregation and large phenotypic variability in both progenies, these are essential for the selection of new genotypes with improved attributes. Eleven red and eleven white genotypes were pre-selected in the Grenache × Tempranillo population based on berry weight, cluster weight, acidity and ripening date. Whilst in Tempranillo × Graciano population, evaluation of twelve pre-selected hybrids including physicochemical and sensory properties of wines, were conducted in 2017 and 2018. Two early ripening selections, TG8 and TG63 were consistently perceived as higher quality than Graciano and Tempranillo, in two very different vintages. Moreover, TG129, a late ripening selection, was perceived as a good option for future climatic conditions. Wines from TG35 or TG128 genotypes provided distinct sensory characteristics (roasted notes) which are valuable for the necessary diversification of the wine market.

Results of this research reveal novel insights into the genetic control of relevant traits for wine grapes, and will be useful for breeding new genotypes with better quality features and adaptation to new consumption patterns. This is the first physicochemical and sensorial evaluation of young red wines elaborated with Tempranillo intraspecific hybrid grapes. Despite an important effect of vintage on sensory properties of wines, selected genotypes were able to produce quality wines with great sensory variability, therefore, confirming that intraspecific hybridization is a useful tool to improve traditional varieties and meet new consumer demands.

Keywords: Tempranillo progenies, QTL, berry size, flower sex, *Vitis vinifera* L.

Resumen

La vid (*Vitis vinifera* L.) es uno de los cultivos más extendidos y de mayor valor económico, debido a su principal producto, el vino, siendo uno de los productos más valiosos en la agricultura en Europa. Los principales ámbitos de mejora para la uva de vinificación son la calidad del vino, la resistencia a enfermedades y la adaptación al cambio climático, debiendo además estas nuevas selecciones satisfacer las nuevas preferencias del consumidor en el mercado. Entre los parámetros más relevantes en las uvas para vinificación están el peso y la composición de las bayas, alto contenido fenólico, alta acidez, fenología temprana o tardía, y productividad moderada con bajo contenido alcohólico. Un menor tamaño de baya es un factor clave que influye en la composición de la uva y presumiblemente mejora la calidad del vino obtenido, debido a la mayor concentración de compuestos aromáticos y fenólicos en la piel de la uva. Además, se estima que la forma final de la baya es predecible en la etapa de desarrollo del ovario, correlacionándose el tamaño final de la baya con la morfología de la flor, que a su vez podría estar influenciada por el sexo de la flor.

El objetivo principal de esta investigación fue desarrollar estrategias para la mejora de la uva de vinificación. Se consideraron dos objetivos específicos: 1) analizar la base genética de los caracteres de interés, como el tamaño y la morfología de las bayas, el sexo de las flores, la productividad y los estadíos fenológicos 2) evaluar la influencia del tamaño de la baya en la composición enológica de progenies segregantes de Tempranillo y clones de Pinot Noir, así como evaluar sensorialmente los vinos derivados de las selecciones de Tempranillo.

Se evaluaron dos progenies segregantes derivadas de Garnacha × Tempranillo (130 genotipos) y Graciano × Tempranillo (151 genotipos) para 26 parámetros relacionados con la baya, flores, semillas, fenología y productividad en hasta cuatro años, evaluándose además la influencia del sexo a nivel fenotípico. Las plantas femeninas mostraron una forma de flor más redondeada, un diámetro de flor más grande, un menor número de semillas y un retraso en la floración y en las fechas de inicio del envero en comparación con los hermafroditas en ambos fondos genéticos. La progenie de Garnacha × Tempranillo se genotipó utilizando la metodología GBS con 4452 marcadores polimórficos tipo SNP. Se construyó un mapa genético consenso con 1296 SNP y 4 marcadores SSR con una distancia de 1540 cM distribuidos en 19 grupos de ligamiento, con un intervalo promedio de 1,2 cM entre marcadores. El mapa de Garnacha constaba de 1011 marcadores que abarcaban 1364,5 cM, mientras que el mapa de Tempranillo resultó ser de 1237,7 cM con 826 marcadores y una distancia promedio de (1,4) y (1,5) cM respectivamente.

Se realizaron análisis de QTL de 26 caracteres, incluyendo morfología de la baya y de la flor, composición del mosto, productividad y parámetros fenológicos en la progenie Garnacha × Tempranillo a lo largo de 4 años 2014-2017, donde también se evaluó la influencia del locus del sexo. Además, a lo largo de dos años se realizaron análisis QTL para caracteres de baya, flor y semilla en la progenie Graciano × Tempranillo. Se encontró una región QTL en el GL17 en la progenie de Garnacha × Tempranillo asociada significativamente con el tamaño de la baya, los rasgos de productividad y los estadíos fenológicos, y en GL7 y GL13 QTL para la morfología de la flor y la fecha de floración, lo que sugiere una estrecha asociación o efectos pleiotrópicos. En la población Graciano × Tempranillo, regiones en GL3 y GL5 resultaron asociadas principalmente al tamaño de la baya y caracteres de semilla. Un QTL en GL5 fue encontrado relacionado con parámetros de baya, semilla y flor en la progenie Graciano × Tempranillo, cubriendo la región del locus *FERONIA*. En la misma población, se encontró un QTL en el GL18 para parámetros de semilla asociado al locus *SDI*. En relación con la morfología de las flores, una

región en el GL11 resultó altamente significativa y estable durante los dos años en ambos fondos genéticos, y se propuso un gen candidato VIT_11s0016g03650 con una función asociada a la morfología del polen. Se encontraron también regiones QTL altamente significativos para la acidez total en GL4, GL12, GL13, GL14 y GL17 en la progenie de Garnacha × Tempranillo. Con respecto a los estadíos fenológicos, en la progenie de Garnacha × Tempranillo, el periodo de envero mostró asociaciones significativas con las regiones genómicas en GL11 y GL17, y la fecha de maduración resultó significativamente asociadas a GL8, GL11 y GL13. En la progenie Graciano × Tempranillo, se detectaron co-localizaciones de QTL para morfología de la flor, caracteres de semilla y estadíos fenológicos en GL3 y GL11. Además, se detectó una región QTL en GL2 para la morfología de la flor, parámetros de semilla, rasgos de productividad y etapas fenológicas (fecha de floración, envero), lo que confirma la influencia del sexo de la flor en la determinación genética de estos caracteres.

La influencia del tamaño de la baya en la composición del vino se estudió en dos progenies segregantes de Tempranillo y en clones de Pinot noir. Consistentemente, los vinos obtenidos de genotipos de tamaño de baya pequeño presentaron una mayor concentración de compuestos fenólico, así como un color más intenso. Se obtuvieron mayores puntuaciones de calidad para los vinos de tamaño pequeño de baya, independientemente del fondo genético y de la vendimia en las progenies de Tempranillo, siendo descritos sensorialmente estos vinos como más dulces, frutales y con una mayor astringencia. Los clones de Pinot Noir presentaron diferencias en la morfología de la baya y en la acumulación de compuestos de nitrogenados en el mosto independientemente de la subregión estudiada. Se demostró que las condiciones ambientales y el portainjerto influyen en parámetros como el tamaño de la baya, composición fenólica y nitrogenadas en los clones de Pinot Noir.

Todos los caracteres estudiados, excepto la forma de la baya, mostraron segregación transgresiva y gran variabilidad fenotípica en ambas progenies, factores esenciales para la selección de nuevos genotipos con características mejoradas. Se seleccionaron once genotipos de uva tinta y once de uva blanca en la población Garnacha x Tempranillo estableciendo como criterios el peso de la baya, el peso del racimo, la acidez y la fecha de maduración, mientras que en la población Graciano × Tempranillo, se evaluaron durante 2 años, 2017-2018, las propiedades fisicoquímicas y sensoriales de los vinos de doce híbridos preseleccionados. Dos genotipos de maduración temprana, TG8 y TG63 fueron percibidos consistentemente como de mayor calidad que Graciano y Tempranillo, en dos añadas muy diferentes. Además, TG129, una selección de maduración tardía, se considera una buena opción dentro del contexto de cambio climático. Los vinos de los genotipos TG35 o TG128 proporcionaron perfiles sensoriales distintos (notas tostadas) interesantes para la necesaria diversificación del mercado del vino.

Los resultados de esta investigación revelan nuevos conocimientos sobre el control genético de caracteres relevantes para la uva de vinificación, y serán útiles para generar nuevos genotipos con una mejor calidad y adaptación a los nuevos patrones de consumo. Esta es la primera evaluación fisicoquímica y sensorial de vinos tintos jóvenes elaborados con uvas híbridas intraespecíficas de Tempranillo. A pesar del importante efecto de la añada en las propiedades sensoriales de los vinos, los genotipos seleccionados pudieron producir vinos de calidad con una gran variabilidad sensorial, lo que confirma que la hibridación intraespecífica es una herramienta útil para mejorar las variedades tradicionales y satisfacer las nuevas demandas de los consumidores.

Palabras clave: poblaciones Tempranillo, QTL, tamaño de baya, sexo de la flor, *Vitis vinífera.*

CHAPTER 1.
GENERAL INTRODUCTION

1. General Introduction

1.1. Economic importance

Grapevine (*Vitis vinifera* L.) is one of the major fruit crops, cultivated along 7,4 mha, (OIV 2019) through temperate and tropical regions around the world. Success of grapevine is partly due to its great adaptability to a wide range of different climates (from oceanic, cold continental, Mediterranean, subtropical, to hyper arid), and latitudes (Schultz & Stoll 2010). However, the ideal growing conditions are met between 30 and 50 degrees, latitude North and 30 and 40 degrees in the Southern Hemisphere, where the most famous and extensive grape producing regions are located (Reisch et al. 2012). Winemaking is the major use of grapes both in terms of quantity and production area, but fresh fruit consume, transformation into raisins and unfermented juice, production of vinegars, spirits, grape concentrates, jams, jellies and grapeseed oil are other of their uses (Myles et al. 2011, Reisch et al. 2012).

Mediterranean countries, where grapes have been grown for thousands of years, are still the world leading wine production and grape cultivation area, being Spain, France and Italy the three major grape growing countries, with 969, 789 and 702 Mha, respectively, representing a third of worldwide production (7400 Mha, OIV 2018). The expansion of Asian vineyards leaded by China which with a production of 875 Mha (OIV 2018) is threating the hegemony of Europe as the first continent in production for first time in the history. Other important regions are eastern regions of Turkey (448 Mha), western regions of the United States of America (430 Mha), and temperate areas of Argentina (219 Mha), Chile (212 Mha), South Africa (125 Mha), being situated New Zealand in the low part of the list (39 Mha) (OIV 2018). In total, around 75 million Tons of grapes were produced worldwide in 2018, and 292,3 Mill hl of wine (OIV 2018). In Spain 92 Denominations of Origin are recognized by the European Union, being DOCa (Denominación de Origen Calificada) Rioja with 65001 ha cultivated and a total of 339290 tons (296174 tons of red and 43116 tons of white grapes) produced, one of the most internationally renowned wine producing regions.

1.2. Taxonomy of Vitis

The Eurasian wild grape (*Vitis vinifera* ssp. s*ylvestris*) is a dioecious, perennial, forest vine extensively grown in the Near East and the northern Mediterranean before its domestication (McGovern et al. 2017). Cultivated grapevines (*Vitis vinifera* ssp. *sativa*) were domesticated from wild populations of *Vitis vinifera* ssp. *sylvestris* which still grown along riverbank forests from Western Europe to central Asia and North Africa (Arroyo-Garcia et al. 2006, Reisch et al. 2012).

Grapevines are members of the *Vitaceae* family, which belongs to *Rhammales* order in the subclass *Rosidae* of Eudicots (Bouquet 2011). *Vitaceae* family is formed by around 1000 different species grouped into 17 different genera, from which only the genus *Vitis*, with two subgenera *Euvitis* and *Muscadinia*, have real agricultural interest (Reisch et al. 2012). *Euvitis* Planch and *Muscadinia* Planch. subgenus *Euvitis* (2n = 2x = 38) contains around 70 different species that are the most important in viticulture (Keller 2010). This subgenus is divided into three major groups of species that widely differ in their utility in agronomy, including Asian and American groups with around 30 species each, and the European or central Asian group that contains the widely cultivated *V. vinifera* L. species (Owens 2008). Among the Asian species, only *V. amurensis* has been domesticated and used for fresh fruit, juice wine and jelly production,

and although it contains high-yielding species they are mostly disease-susceptible. (Keller 2010). The American species, including *V. aestivalis, V. cinerea, V. labrusca, V. riparia, V. rupestris, or V. berlandieri* have been extensively used to produce rootstocks and fruiting cultivars characterized by pest and disease resistance but producing low yield and low-quality fruits (Owens 2008).

Although hybrids between the subgenera are usually sterile due to the difference in chromosome number (Reisch et al. 2012); hybrids between species within a subgenus are normally fertile and many interspecific hybrids between *Euvitis* species have been developed as scion and rootstock cultivars. In fact, most commercial grape cultivars belong to the species *V. vinifera*, cultivated grafted on varieties or hybrids of American *Vitis* species used as rootstocks due to their tolerance to diseases or cold temperature (Keller 2010). Few interspecific hybrid cultivars, obtained from crosses of *V. vinifera* with other species (e.g.: *V. labrusca, V. amurensis, V. riparia, V. rupestris, V. aestivalis*), are important in some local regions (Reisch et al. 2012), for cold and humid climates, or for disease resistance, but are generally considered lower quality specimens.

1.3. Historical origin and cultivar evolution

The cultivated grapevine (*Vitis vinifera* ssp. *sativa*) comes from its wild ancestor (*Vitis vinifera* ssp. *sylvestris*) after several domestication events (McGovern et al. 2017). Current cultivated grapevine shows important modifications compared to its predecessor, including the change from dioecy to hermaphroditism, the increase in the number and size of berries per bunch and modifications in seed morphology (This et al. 2006, Picq et al. 2014, Houel et al. 2013). Zhou et al. 2019, found that the diversity level of *V. v. sativa* samples is 94 % that of *V.v. sylvestris*, a far higher ratio of cultivated-to-wild diversity than in other species such as maize (83 %), rice (64 %), soybean, cassava (71 %), or tomato (54 %).

Chemical findings in pottery fabrics of Georgia in the South Caucasus region, belonged to the early Neolithic period provide the first biomolecular archaeological evidence for viticulture and wine from the Near East, at 6000 – 5800 BC. The discovery was also confirmed by climatic, botanical and environmental analysis, being grape pollen and epidermal remains associated with that date and the pots found (McGovern et al. 2017). Humans spread cultivars first to close regions such as Egypt (Myles et al. 2011) and later to distant Mediterranean regions like Greece, both coasts of the Italian and Iberian peninsulas and the north of Africa (This et al. 2006). Secondary events of domestication and spontaneous hybridizations took place among selected individuals and wild progenies (Arroyo-García et al. 2006, Sefc et al. 2003), increasing crop variability. Spanish, Dutch and French missionaries introduced the European varieties in America as seeds or cuttings around the 16th century, and varieties also reached South Africa, Australia and New Zealand at the beginning of the 19th century (This et al. 2006). Most of the crop diversity generated after all this process of expansion was drastically reduced in European vineyards due to the arrival of phylloxera aphid. Differential selection of genotypes for table and wine during domestication led to a significant phenotypic diversity of current varieties, being cultivars with large, fleshy berries and loose bunches selected for their use as table grape varieties, whereas cultivars with smaller, more compact bunches bearing smaller and juicier berries were preferred for winemaking (Bacilieri et al. 2013, This et al. 2006). Thus, genetic stratification of modern cultivars has been related to human interests and geographical factors, (Wolkovich et al. 2018)

by linking morpho-geographic grouping and haplotypes defined by nuclear and chloroplast DNA (Arroyo-García et al. 2006).

The first classification made by Negrul (1946) differentiated cultivars into three groups: *proles occidentalis*, *pontica* and *orientalis*, attending to bunch and berry morphological differences and geographical origin. The *proles occidentalis* was characterised by wine cultivars of Western European origin with compact and small bunches and berries such as "Riesling", "Pinot ", "Sauvignon". The *proles orientalis* consisted of table cultivars from Central Asia with large and loose bunches and fleshy berries including "Muscat d"Alexandrie", "Sultanine", whilst *proles pontica* included a group with intermediate characteristics as displayed in "Vermentino" or "Clairette" varieties (Levadoux 1956). Bacilieri et al. (2013) also identified different levels of stratification attending to geographic origin (Iberian Peninsula, West and Central Europe, Balkans and East Europe) and use (wine and table cultivars). In a similar approach, Emanuelli et al. (2013) classified 1659 *V. sativa* into four different groups with a set of SSR markers; the first integrated by Italian / Balkan wine cultivars, the second with Mediterranean table/wine cultivars, the third with the Muscats varieties, and the last group including Central European wine grapes.

DNA fingerprinting allowed to estimate the number of different *V. vinifera* genotypes cultivated across the globe (This et al. 2006), in between 6000 and 10000, being many of them closely related (Myles et al. 2011). The real number is difficult to determine due to the existence of many synonyms (different names for the same cultivar, like "Sultanina" and "Thompson seedless") and homonyms (identical name for different varieties; such as Malvasía) (Cattonaro et al. 2014). Genetic variability among cultivars is related to variation in agronomical traits such as ripening time, yield, berry size or resistance/tolerance to biotic and abiotic stresses (Duchene 2016).

Winemaking varieties are the result of hybridization (spontaneous or artificial) or somatic mutations. Mutations affecting only some of the cell layers of plant tissues give rise to "chimeras" such as Pinot Gris and Pinot Meunier (Franks et al. 2002). Moreover, somatic mutations caused by small changes in the genome generate new varietal forms within a variety, differing agronomically or morphologically from the original ("Grenache Blanca", "Grenache Gris", and "Grenache Peluda" or "Tempranillo Royo" and "Tempranillo Blanco") (García-Brunton et al. 2018). However, the most famous *V. vinifera* cultivars are the result of intraspecific hybridizations being "Müller-Thurgau", "Alicante H. Bouschet", "Cabernet Sauvignon", "Chardonnay", or "Merlot" in fact descendants of other known varieties (Duchene 2016). Tempranillo, the most relevant variety in Rioja´s viticulture, is also the result of hybridization between "Albillo Mayor" and "Benedicto" (Ibañez et al. 2012) like other well-known Spanish varieties such as "Palomino", "Malvasia", "Moscatel", "Torrontés", or "Hebén". (García-Brunton et al. 2018).The extent of parentage among grapevine cultivars is as surprisingly high that in the study of the relationship among 2344 unique genotypes of the INRA "Domaine de Vassal" grape germplasm repository with molecular markers, Lacombe et al. (2013) identified only 276 genotypes with no direct relationship with any other genotype in the collection. Nevertheless, they could elucidate the complete parentage of 828 cultivars, indicating that sexual reproduction, due to chance or controlled by man, is a major driver of genetic diversity in cultivated grapevine (Duchene 2016).

Unfortunately, wine market globalization, variety-oriented wine labelling and the increasing demand of healthy plant material have led to genetic erosion of landraces in the cultivated grapevine (Wolkovich et al. 2018), with many of the traditional and local cultivars almost disappeared, and some of them only found in germplasm collections (This et al. 2006). In the current scenario, only five highly appreciated wine cultivars: "Cabernet Sauvignon",

"Merlot", "Tempranillo", "Syrah", and "Grenache Tinta", make up for the production of red wines, while "Airén", "Chardonnay", "Sauvignon Blanc", "Trebbiano Toscano" and "Welschriesling" (syn. "Grasevina") are the basis for white wine production (Anderson 2013). On top of that, New World regions are also engaged with those "international varieties" that represent only 1 % of the total genetic diversity but cover more than 80 % of the planted hectares in Australia and New Zealand, being 78 % in Chile and 70 % United States (Wolkovich et al. 2018). In Spain, "Tempranillo" cv. represents 21 % of the vineyard surface-area (201081 has) and 41 % of the area dedicated to red varieties, increasing the surface planted between 2000 and 2012 by 75 % from around 90000 to 200000 ha (MAPA 2016). Besides Tempranillo, other six red varieties, "Bobal" (60301 has), "Grenache" (61372 has), and "Monastrell" (42500 has) followed by "Cabernet Sauvignon", "Syrah" and " Grenache tintorera" account for 90 % of the total global surface of red varieties and 46 % of the total vineyard area (García-Brunton 2018). In Rioja, the number of cultivated varieties have decreased from 44 in 1912 to 7 in 2000, with only three varieties (Tempranillo, Grenache, Graciano) covering 90 % of the total area under cultivation in 2019 (Riojawine https://www.riojawine.com).

1.4. DOCa Rioja varieties: Tempranillo, Grenache and Graciano

Rioja total winegrape vineyard area is 65001 ha, of which Tempranillo, a native cultivar to La Rioja (and Aragón) covers 79.8 %, followed by Grenache 7.8 % and Graciano 2 % (www.riojawine.com 2019).

Tempranillo is oenologically very versatile, capable of producing wines that can withstand long ageing periods, with a good balance of alcoholic strength, colour and acidity. From a sensorial point of view, Tempranillo aroma is normally characterized by banana, clove, toasty notes and roses (Ferreira et al. 2000). In ampelographic terms, Tempranillo shares characteristics of Albillo Mayor and Benedicto, being more similar to Benedicto in global terms (Ibañez et al. 2012). The three cvs, are characterized by mature leaves with seven lobes, a pentagonal blade shape, green shoots with red lines on both sides, and globose berries with low weight and neutral flavour (Ibañez et al. 2012). It is also defined by uniform fruit set and dark-blue berries with thick skin (Cervera et al. 2002). Agronomically, it sets well but is quite susceptible to pests and diseases and performs poorly under drought and high temperature conditions. Its name comes from the Spanish *temprano* (early) because of its short ripening cycle (www.riojawine.com). Among the synonyms, complete similarity with Cencibel, Tinto de Madrid, Tinto del País and Tinto Fino and more distant resemblance with Tinto de Toro and Ull de Llebre have been reported (Maul et al. 2014, http//: www.vivc.de).

Grenache is also a variety native to Spain, being also the most extensively grown variety in the world. According to OIV description, it is characterised by a medium-short length of leaf, with 1-2 clusters per shoot, medium cluster length and weight. Clusters contain a medium number of berries of small size and length, very slightly coloured flesh and neutral flavour. Grenache musts are characterised by high sugar content and medium total acidity (OIV 2018). Sensorially, it produces very aromatic wines characterised by floral scents as violet and fresh fruit notes as strawberry or plum (Ferreira et al. 2000). It is considered a robust variety able to withstand periods of drought, and moderately resistant to pests and major vine diseases, such as grape rust mite and powdery mildew, which explains its popularity among growers all over the world, despite being a shatter-prone variety. Synonymies found in ampelographic collections around the world include: Abundante, Alicante, Cannonaddu, Cannonaddu Nieddu, Cannonao, Cannonau

Selvaggio, Canonazo, Carignane Rosso, Garnaccho Negro, Garnatxa País, Gironet, Granaccia, Granaxa, Grenache Rouge, Lladoner, Retagliad Nieddu, Rivesaltes, Rousillon Tinto, Rousillon, Tinto Aragonés, Tinto Navalcarnero, Uva di Spagna.(Maul et al. 2014, http//:www.vivc.de).

Grenache complements Tempranillo cv. with its robustness, intense aroma and freshness, its resistance to drought and pests or diseases, whilst Tempranillo presumably improves Grenache in agronomic features: yield or fertility and oenologically versatility: deep wine colour and phenolic content, and a good balance between alcoholic strength and aging potential.

Graciano is a red grape cultivar native to La Rioja region whose cultivation is very restricted in other areas. Agronomically, shows low productivity and long ripening cycle, being quite resistant to downy and powdery mildew (www.riojawine.com). Among the synonyms found Morratel (France), Xeres (California) and Tinta Miúda (Portugal) (www.riojawine.com) with 78 synonyms gathered in the Vitis International Variety Catalogue (VIVC) (Maul et al. 2014). It delivers vivid red colour wines with a marked acidity and polyphenolic content, very aromatic ideal for ageing, and normally used to improve the characteristics of Tempranillo, giving higher colour intensity and aroma to the mixture (Escudero-Gilete et al. 2010).

1.5. Breeding evolution

The heterozygous nature of grapevine is a complicating feature for any effective breeding program (Adam-Blondon et al. 2004), on the other hand that enables producing offspring with a wide range of variability from crosses between different parental varieties. Old grape varieties carry deleterious alleles that exhibit pronounced inbreeding depression after selfing or sibling mating (This et al. 2011).

Grape breeding is a double face effort, whilst table and raisin grape markets are very receptive to new cultivars, wine industry is highly traditional. Breeding wine varieties is very restricted in European viticulture, especially in the Mediterranean, due to different regulations that ban the introduction of new wine making varieties in the *Denominaciones de Origen* system. Furthermore, grapevine is a perennial crop with a short juvenile period that requires time and space for phenotypic evaluation. In wine grapes, single seedling vines produce small quantity of fruit that needs to be transformed into wine before being evaluated, which complicates the process. Breeding efficiency depends on the screening methods used for fruit quality, yield and disease or climatic resilience. Moreover, little is known about the inheritance of wine-quality parameters, probably quantitative in nature and strongly influenced by environmental conditions (Riaz et al. 2007). However, the need for genotypes able to face new challenges such as plant diseases and climate change, has prompted recently the development by hybridization of new selections with optimal agronomic and oenological characteristics that maintain wine typicity. Since 2013, registration of new wine grape varieties has focused on disease-resistant genotypes, such as “Solaris” or “Cabernet Cortis” in Italy, implying a step forward in the regulation of hybrid varieties.

Plant breeding and genetics research is transitioning from a data-poor to a data-rich environment. The long and cost-consuming process of obtaining a new variety based in a conventional breeding program is being overcome by alternative methods in the recent years seeking to identify genes for desirable traits. These techniques are based on the use of marker-assisted selection (MAS) (Töpfer et al. 2011), genomic selection (GS) (Fodor et al. 2014) or Next generation sequencing (NGS) technologies. NGS of crop plant genomes, is revolutionizing the

field as newly abundant data enable and facilitate the discovery and use of millions of single nucleotide polymorphisms (SNPs) in diverse genomes (Huang et al. 2012, Xu et al. 2012). An important landmark in grapevine genetics was the complete sequencing of two grapevine genomes: the near homozygous "Pinot noir"-derived inbred line PN40024 (Jaillon et al. 2007) and its update by Canaguier et al. (2017); and the heterozygous cultivar "Pinot noir" clone ENTAV115 (Velasco et al. 2007). The publication of the grapevine reference genome sequence has enabled the prediction of gene sequences, the annotation of the grapevine genes (Grimplet et al. 2012) and the identification of single nucleotide polymorphisms (SNPs), which have become the most widely used on high-quality genetic map construction (Zhang et al. 2015). These SNP markers can be identified from short reads generated by NGS, either by aligning to a reference genome or by de novo assembly (Nielsen et al. 2011).

Following the publication of the PN40024 genome in 2007, no genome reference of equivalent or greater quality has been released for *V. vinifera*. A *de novo* approach was adopted to assemble the genome sequence of Thompson Seedless, a ubiquitous multipurpose cultivar. The genome of "Sultanina" table variety has also been fully sequenced (Di Genova et al. 2014), representing a new opportunity for the identification of genes related to the historical and morphological divergence existing between wine and table cultivars. In wine grape cultivars, Da Silva et al. (2013), proved a reference-based assembly approach, which resulted successful assembling multiple Arabidopsis genotypes, but failed to reconstruct specific sequences with Tannat variety and over 10 % of the gene space was not represented in the assembly, illustrating that the genomic sequence of one cultivar is not enough to represent the total variability of the species (Minio et al., 2017). Thanks to the discover of the FALCON-unzip diploid-aware software for the genome reconstruction, the Cabernet Sauvignon (Minio et al. 2017), Chardonnay (Chin et al. 2016), and Carmenere (Minio et al. 2019) assemblies are able to represent their haplotype diversity, since this strategy produced genome assemblies more contiguous and complete than PN40024 and include haplotype-specific gene sequences that are endemic to the highly heterozygous species (Cantu & Walker 2019). Besides, recently, a high-quality, diploid-phased Chardonnay genome assembly was produced from single-molecule real time sequencing, and combined with re-sequencing data from 15 different Chardonnay clones (Roach et al. 2018).

Among the NGS technologies the recent development and availability of different genotype by sequencing (GBS) protocols provided a low-cost approach to perform high-resolution genomic analysis of entire populations in different species (Crossa et al. 2013). GBS method is a powerful and useful method to obtain genome-wide variability information for populations composed by hundreds of individuals (Crossa et al. 2013, Spindel et al. 2015, Perea et al. 2016), delivering large numbers of marker genotypes with potentially less ascertainment bias than standard single nucleotide polymorphism (SNP) arrays (Crossa et al. 2013).

GBS protocols start with a digestion of the DNA using one or more known restriction enzymes. Then, fragments of suitable lengths (less than 800 bp) are ligated to adapters, amplified and sequenced in a high throughput Illumina platform. Another advantage of this method is that multiple samples can be sequenced in one single lane adding appropriate barcodes (Elshire et al. 2011). After this step, sequenced reads are ready to be demultiplexed and either analysed de-novo or aligned to a reference genome if available (Perea et al. 2016). The most interesting characteristic of this protocol is that although a relatively small portion of the entire genome is sequenced, it is reasonably well distributed and reproducible, what enables to identify and genotype thousands of genomic variants across the genome of different samples. For that reason, this technique is becoming the chosen method for several applications in plant genomics and plant

breeding (Myles et al. 2013), such as the construction of high-density genetic maps (Hyma et al. 2015, Smith et al. 2018, Teh et al. 2017), genetic mapping of complex traits through Genome-Wide Association Studies (GWAS) (Crossa et al. 2013) and estimation of breeding values in genomic selection (Spindel et al. 2015).

A key component of any GBS protocols is the bioinformatics pipeline required to analyze the reads and to obtain polymorphic sites within the sequenced population. Custom packages such as Tassel GBS pipeline (Glaubitz et al. 2014) have been developed specifically for analysis of the types of reads produced by GBS technologies Tassel in particular takes advantage of the nature of GBS reads to perform a highly efficient calculation of genomic variants. Widely used packages as Samtools or GATK for variant detection and genotyping have been used to analyse GBS data (Perea et al. 2016). The main advantage of these methods over previous approaches is that they can still work in the absence of a reference genome. NGSEP (Next Generation Sequencing Experience Platform) currently provides a great balance between completeness, accuracy, efficiency and usability (Perea et al. 2016).

GBS holds the potential to close the genotyping gap between references of broad interest and mapping/breeding populations of local or specific interest. Unlike other high-density genotyping technologies which have mainly been applied to general interest "reference" genomes, the lower cost of GBS makes it an attractive tool of saturating mapping and breeding populations with a high density of SNP markers (Spindel et al. 2013). Results have shown that this methodology is efficient for genotyping a variety of species, including those with complex genomes such as barley (Poland et al. 2012), oats, (Huang et al. 2014), onion (Jo et al. 2017) and grapevine (Yang et al. 2016, Smith et al. 2018, Guo et al. 2019).

The first application of GBS in grapevine was done by Barba et al. (2014), constructing high-resolution parental linkage maps in an interspecific *V. rupestris* × *V. vinifera* segregating population. More recently, high density genetics maps have been elaborated merging two (Teh et al. 2017) or more (Tello et al. 2019) populations. The application of GBS and other NGS technologies has enabled the efficient discovery and genotyping of SNPs in grapevine, resulting in the detection of a massive number of markers to detect phenotype – genotype associations in interspecific segregating populations (Chen et al. 2015, Zhu et al. 2018) and in grapevine diversity panels (Guo et al. 2019).

1.6. QTL analysis

The identification of genotype-to-phenotype associations is essential in plant breeding. Genetic maps have been widely used to identify genes responsible for grapevine composition and development. Traditional bi-parental mapping populations continue to play an important role in gene discovery, and both bi-parental and multi-parental breeding populations remain the foundation of many plant breeding programs (Almeida et al. 2013, Zhu et al. 2018).

Considerable progress has been made in the identification of molecular markers and the construction of molecular linkage maps in grapevine. A great step forward has been made between the first molecular map built from a 60 F_1 progeny from the cross "Cayuga White" × "Aurore" generated in 1995 (Lodhi et al. 1995) and the last high density multiparent map developed in 2019 using 10 subpopulations (Tello et al. 2019).

At the beginning qualitative traits were studied, so the first genetic localizations of traits were based on the observation of their segregation as presence or absence. In grapevine, the major genes responsible for qualitative traits are sex determinism (Dalbó et al. 2000, Margueritt et al. 2009, Fechter et al. 2012, Battilana et al. 2013, Zhou et al. 2018), and berry color (Doligez et al. 2002, Mejía et al. 2007). With the application of genome-spanning genetic maps, the polymorphic qualitative traits detected in a segregation progeny can be positioned in relation to molecular markers. However, many agriculturally important traits such as berry weight (Fanizza et al. 2005, Cabezas et al. 2006), seedlessness (Doligez et al. 2002, Costantini et al. 2008), flower morphology (Margueritt et al. 2009), total sugar content and total acid content (Viana et al. 2013, Chen et al. 2015), timing of flowering, veraison, and ripening (Fischer et al. 2004, Costantini et al. 2008, Duchêne et al. 2012) are controlled by many genes and are known as quantitative or polygenic traits. Quantitative Trait Loci (QTL) are the regions within genomes that contain genes associated with those traits, and their identification was enabled by the development of DNA (or molecular) markers early in the 1980s (Collard et al. 2005). Initially, the identification of QTL was mainly based on linkage mapping techniques, where polymorphisms between two parents were detected in a segregating population, and the linkage of a region to a given phenotype was determined by genotyping recombinants exhibiting phenotypic variation for the trait of interest. Thus, the genetic control of major traits in grape, such grape size, phenology stages, must composition, has been explored via simple sequence repeat (SSR) markers in biparental populations (Constantini et al. 2008, Fechter et al. 2014, Ban et al. 2016, Bayo-Canha et al. 2019). Nowadays thousands to millions of markers are developed by the emerge of next generation sequencing technologies improving mapping coverage and resolution (Deschamps et al.2012). As a result, association analyses have been also performed in grapevine using GBS, starting by Barba et al. (2014) in the study of powdery mildew resistance (Guo et al. 2019), downey mildew resistance (Saptoka et al. 2019) or berry weight, cluster size, berry flavour, malic acid, total soluble solids (Yang et al. 2016). These works could establish the genomic regions that influence a particular trait within different grape mapping populations with common markers, being applicable to establish the relationships of QTL in different genetic backgrounds.

Among the methods for detecting QTL, Simple Interval Mapping is one of the most powerful, since instead of analysing single markers, uses linkage maps and interval analyses between adjacent pairs of chromosomes simultaneously (Lander & Botstein 1989). Several public and private software packages are available to perform QTL analysis; among the most used in grapevine are Cartographer (Basten et al. 2003), WinQTLCart (Wang et al. 2004) and MapQTL (Van Ooijen 2009).

1.7. Grapevine reproductive cycle

In temperate regions, grapevine completes the reproductive developmental cycle over two consecutive growing seasons separated by a dormancy period between autumn and spring (Carmona et al. 2008). A typical trait of *Vitis vinifera* is the simultaneous formation of both vegetative and reproductive forming organ primordia by the same apex (Boss et al. 2003). In spring, every sprouting bud gives rise to a stem and the first-formed bud in the leaf axil produces a lateral shoot that will carry bunches in the second season (Carmona et al. 2008). In the axil of that lateral shoot, a latent bud will be formed and there will take place floral initiation and early stages of inflorescence development (Carmona et al. 2008). During the flowering process the first two to three lateral meristems have the potential to differentiate as inflorescences while the

following lateral meristems produced will start differentiation as tendrils (Lebon et al. 2008). By the end of the summer these buds enter in a dormant state, allowing the possibility to resume growth under more favourable conditions the second year (Díaz-Riquelme et al. 2012). The primary latent bud, if fruitful, contains a future shoot with inflorescence meristems, tendril and leaf primordia and in the case of non-fruitful canes (originated from non - fruitful buds), no lateral structures develop (Carmona et al. 2008). At this stage, carbohydrate physiology of the whole vine during the period of inflorescence initiation determines the number of bunches that will emerge the following year. Once winter comes to its end, this dormant period finishes and different developmental processes start generating the elongation of rachis and lateral branches, and the differentiation of secondary and tertiary branches, that form the racemes characteristic of grapevine inflorescences. (Carmona et al. 2008). In this stage the formation of floral meristems also takes place, producing flowers with their sexual organs, completed only a few days before anthesis. Besides, the terminal flower develops first, then the lateral ones and finally, the most basal (Carmona et al. 2008, Keller 2010).

Grapevine reproductive cycle is different in cultivars which present hermaphroditic flowers, and pollination is made by self - fertilization, compared with wild plants that are dioecious, requiring crosspollination (via either wind or pollinators).Thus, *Vitis vinifera* species display three types of flowers: males and females in *V. v.ssp.sylvestris* and hermaphrodites in the cultivated subspecies *V. v. ssp sativa*. Flowers can be perfect (hermaphroditic), imperfect male (female sterile or staminate) or imperfect female (male sterile or pistillate), with fused petals that separate at the base, forming a “calyptra” or cap (Cattonaro et al. 2014). Male flowers are characterized by having long erect stamens and a reduced carpel without style or stigma, but with nectaries and ovaries. Female flowers have a complete carpel with style and stigma but short and reflexed stamens with sterile pollen (Caporali et al. 2003). Berries produced by female plants are described as small, dark in colour, and sweet enough to attract birds, contributing to seed dissemination (This et al. 2006). Male, female, and hermaphroditic flowers are not visually attractive to insects as the flowers are small and the petals drop at anthesis (Carmona et al. 2008). At early developmental stages, male and female flowers are morphologically indistinguishable from a hermaphrodite flower, becoming unisexual only at later development stages (Ramos et al. 2014).

Morphologically, hermaphroditic flowers are formed by sepals, petals, androecium and gynoecium, which arrange in concentric rings (or whorls) from the outside to the inside (Vasconcelos et al. 2009). Sepals (normally five) constitute the calyx, and they are located at the base of the flower to protect it in the early stages of development (Keller 2010), and petals are fused by epidermal cells, forming the calyptra. The androecium is normally comprised of five stamens, each one composed of a long filament ending in a bilocular anther containing pollen sacs, which contain pollen grains. The gynoecium (or pistil) is located on the central part of the flower, its inner cell wall develops into the septum, which is the central part of the style through which the pollen tube will grow. The ovary is the enlarged area at the base of the style, and it protects the ovules (located in the ovary locules) from desiccation and physical injury (Keller 2010, Lebon et al. 2008, Vasconcelos et al. 2009). Pollination usually occurs by pollen grains originated in the flower own anthers (Keller 2010), which are deposited on the stigma, in the upper part of the pistil.

Flower formation happens during spring, bud break is preceded by the activation of all structures in the latent bud, especially the differentiation of inflorescences and the first steps of floral organ development (Lebon et al. 2008). There is an order of organ appearance that is similar

to all angiosperms: five sepals appear first and form the calyx, then five petals form the corolla, followed by five stamens and then two carpels that generate the pistil. The calyx has a ring feature (Gerrath 1993) that protects the internal organs from environmental fluctuations at the early stages of bud break, and the cap (formed by the join of petals and sepals) protects the fertile organs, until it falls at anthesis following the growth of stamen filaments. The gynoecium originates from the fusion of two carpels, and in each locule, two anatropous ovules develop and are inserted into the septum. Inflorescence and flower formation in grapevine are such a complex process that some authors have described it with 22 successive stages encoded by numbers from 0 to 50 based on the external inflorescence characteristics (Lorenz et al. 1994, Coombe 1995). For example, anthesis occurs at stage 19 and continues for about one week, and after cap fall take place the following stages: full bloom (stage 23) is reached when 50 % of flower caps have fallen, whereas stage 25 is reached when 80 % of caps have fallen. Stage 27 marks the onset of berry development from the fertilized ovules, stamens then degenerate and the young berry is then visible (Lebon et al. 2008). Interestingly, the kinetics of male and female reproductive development depend on variety and are not necessarily synchronous with the developmental scale previously mentioned. (Lebon et al. 2008). For example, both male and female meiosis occur one week earlier in Pinot noir than in Gewurztraminer and in Pinot noir, meiosis takes place between stages 12 and 15 in anthers and between stages 15+2 days and 15+8 days in ovules (Lebon et al. 2008). Meiosis is a key point in the accomplishment of sexual reproduction, where anthers and ovules show particular sensitivity to various kinds of stress, and even a lack of sugar could conduct to its abortion (Lebon et al. 2008).

Fruit development is triggered by pollination and fertilization processes. In fleshy fruits, such as tomato or grapevine, berries develop from an ovary after fertilisation. The ovary wall turns into the pericarp, formed by three distinct cell layers: the epicarp (skin); the mesocarp (flesh); and the endocarp (cell layers in contact with the seeds). (Coombe 1976, Ollat et al. 2002). Seeds are in the endocarp and (as in the berry mesocarp) it is possible to distinguish two different seed tissues: an internal hypodermis formed by a few cellular layers and an internal epidermis (Carmona et al. 2008). Berry growth follows a double-sigmoidal pattern with two growth stages (berry formation and berry ripening) separated by a lag phase of slow or no growth (Coombe & McCarthy 2000, Robinson & Davies 2000). The first stage begins immediately after flower pollination, during stage I, berry growth is due to cell mitotic division and cell expansion. Approximately 4 – 6 weeks post-anthesis, cell division ceases and only cell expansion subsists. Coinciding with this rapid growth is the biosynthesis of phenolic compounds, such as tannins and hydroxycinnamates, and organic acids, such as tartaric and malic acid, reaching their maximal concentration at the end of this first stage. (Bindon et al. 2013, Coombe & McCarthy 2000). Besides, at the end of this stage, all seed tissues are formed. Stage II corresponds to a slow growth phase that ends with veraison (the onset of ripening). During this phase, sugars initiate its accumulation reaching its maximal concentration at the end of the third stage. Finally, berry growth restarts in stage III but only through cell enlargement (Coombe 1976, Ojeda et al. 1999). Is in this final stage, when anthocyanins and aroma compounds are accumulated in berry skin (Bindon et al. 2013, Coombe & McCarthy 2000, Zamboni et al. 2010) and the berry experiences a second period of rapid cell expansion as the pericarp grows to its final size. Many changes in berry metabolism happen during this process: accumulation of sugars, decrease in organic acid concentration, and production of secondary metabolites, so berry size and composition will differ depending on the stage of development. (Wong et al. 2016). Grapevine flowering and fruiting developmental processes are not only genetically determined, but are markedly influenced by environmental variations and management practices (Bindon et al. 2008).

1.8. Grape quality and climate change adaptation

The statement that good wine production requires good quality grapes is a crucial dogma in wine industry. Quality is a difficult term to define and is usually linked to 'grape composition' as the metabolite composition of grapes and wine can be measured and quantified (Carmona et al. 2008). Thus, berry quality is closely linked with the presence of sugars, acids, anthocyanins, tannins (Holt et al. 2008, Rolle et al. 2015, Gil et al. 2015, Wong et al. 2016), and phenolic and volatile compounds (Gerós et al. 2012), most of them accumulate in the skins and seeds (Barbagallo et al. 2011, Downey et al. 2006).

Skin-to-flesh ratio influences grape composition and quality with higher concentrations of phenolic compounds in small berries (Gil et al. 2015). However, the direct relationship between berry size and wine quality is still highly debated (Friedel et al. 2016, Xie et al. 2018). Several studies reported that berry size had no influence on grape and wine quality, while viticulture practices such as pruning (Holt et al. 2008, Roby & Mathews 2004), and environmental conditions (Van Leeuwen et al. 2017) are major drivers in vine metabolism, hence grape composition (Dai et al. 2011) not berry size per se (Xie et al. 2018).

One of the limitations in the study of berry size and composition is variability. Mean and range values of both parameters are the result of complex interactions among genotype, environmental factors, such as temperature or light, their interactions, and cultural practices (Keller 2010). Variability is present within berries, among berries within a cluster, among clusters on a vine, and among vines within a vineyard (Dai et al. 2011). Sink competition at the tip of a cluster produces lower weight berries than in the centre or shoulder (Tarter & Keuter 2005). Berry weight shows high genetic diversity within the *Vitis* genus, ranging from < 0.5 to > 10 g (Houel et al. 2013).

Among viticultural practices, crop thinning has been found to be a useful tool in the improvement of berry composition by increasing anthocyanin and polysaccharide levels in Syrah (Gil et al. 2013) or increasing color, currant aroma, and astringency of Pinot Noir wines (Reynolds et al. 1996). A decrease in vine vigour is thought to improve final wine quality, by increasing grape-derived compounds, such as anthocyanins (Koundouras et al. 2006, Song et al. 2014), or other phenolics (Schreiner et al. 2013), due to more open canopies and more exposed clusters. Leaf removal is estimated to modify canopy microclimate (sunlight exposure), in a cool-climate Pinot Noir region increasing the levels of grape-derived volatile compounds (Feng 2014). Given that those cultural practices have a repercussion on the final wine aroma and sensory characteristics, wine growers may take advantage of adapting vine management to the specific region and annual weather conditions in order to improve wine quality.

In the last 30 years, a significant change in grape composition has been observed due to climate change. In the future, countries like Spain, and especially warm and semi-arid regions in the south east, will suffer the effects of temperature rise, and the increased atmospheric water deficit and evaporation rate that will make difficult to maintain quality and productivity (Fraga et al. 2013, Resco et al. 2016, Savé et al. 2017). Other visible consequences of climate change will be the advancement in phenology periods like sprouting, veraison and maturation, promoting harvests of up to 20 days earlier in some Mediterranean regions (Webb et al. 2008). Consequently, berry ripening will occur earlier in summer, under higher temperatures, having a significant impact on berry quality. High temperatures accelerate pulp maturity and cause a decrease of grape acidity, mainly because of a faster degradation of malic acid (Sweetman et al. 2014). An excess of sugar content in berries (Fraga et al. 2013), conducts to a higher ethanol content, a greater

aroma volatility, lower anthocyanin content and hence less colour (Resco et al. 2016). In contrast, wine-growing regions at high latitudes where achieving a correct level of ripeness is the limitation for high-quality wines, will be favoured. This challenge can be faced by introducing more variability at the cultivar level with better adaptation to the new climatic conditions.

In 2020, OIV has estimated a total wine production of 260 Mill. hL, meaning a 10 % decrease relative to 2018, that in Spain would reach a 25 % drop, due to the changing climatic conditions. Even though moving vineyards to higher altitude areas seems a good approach, developing varieties better adapted to this new scenario seem to be the best long-term strategy. Therefore, grapevine breeding programs involving the hybridization of heterozygous premium varieties and the further selection for one or a few of the best hybrids using the recently available NGS tools may succeed in developing high - quality wines preserving tipicity in the future climate change context.

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CHAPTER 2.
OBJECTIVES

2. Objectives

Wine making is a dynamic process that must be adapted to changes such as global warming and new consumer interests. In the context of breeding for grape quality; two intraspecific populations obtained from crosses between three of the most relevant red wine Spanish varieties, Grenache, Graciano, and Tempranillo were studied with two main objectives:

1. Identification of the major genetic determinants of quality traits such as berry size and shape and the different traits contributing to them.

    - Evaluate phenotypic segregation of relevant traits related to phenology stages, productivity, berry and flower morphology, seed-related traits and must composition in the progenies, and conduct a pre-selection of hybrids with improved characteristics.

    - Construct a high-density genetic map of Grenache ×Tempranillo progeny using GBS technology and identify QTL for berry, flower, seed, productivity, must composition and phenology stages on the genetic maps.

    - Analyze the influence of flower sex in flower morphology, berry size, seed parameters, productivity, must composition and phenological stages.

2. Characterization of oenological composition of Tempranillo segregating progenies and Pinot Noir clones.

    - Assess of the influence of berry size in must and wine composition and quality parameters in two well-known wine regions, La Rioja (Spain) and Marlborough region (New Zealand).

    - Perform sensory profiling of wines derived from twelve Graciano × Tempranillo selections, and identify premium genotypes for a climate change scenario.

CHAPTER 3.

GENETIC ANALYSIS OF MORPHOLOGICAL, AGRONOMICAL AND PHENOLOGICAL TRAITS.

STUDY OF SEX INFLUENCE

CHAPTER 4.

EVALUATION OF WINES DERIVED FROM TEMPRANILLO INTRASPECIFIC HYBRIDS AND PINOT NOIR CLONES

4.2. Sensory profiling and quality assessment of wines derived from Graciano x Tempranillo selections

Abstract

Wine production is a dynamic process that must be adapted to changes such as global warming and new consumer interests. Obtaining new cultivars by hybridization of traditional varieties is a promising approach with great potential to produce wines that are able to preserve regional typicity, together with adaptability to both evolving market preferences and distinct environmental scenarios.

In this research, wines from twelve Graciano × Tempranillo selections were analyzed in two consecutive years. Sensory properties and quality were evaluated by a trained panel and a group of wine experts, respectively. Quality was positively correlated with anthocyanin and phenolic content ($r = 0.8$, $r = 0.7$, $p < 0.01$, respectively). Wines presented high sensory variability differing in eight attributes in each vintage. Two high quality selections, TG8 and TG63 consistently improved Tempranillo and Graciano specimens, presenting high color intensity, acidity, and positive aroma related to red fruit. Furthermore, TG129 a late-ripening genotype with high polyphenol content and fruity aroma, and other selections with roasted or dried fruit aroma notes appear as potential cultivars suitable to satisfy distinct consumer demands in the context of global warming.

4.3. Variability in berry traits, must and wine composition in Pinot Noir clones in the region of Marlborough in New Zealand

Abstract

New Zealand is considered one of the New World best regions for the production of high-quality Pinot Noir (PN) grapes and wines. In an increasingly competitive market, the offer of a wide range of wines better adapted to consumers can be a hallmark for these new regions. Know the potential variability on quality parameters of Pinot Noir clones and how berry size and environmental conditions could influence on them was the objective of this research. First of all, a preliminary study using 8 different clones to select 3 (PN_115, PN_ Abel and PN_UCD5) in base on berry size. Samples were collected from 3 different vineyards, located in two different subregions, and with two different rootstocks. Morphological traits of berries and seeds, and chemical parameters of berry extracts, musts and wines were measured. Significant differences were found among clones in berry morphology (PN_115 presented the lowest berry size) or aminoacid accumulation in musts, being the concentration of these compounds higher in PN_UCD5 independently of the sub region studied. The influence of berry size on studied variables was weak, smaller berries presented higher total acidity than bigger berries. In the warm and humid subregion, with poor fertile soil conditions (Wairau valley), berry weight was smaller, amino acid accumulation lower, instead phenolic composition and colour index in must were higher. Rootstock effect was also observed, the RSK 101-14 produced higher seed weight, pH, and phenolic and nitrogen composition in musts.

Introduction

Wine grapes are one of the most valuable perennial crops in the world (FAO 2018). Wine quality relies on high quality grapes (Nimii et al. 2018), which are influenced by several factors as viticultural management, selecting suitable varieties/clones; and winemakers supervising the correct fermentation of grapes. Grape quality has traditionally been associated to berry size, with small berries leading, in theory, to high quality wines. However, the relationship between berry size and berry composition has been matter of debate, between supporters (Rolle et al. 2015, Wong et al. 2016) and detractors (Roby et al. 2004, Walker et al. 2005).

Grape berry weight shows high genetic diversity within the *Vitis* genus, ranging from < 0.5 to > 10 g (Houel et al. 2013) and varies among clones of a given cultivar (Dai et al. 2011). Grape berry composition is a highly complex trait under the control of complex interactions among genotype, environment and cultural practices, also showing a high genetic diversity. As a result, different clones have the capacity to produce wines with different chemical composition. Thus, distinct color, and aromatic profile was found between Albariño clones (Zamuz et al. 2007) or phenolic content in Monastrell (Gómez-Plaza et al. 1999, 2000), Cabernet Sauvignon (Burin et al. 2011), Pinot Noir (Schueuermann et al. 2018), Cabernet Franc, Cabernet Sauvignon and Merlot (Forveille et al. 1996).

Grape berry and must composition variability may be the result of berry-to-berry differences within a bunch; bunch-to-bunch differences on a shoot; shoot-to-shoot differences on a vine; or vine-to-vine differences in a vineyard (Trought et al. 2017). Many studies have found internal variability among clusters from the same vine for different physical and chemical

parameters (Tarter et al. 2005, 2008, Trought et al. 2017). Bunch position on the shoot and shoot position on the cane influence phenology, with shoots from distal buds and inflorescences developing earlier, and advancing fruit ripeness. In lower cane node positions a greater number of shoots with looser bunches were found whilst at higher leaf node positions bunch size was smaller and berry size greater (Martin & Vasconcelos unpublished data). Even berries from the tip and shoulder of the same cluster exhibited different aroma profiles (Noguerol-Pato et al. 2012). These results appear very important for quality modelling because both bunch and berry size are strongly and significantly related to the leaf node positions count of the basal bunch.

The influence of environmental and climatic factors as temperature and pluviometry in grape composition has been widely observed in many wine regions (Resco et al. 2016). These factors linked to others such as the variety, rootstock and viticultural management determine the chemical composition of the berry, influencing variables such as the accumulation of nitrogen or phenolic compounds (Gutiérrez-Gamboa et al. 2017, Vidal et al. 2017).

In this context, in the present research it was assessed the influence of berry size, environment conditions and rootstock on berry, must and wine composition in Pinot Noir clones. First, a pilot study was made to select clones in base on berry size. This study also provided information about Pinot Noir genetics influence berry morphology and must composition. Two assays were made in different subregions to prove the following hypotheses:

- **Study 1.** PN clones differing in berry size will present differences in grape, must and wine composition.

- **Study 2.** Different environmental conditions will affect berry morphology, must and wine characteristics of different PN clones.

Material and Methods

Climatic data and subregion characteristics

Marlborough wine region is formed by three sub-regions: Awatere Valley located at south - east (30 % of vineyard area), Wairau Valley (45% of plantings) and the Southern Valleys zone (25 %) (Figure 4.3.1). In order to maximize differences, the first two sub-regions were chosen for this work. Awatere valley is characterised by free - draining soils fertile soils formed from loam and alluvial gravel and vines grow deep root systems. Climate is greatly influenced by the ocean, characterised by intense sunlight cooled by ocean winds, (Table 4.3.1) and often with a degree of elevation, which promotes a delay in harvests in comparison to Wairau Valley (https://www.nzwine.com).

Wairau Valley is a wide river valley that follows the Wairau River, and is separated in its upper part from the city of Nelson by Richmond Mountains, and the Wither Hills in the south protect the valley from harsh weather from the south - east. The effect of mountains and hills create a Foehn effect, with the west being subjected to wet weather, whilst Blenheim city enjoys the warmth and sunshine. Soils are not very fertile, located along the river terraces, being usually shallow, formed by clay, silt and stones which aids fast-draining. As a result, it has a warmer, more sheltered climate than in the Awatere Valley (https://www.nzwine.com). Full details of the

regional weather conditions are available on the Marlborough Research Centre web page (http://www.mrc. org.nz/category/weather-data/).

Figure 4.3.1. Subregions of Marlborough region. A. Southern Valleys, B: Wairau Valley and C: Awatere Valley. Source: https://www.nzwine.com/

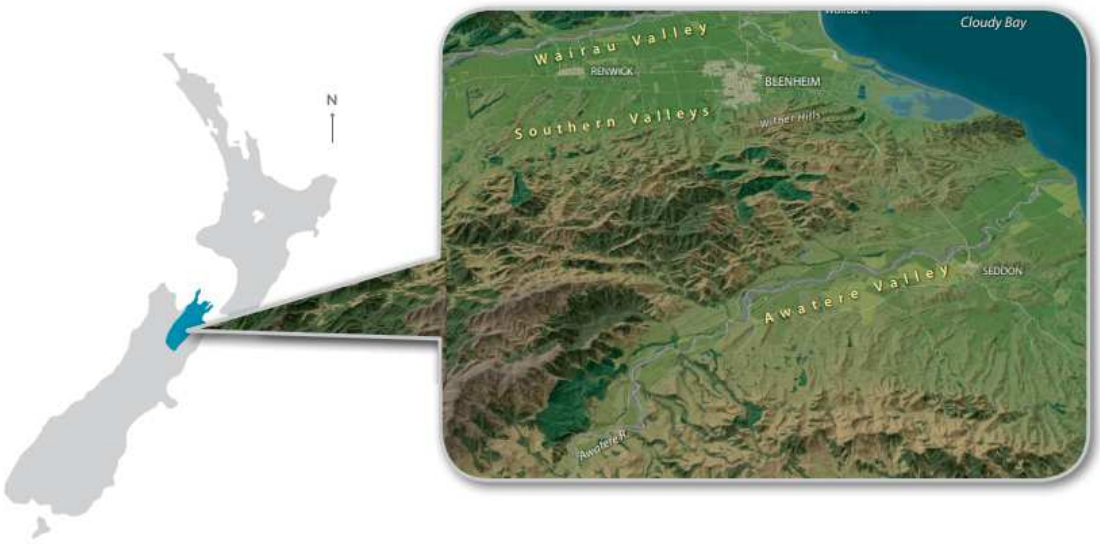


Table 4.3.1. Climatic data of Awatere and Wairau valley during September 2017 to April 2018.

	Period Sept-April	Awatere	Wairau
Rain (mm)	Total rain	675	487.8
Humidity	RH	63.9	71.8
	Max mean	26.9	28.2
T[a]	Min mean	3.8	5.4
	Max	30.5 (Jan. Feb)	32.5 (Jan)
	Min	-0.7 (April)	0.3 (April)
Radiation	Mean day	401.6	548.7
(MJ/m2)	Max	474.0 (Sept)	790.1 (Dec)
	Min	167.1 (March)	320.0 (April)

Plant material

The three studies were conducted in commercial vineyards were vines grown using a Double Guyot, bilateral 12 - node canes training system. Pest and disease management followed Sustainable Winegrowing New Zealand guidelines (http://www.nzwine.com). On April 2018, twenty-five cluster samples of each clone were harvested separately from both the north (exposed) and south (shaded) sides of the same vine in cane node position 2, inflorescence leaf node position 4. Careful cluster selection was undertaken to ensure the clusters were picked from the correct position on the vine. Not field replicates were collected in the Pilot Study, three was the number of replicates in Study 1 and Study 2, where seventy-five clusters per clone were picked.

A Pilot study was conducted with eight different PN clones with the aim to select three with the greatest differences in berry morphology (Table 4.3.2). Three of them were assessed for Studies 1 and 2 for Studies 2 in two different regions and grafted in two different rootstocks. Vines of 25-year-old grafted onto 3309 rootstock were used for Pilot study and Study 2, being the spacing between vines of 1.5 × 1.25 m and 2.2 × 1.44 m, respectively. Study 1 were conducted in Wairau subregion grown on 101 - 14 rootstock, with a spacing of 1.5 × 1.22 m.

Table 4.3.2. Summary of the main features of the studies conducted.

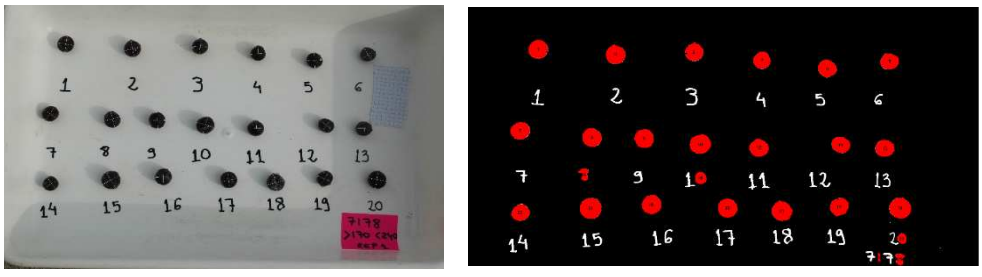
Study	Clones	Rootstock	Subregion	Plot
Pilot Study	PN_UCD5, PN_115, PN_Abel, PN_777, PN_Am, PN_Mariafeld, PN_667, PN_UCD6	3309	Wairau Valley	Clayvin vineyard (Giesen)
Study 1	PN_UCD5, PN_115, PN_Abel	110-14	Wairau Valley	Bankhouse vineyard (Indevin)
Study 2	PN_UCD5, PN_115	3309	Awatere Valley	Ballochdale vineyard

Berry and seed parameters

Berry morphology and berry extracts

Berry data were collected on April 2018 at ripening stage when random grapes picked from the top, medium and bottom of the clusters reached technological maturity (21º Brix). At harvest date, 200 whole berries from each clone were sampled from representative clusters and mean berry weight (g) was calculated. Berries were after frozen at - 20ºC to assess berry morphology and for subsequent extraction and analysis. In 40 berries per plant, length and diameter (mm) were measured in ImageJ software after photographs were taken (Figure 4.3.2), and shape coefficient was calculated as the ratio between length and diameter (Houel et al. 2013). Measures were analyzed in replicate.

Figure 4.3.2. Picture of the berries before and after being analyzed.



With the remaining 160 berries, 2 extracts were made for each clone, following the protocol guide showed in AWRI Grape Portal (http://www. https://www.awri.com.au/), and consists in the extraction of colour and phenolics using a solution of 1.0M Hydrochloric Acid and Acidified 50% v/v ethanol. Total anthocyanin content (mg/L), monomeric anthocyanin content

(mg M3G/L), total phenolics (AU), total polyphenolic and colour indexes (AU) were measured by spectrophotometric assays.

Seed analysis

Mean seed number per berry (SN) and mean seed fresh weight (SW, mg) were obtained in duplicate from a sample of 20 berries per clone randomly selected. Seed mass was also measured by digital image with ImageJ software (Figure 4.3.3). The number of seeds per g of marc was also measured in a representative sample of 20 g. Measures were analyzed in replicate.

Figure 4.3.3. Picture of the seeds before and after being analyzed.

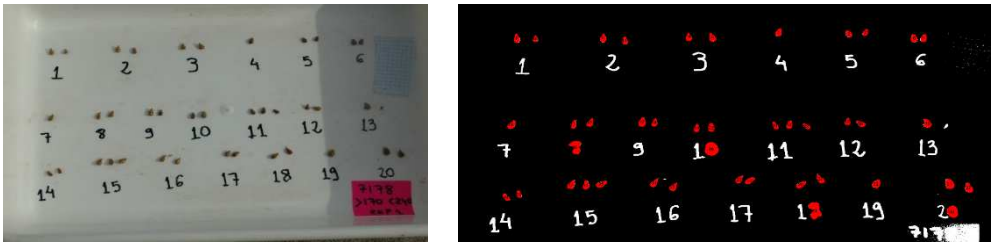


Image analysis techniques were confirmed as a useful tool for the measurement of phenotypic characteristics of berry and seeds as it was previously confirmed in other works (Wycislo et al. 2008, Rodriguez-Pulido et al. 2012). In the present study, image analysis was confirmed to be a lower time-consuming tool in the measurement of berry morphology, since berry area, volume and mass obtained correlated to berry weight and berry length or diameter.

Analysis of phenolics in grapes and wines

Direct optical density analysis was used to estimate the concentration of phenolic compound using the Somers Colour essay (Somers & Evans 1977). With this method Total anthocyanins (mg / L), colour density (AU), Hue, and Total phenolics (AU) were estimated from absorbance lectures. Total phenolics were estimated by the magnitude of absorbance at 280 nm. Absorbance at 420 nm gives an estimate of the concentration of yellow/brown pigments (mainly tannins) but also some oxidative phenolic breakdown products under natural wine pH / SO_2 conditions. Absorbance at 520 nm gives an estimate of the concentration of all red coloured pigment present under natural wine pH / SO_2 conditions (Somers & Evans 1977). Besides, total phenolics in musts were also evaluated by Folin - Ciocalteu method and expressed mg GAE / L.

Monomeric anthocyanins (mg M3G / L) were quantified by the pH difference method (Lee et al. 2005), which is a rapid and simple spectrophotometric method based on the chromophore anthocyanin structural change between pH 1.0 (colored) and 4.5 (colorless). Monomeric anthocyanin pigments reversibly change colour with a change in pH. The coloured oxonium form exists at pH 1.0, and the colourless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. Concentration is expressed on a malvidin-3-glucoside basis.

Nitrogen compounds and organic acid determination

Amino acid profiles were quantified on an Agilent 1200 series HPLC using a gradient elution programme of phosphate/borate buffer (10 mM each, pH 8.2) and organic solvent (MeOH: MeCN: H_2O, 45:45:10) on a Phenomenix Kinetix C18 column (5 um, 240_4.6 mm) as is described in Martin et al. (2016). Primary amino acids were derivatised online with o-phthalaldehyde and 3-mercaptopropionic acid and detected by fluorescence (340 nm excitation, 450 nm emission). Samples were treated with iodoacetic acid to aid in the reduction of cysteine. Secondary amino acids derivatised online with 9-fluorenylmethyl chloroformate and detected by fluorescence (260 nm excitation, 315 nm emission). A standard mix of 17 amino acids was purchased from Agilent. All standards and samples contained the internal standards sarcosine (100 mg / L) and a-aminobutyric acid (100 mg / L). All samples were diluted fourfold in water and filtered through a 0.45-um syringe filter before injection. All samples were run in duplicate and quantified on a four-point standard curve (R2 > 0.98) (Henderson & Brooks 2010). Ammonium was analyzed to assess the YAN requirements of the ferments, being quantified by enzymatic assay (Vintessentials Laboratories, Victoria, Australia).

Tartaric and malic acids were quantified on a Shimadzu Prominence, high performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) system using isocratic elution with a phosphate buffer (140 mM, pH 2.4) on an Allure Organic Acids Restek column (5 um, 240 _ 4.6 mm) as is described in Martin et al. (2016). All samples were diluted ten-fold in a solution containing thiourea as an internal standard and filtered through a 0.45-um syringe filter before injection (Shi et al. 2011). All samples were run in duplicate and quantified on a five-point standard curve. The correlation coefficient (R^2) of actual vs predicted concentration was > 0.98.

Must analysis

Must samples were subjected to a range of primary metabolite analyses. Soluble solids concentrations (° Brix) were determined with an Atago refractometer PAL-1 (Atago Co. Ltd, Japan). Titratable acidity (TA) and pH were determined on a Mettler Toledo T70 autotitrator (Mettler Toledo, Columbus, OH, USA) using an equivalence point titration. Aqueous sodium hydroxide (0.1 M) was used as titrant and TA was expressed as tartaric acid equivalents. TSS (measured as _Brix) was determined using a Mettler Toledo RM40 refractometer (Iland et al. 2004). Samples were centrifuged or filtered before analysis and analyses were carried out in duplicate. Spectrophotometric assays were run on a Molecular Devices Spectramax 384 Plus, UV transparent 96 well microplate.

Winemaking

At harvest (on April 2018) fruit from three field replicates of Studies 1 and 2 were combined, to give three fermentation replicates. Clusters were processed using the standard Plant and Food Research (PFR) winemaking protocol. Samples were chilled overnight at 10 ºC and then crushed in a manual crusher (Marchisio Cervino 400 / 600 kg / H). A standard sulphur dioxide (SO_2) quantity (40 ppm) was added as potassium metabisulphite at crushing. Musts were cold soaked for 3 days at 6 °C and then warmed to 18 °C and inoculated with RC212 yeast (Lallemand, Denmark) (rate 250 mg/·L). Grapes of each clone were fermented in triplicate at 25 °C and di-ammonium phosphate (DAP) was added where yeast available nitrogen (YAN, mg N / L) concentrations were below 250 ppm. Ferments were plunged three times a day. Fermentation soluble solids concentrations (measured as °Brix) were monitored daily using a portable density meter (Anton-Paar DMA 35, Austria) and when residual sugar was less than 2 g / L as determined

by Clinitest® (Bayer, USA), ferments were given three days of post-fermentation maceration before pressing. Ferments were pressed in a compressed air operated 6-kg sample press (Stainless Steel Systems, Blenheim, New Zealand) under a cover of carbon dioxide (CO_2). A pressing regime of two minutes at 1 Bar followed by another two minutes at 2 Bar was applied. Wine was settled for one week and then racked off yeast lees. An addition of 50 mg / L SO_2 (as potassium metabisulphite) was made.

Wine samples were analyzed for pH, total phenolics and titratable acidity as for juice analysis one month after bottling. Reducing sugars (g / L) were quantified by an enzymatic assay kit (Megazyme International, Ireland). Alcohol (%) was measured using an Anton Paar wine alcolyzer (Anton-Paar, Austria). All measurements were taken in duplicate from each of the three fermentation replicates and variation was < 0.02 % v / v.

Statistical analysis

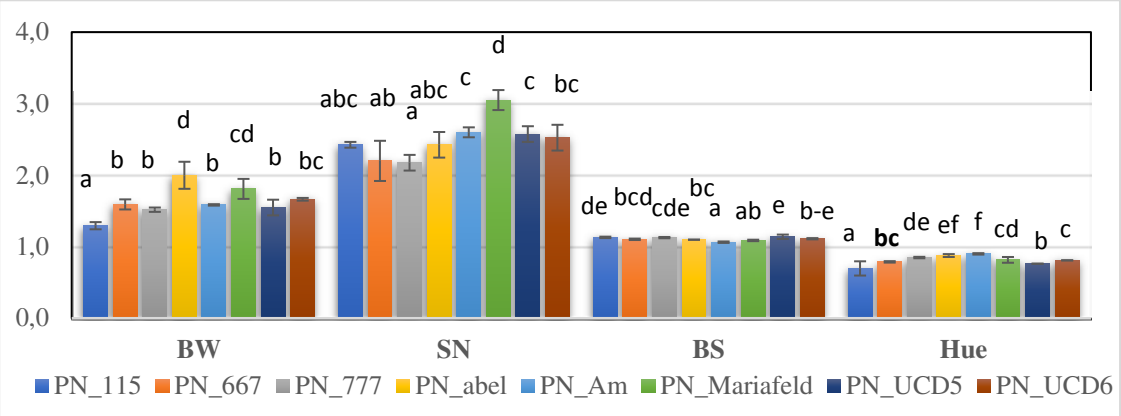
Normality distribution was checked by the Kolmogorov-Smirnov test. Data that significantly deviated from normality were analyzed by non-parametric Kruskal-Wallis test. ANOVA analysis performed with LSD test were carried out to detect differences between clones, plots and rootstocks. A MANOVA test was conducted to detect interactions between clone, rootstock and plots factors on the traits analyzed. Analysis were conducted with SPSS v.25. Principal Component Analyses (PCA) among the samples of each study were calculated using PAST software.

Results

Pilot study

Samples from eight PN clones were collected from the same position in the vine to study differences in berry, seed and must parameters. Clones presented statistical differences in berry morphology, seed traits and phenolic compounds of berry extracts as well as musts parameters (Supplementary material 4.3.1.). Figure 4.3.4 shows the main statistical differences of the parameters analysed. PN_Abel presented the highest berry weight (2.0 ± 0.19) and PN_115 the lowest (1.29 ± 0.05), both were selected to perform the two following studies along with PN_UCD5 because it showed intermediate values. PN_Mariafeld presented the highest number of seeds (3.05 ± 0.14), whilst PN_777 the lowest (2.18 ± 0.11). Regarding berry extracts, PN_115 presented the lowest value in Hue (0.7 ± 0.03) and PN_Am the highest (0.9 ± 0.06).

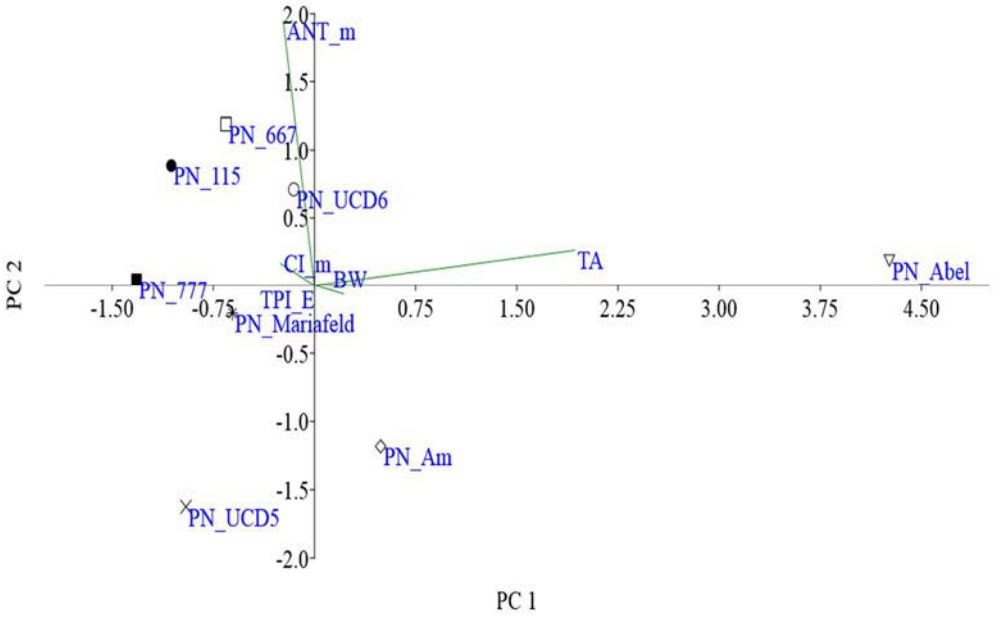
Figure 4.3.4. Differences between PN clones studied for berry weight (BW), seed number (SN), berry shape (BS) and Hue.



In musts, the widest ranges among clones were obtained for total acidity (11.3 g / L in PN_UCD6 and 5.9 g / L in PN_Abel), content of ammonium (149.4 mg / L in PN_777 and 78.1 mg / L in PN_UCD5), colour intensity (2.8 AU in PN_777 and 1.3 AU in PN_Mariafeld) and tonality (4.4 in PN_Am and 2.3 in PN_777). Major nitrogen compounds were arginine, alanine and proline while glycine, tyrosine, methionine, phenylalanine and lysine with the lowest concentration (Supplementary material 4.3.1.).

With the aim to identify the features that best described each clone, a PCA was performed including the traits analysed (Figure 4.3.5). Clones resulted clearly separated in the dimensional plot. Total variance explained by the first two PCs was 95.1 %.; PC1 explained 73.5 % with anthocyanin (ANT) and colour index (CI) discriminating among clones in the biplot. Thus, PN_115 and PN_667 were located in the negative side of first dimension mainly influenced by ANT, whilst PN_Abel and PN_Am were located in the opposite side. Berry weight (BW) and musts total acidity (TA) discriminated samples in PC2, where PN_Am and PN_UCD5 where located in the negative side.

Figure 4.3.5. PCA plot considering clones and the traits analyzed.



Relationship between berry size and chemical composition of grape, must and wine

Study 1. Wairau Valley

The aim of the study was to assess the influence of berry weight on berry, must and wine composition of three PN clones previously selected. PN_Abel, presented the highest berry weight and higher number of seeds with lower seed weight compared to PN_UCD5 and PN_115. In berry extracts, differences in colour index and total phenolics were found, being higher in PN_Abel compared with PN_115. PN_UCD5 showed significant differences in berry diameter, weight, area and volume relative to PN_Abel and PN_115 (Table 4.3.3.).

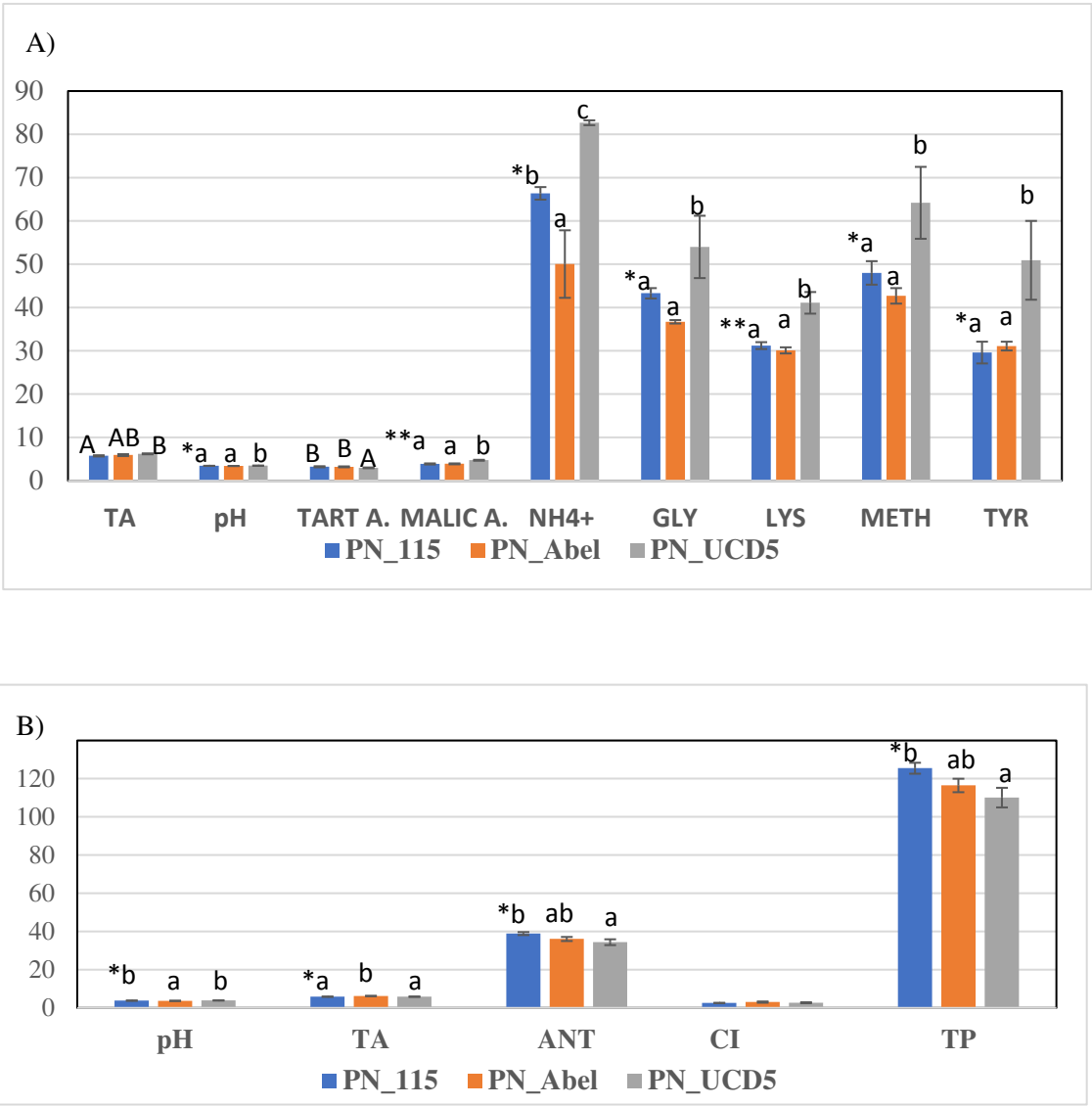
Table 4.3.3. Summary of berry, seed and berry extracts parameters in PN_115, PN_Abel and PN_UCD5.

		PN_115	PN_Abel	PN_UCD5
BERRIES	**Berry length (mm)**	**15.0 ± 0.3a****	**16.4 ± 0.1b**	**15.3 ±0.2a**
	Berry diameter (mm)	**13.4 ± 0.2a****	**15.1 ± 0.04c**	**14.0 ±0.2b**
	Berry shape	**1.1 ± 0.02b ***	**1.09 ± 0.01a**	**1.10 ± 0.01a**
	Berry weight (g)	**1.26 ± 0.05a****	**1.83 ± 0.1c**	**1.50 ± 0.04b**
	Berry area	**157.6 ± 4.6a****	**195.3 ± 0.7c**	**168.4 ± 4.6b**
	Berry volumen	**1409 ±55a****	**1980 ± 6c**	**1580 ± 62b**
	Berry mass	**11030 ± 477a***	**13857 ± 679b**	**12674 ±524b**
SEEDS	**Seed nº/g marc**	5.7 ± 0.3	5.9 ± 0.8	4.9 ± 0.3
	Seed weight (mg)	**53.8 ± 3ab***	**49.6 ± 0.3a**	**57.0 ± 1.1b**
	Seed number	**1.5 ± 0.1a****	**2.5 ± 0.1b**	**1.5 ± 0.3a**
	Seed mass	**1467 ± 239**	**1538 ± 101**	**1648 ±43**
BERRY EXTRACTS	**º Brix**	17.8 ± 0.3	18.6 ± 1.0	17.9 ± 0.4
	Colour Index	**1.0 ± 0.1a***	**1.8 ± 0.5b**	**1.2 ± 0.2a**
	Monomeric Anthocyanins	**47.0 ± 4.7a***	**52.7 ± 1.8ab**	**58.0 ±2.6b**
	Total Anthocyanins (mg/L)	60.4 ± 3.9	64.6 ± 2.7	69.0 ± 4.4
	Colour Density (AU)	1.6 ± 0.2	1.8 ± 0.0	1.8 ± 0.0
	Hue	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.0
	Total Phenolics (AU)	**7.5 ± 1.4a***	**9.6 ± 0.9b**	**8.7 ±0.4ab**

** reflects statistical differences at 0.01 level and * at 0.05. Differences are highlighted in bold.

In musts, PN_Abel had the lowest values in ammonium. PN_UCD5 was significantly distinct from the other clones, with higher pH and malic acid content, and highest content of glycine, lysine, methionine and tyrosine amino acids (Figure 4.3.6 A). Wines derived from the smallest berry size clone (PN_115) presented higher pH and lower total acidity than the larger berry size clone PN_Abel (Figure 4.3.6 B). Thus, variables related to colour and phenolic compounds only presented differences associated to berry size in berry extracts.

Figure 4.3.6. Differences in must (A)and wine parameters (B) between PN_115, PN_UCD5, PN_Abel.



Abbreviations: TA Total acidity (g / L), TART A. Tartaric acid (g / L), MALIC A. Malic Acid (g / L), GLY Glycine (mg / L), LYS Lysine (mg / L), METH Methionine (mg / L), TYR Tyrosine (mg / L), ANT Total Anthocyanin content (mg / L), CI Colour intensity and TP Total phenolics (mg GAE / L). ** reflects statistical differences at 0.01 level, * at 0.05.

Study 2. Awatere Valley

This study focused on the influence of berry weight on berry and wine composition of PN_ UCD5 and PN_115 clones collected in Awatere Valley sub-region, with distinct climatic and edaphic features compared to the first two studies. Clones presented statistical differences in seed and berry traits (Table 4.3.4), and in several berry extracts and musts traits. PN_ UCD5 showed higher berry length, weight and area than PN_115; and presented higher seed number and weight. Total phenolic content in berry extracts was higher in PN_115 and in musts only pH was significantly lower.

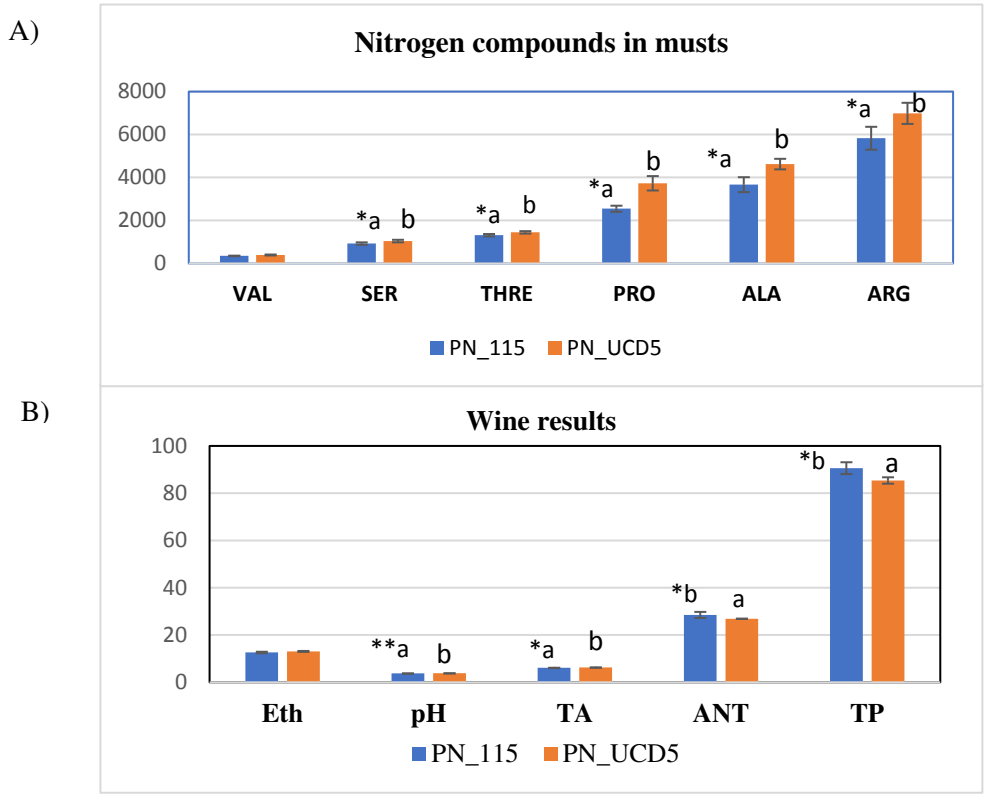
Table 4.3.4. Summary of seed, berry and must parameters in PN_115 and PN_UCD5.

		PN_115	PN_UCD5
BERRIES	**Berry length (mm)**	**16.4 ± 0.6 ***	**17.2 ± 0.5**
	Berry diameter (mm)	**14.7 ± 0.5***	**15.8 ± 0.6**
	Berry shape	1.1 ± 0.0	1.1 ± 0.0
	Berry weight g)	**1.7 ± 0.0****	**2.2 ± 0.1**
	Berry area	**190.5 ± 13.2***	**213.2 ± 13.2**
SEEDS	**Nº seeds/g marc**	5.0 ± 0.6	5.0 ± 0.7
	Seed nº/ berry	**1.7 ± 0.1****	**2.3 ± 0.2**
	Seed weight (mg)	**35.5 ± 7.4***	**41.1 ± 1.4**
BERRY EXTRACTS	**Monomeric Anthocyanins (mg/L)**	44.3 ± 4.1	43.3 ± 1.4
	Total Anthocyanins (mg/L)	58.9 ± 2.6	56.4 ± 1.7
	Total phenolics (AU)	**9.2 ± 0.6***	**7.7 ± 0.5**
MUSTS	**ºBrix**	21.9 ± 0.3	22.4 ± 0.6
	Total Acidity (g/L)	7.5 ± 0.3	7.4 ± 0.1
	pH	**3.35 ± 0.01****	**3.41 ± 0.01**
	$NH4^+$ (mg N / L)	93.4 ± 6.3	104.8 ± 10.3
	YAN (mg N / L)	383.0 ± 23.8	434.8 ± 30.0
	Glucose + Fructose (g / L)	224.5 ± 2.9	229.1 ± 8.8
	Colour intensity (AU)	**1.0 ± 0.2***	**0.7 ± 0.1**
	Tonality (AU)	4.1 ± 0.5	4.7 ± 0.7

** reflects statistical differences at 0.01 level, and * at 0.05. Differences are highlighted in bold.

Main differences among clones were found in amino acids content (Figure 4.3.7 A), having PN_UCD5 higher accumulation of arginine, alanine, serine, threonine and proline, as reported in Wairau Valley subregion. Results for other amino acids are presented in Supplementary material 4.3.2. In wines, PN_115 presented lower pH and TA, and higher total phenolics and anthocyanin content than PN_UCD5 (Figure 4.3.7 B). Therefore, in this study differences between clones were consistent in berry extracts, musts and wines.

Figure 4.3.7. Differences in nitrogen compounds (A) and wine parameters (B) in Awatere sub-region in PN_115 and PN_UCD5.



Abbreviations: VAL Valine (mg / L), SER Serine (mg / L), THRE Threonine (mg / L), PRO Proline (mg / L), ALA Alanine (mg / L), ARG Arginine (mg / L), Eth % Ethanol, TA Total acidity (g / L), ANT Total anthocyanins (mg / L). *show statistical differences at 0.05 level.

Influence of environment in berry morphology, must and wine characteristics

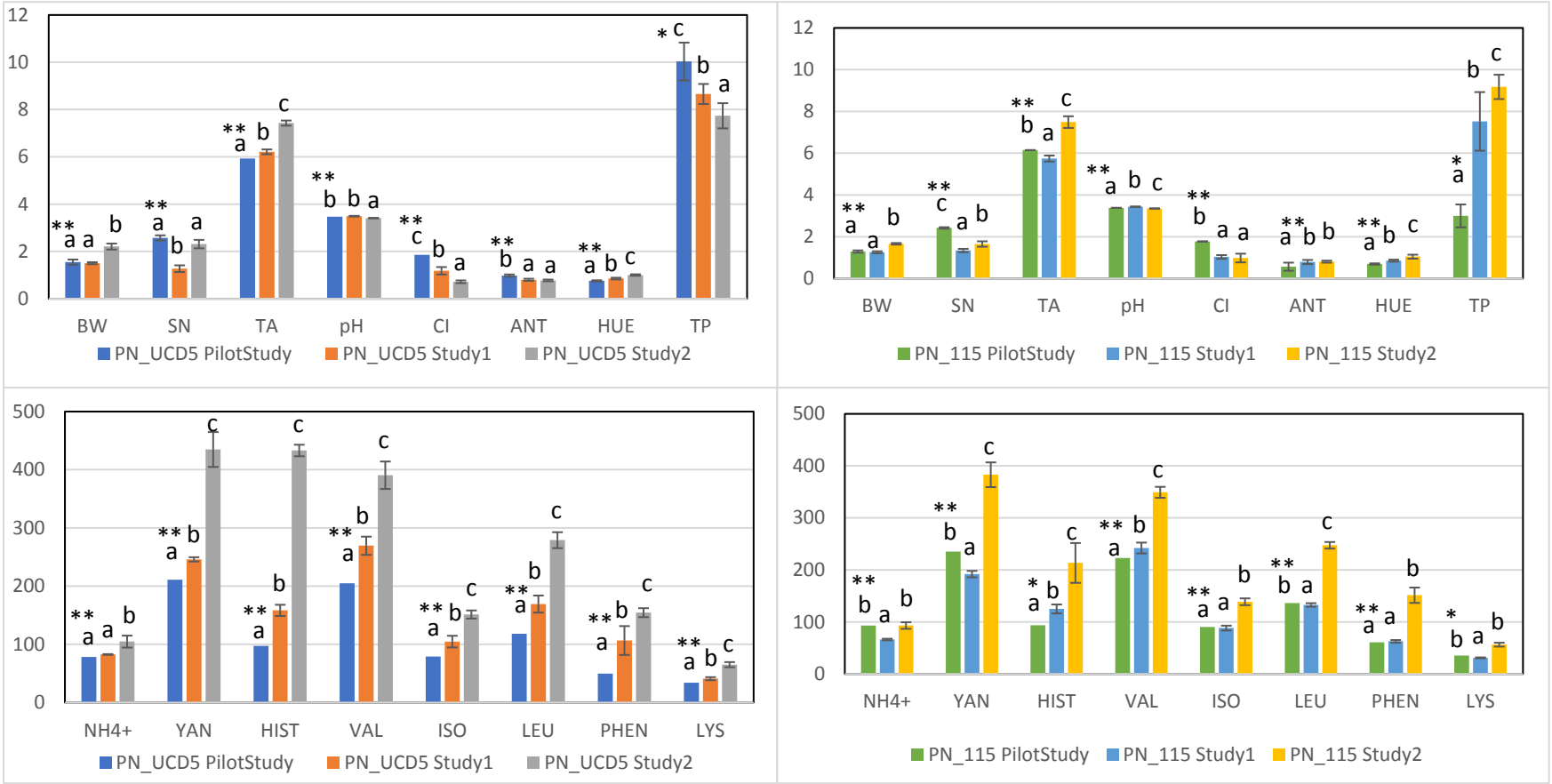
A MANOVA was performed to evaluate the influence of sub region and rootstock in clones PN_115 y PN_UCD5, and to assess the interactions between these factors (Table 4.3.5). Sub region was significant for most of the parameters, whilst rootstock had more influence in must variables. Few interactions were found among these factors, anthocyanidins and total phenolics of berry extracts, pH, YAN and anthocyanidins in musts, and amino acids as leucine showed interactions between clone and rootstock and with sub region factor.

Table 4.3.5. MANOVA results considering clone (PN_115 and PN_UCD5), subregion (plot) and rootstock (RSK) factors.

	Factor	Clone		Plot		RSK		Clone x Plot		Clone x RSK	
	Trait	F	Sig	F	Sig	F	Sig	F	Sig	F	Sig
BERRIES / SEEDS	Berry weight	73.8	0.00	88.5	0.00	ns	ns	7.0	0.03	ns	ns
	Seed number	8.7	0.02	30.1	0.00	148.8	0.00	ns	ns	ns	ns
	Seed weight	8.9	0.02	ns	ns	6.6	0.03	ns	ns	ns	ns
	Anthocyanins	6.0	0.02	ns	ns	ns	ns	32.0	0.00	26.0	0.00
	Total phenolic index	7.1	0.01	5.5	0.03	ns	ns	ns	ns	ns	ns
	Colour Intensity	5.6	0.03	4.5	0.04	ns	ns	ns	ns	ns	ns
	Hue	ns	ns	73.9	0.00	13.4	0.00	ns	ns	ns	ns
	Total phenolics	ns	ns	ns	ns	ns	ns	23.0	0.00	11.1	0.01
MUSTS	º Brix	ns	ns	131.4	0.00	25.2	0.00	9.7	0.01	ns	ns
	Total acidity	ns	ns	167.8	0.00	ns	ns	ns	ns	ns	ns
	pH	96.7	0.00	33.0	0.00	27.6	0.00	5.8	0.04	11.1	0.01
	NH_4	9.0	0.01	17.4	0.00	21.2	0.00	16.8	0.00	ns	ns
	YAN	15.4	0.00	282.0	0.00	ns	ns	9.1	0.01	40.7	0.00
	Anthocyanins	6.9	0.03	10.3	0.01	70.0	0.00	11.4	0.01	18.9	0.00
	Total phenolic index	ns	ns	79.0	0.00	46.3	0.00	ns	ns	ns	ns
	Colour intensity	ns	ns	141.3	0.00	75.6	0.00	ns	ns	ns	ns
	Hue	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Total phenolics	6.3	0.03	7.2	0.02	82.0	0.00	10.4	0.01	17.6	0.00
	Serine	7.1	0.03	125.3	0.00	ns	ns	ns	ns	ns	ns
	Histadine	ns	ns	9.0	0.02	ns	ns	ns	ns	ns	ns
	Glycine	ns	ns	45.7	0.00	ns	ns	ns	ns	ns	ns
	Threonine	17.4	0.00	223.1	0.00	ns	ns	ns	ns	ns	ns
	Arginine	14.6	0.01	154.5	0.00	ns	ns	ns	ns	ns	ns
	Alanine	23.8	0.00	198.0	0.00	8.8	0.02	5.7	0.05	ns	ns
	Tyrosine	24.5	0.00	174.4	0.00	ns	ns	6.0	0.04	ns	ns
	Valine	5.7	0.05	156.0	0.00	8.7	0.02	5.7	0.05	ns	ns
	Methionine	ns	ns	15.2	0.01	ns	ns	ns	ns	ns	ns
	Phenylalanine	ns	ns	83.2	0.00	ns	ns	ns	ns	ns	ns
	Isoleucine	5.7	0.05	118.1	0.00	ns	ns	ns	ns	5.8	0.05
	Leucine	14.6	0.01	291.6	0.00	6.3	0.04	9.7	0.02	8.6	0.02
	Lysine	13.9	0.01	92.1	0.00	ns	ns	ns	ns	ns	ns
	Proline	6.7	0.04	88.8	0.00	ns	ns	29.0	0.00	ns	ns

In Figure 4.3.8 differences between plots and rootstocks in both clones were showed; it was noteworthy the high amino acid contents obtained in musts from Awatere sub region berries. Other parameters as total acidity, berry weight and Hue resulted also higher in Awatere sub region independently of the clone, whilst total phenolics presented a different behaviour depending on the clone.

Figure 4.3.8. Summary of the main differences in PN_115 and PN_UCD5 in the different studies.



Abbreviations: BW Berry weight (g), SN seed number, TA Total acidity (g / L),
CI colour index, ANT anthocyanins (mg / L), TP Total phenolics (UA), HIS Histadine (mg / L), VAL Valine (mg / L) ISO Isoleucine (mg / L), LEU Leucine (mg / L) PHEN Phenilalanine (mg / L) LYS Lysine (mg / L).
** reflects statistical differences at 0.01 level, * at 0.05.

Discussion

Significant differences between PN clones were detected in berry morphology, seed parameters, must and wine composition. Our results support other studies performed in different cultivars (Burin et al. 2011, Schueuermann et al. 2018) which show that clones from the same grape variety can differ in must and wine composition, relevant for industrial use. Clonal selection with a focus on berry size is presumably a useful tool in grapevine genetic improvement to produce wines with distinct colour, aromatic profile and phenolic content. Remarkably, PN_UCD5 must amino acid content was almost as twice as that of the other two clones studied. Must nitrogen components play a key role on wine quality by affecting yeast growth during alcoholic fermentation (Gutierrez-Gamboa et al. 2018), and the link with volatile compounds produced in wine (Valdés et al. 2019). Gutierrez- Gamboa et al. (2018) found that Carignan Noir can be considered as a proline accumulator cultivar, in parallel, Pinot Noir could be regarded as an arginine accumulator variety. Schueuermann et al. (2018) reported arginine and proline as the two more abundant compounds in musts of PN_ 115 and PN_777.

In the present work, smaller berries bore lower number of seeds as previously reported (Walker et al. 2005, Gil et al. 2015), although no relationship between berry and seed size was obtained. Extracts from the smaller berry clones had lower anthocyanidins and phenolic contents and a low colour index. In wines, lower acidity was associated with clones with smaller berries. Barbagallo et al. (2011), and Friedel et al. (2016), also reported wines from small berries having less total acidity, however, Poni et al. (2009), Barbagallo et al. (2011), reported that smaller berries can retain more acidity in terms of pH. Anthocyanidin and phenolic compounds in wines were not related to berry size as no differences between PN_115 and PN_Abel were found. However, PN_UCD5 presented less content than PN_115. That points out that phenolic content in red grapes is largely dependent not only on cultivar and species (Dai et al. 2011), but also on clones.

Environmental factors such as climate, soil characteristics and viticultural practices, as the vine rootstock, caused variability for most of studied traits. Berry shape or total acidity variables were not altered neither by the plot nor by the rootstock. Comparing the two sub regions, berry weight was smaller in Wairau Valley, most likely provoked by water deficit (Roby et al. 2004) and/or high temperatures (Holt et al. 2008), as Wairau is warm and humid with poor fertile soils. Anthocyanidins, phenolic compounds and colour index in musts were higher in this region, as well as wine phenolic content. The moderate stress caused by higher temperatures and low soil fertility could have increased the accumulation of these compounds, since they are involved in the stress response (Koundouras et al. 2006, Schreiner et al., 2013). In studies with Pinot Noir variety, warm temperatures during early berry development increased phenolic concentrations (Nicholas et al., 2011, Blank et al. 2019). Clones from Awatere Valley presented higher accumulation of nitrogen compounds, in agreement with Gutierrez-Gamboa et al. (2018), who found the highest nitrogen compound concentration in grapes in the coolest plot leading to a faster alcoholic fermentation. Therefore, presumably low night temperatures before harvest could lead to a higher synthesis of amino acids.

Rootstock 3309 positively stimulated seed weight, pH, anthocyanidins and phenolic compounds in must compared to 101-14, but not influenced berry parameters. Also nitrogen compounds were higher; as previously reported (Gutierrez-Gamboa et al. 2018) rootstock seem to have some influence in the accumulation of these compounds. A high amount of amino acids can lead to an improvement in wine aroma, due to the fact that certain amino acids are precursor of volatile compounds.

Conclusions

In this work, variability in morphologic and chemical composition of berry, must and wine was found among Pinot Noir clones. Chemical composition of must and wine was mainly determined by the clone. According to the results obtained, clonal selection may result in differences in Pinot Noir wine quality better adapted to different environments. The influence of berry size on studied variables was weak, grapes with lower berry size presented in berry extracts lower content of anthocyanidins or total phenolic compounds, parameters related to quality. In wines could be only related to acidity parameters. On the other hand, berry weight was smaller at the plot with warmer and humid conditions and lower soil fertility, where anthocyanidins, phenolic compounds and colour index resulted increased. Remarkably, the cooler environment presented an increase of nitrogenous compounds. Berry size was not influenced by rootstock, however variability in chemical composition of berries, musts and wine were related to vine rootstock. Results are useful in order to design different wine styles at different plots based on different clones. An evaluation of the aromatic compounds derived from the amino acids detected in musts should be further studied.

Supplementary material

Supplementary material 4.3.1. Summary of the parameters studied in the eight PN clones in Pilot study.

		PN_115	PN_667	PN777	PN_Abel	PN_Am	PN_Maria	PN_UCD5	PN_UCD6
Berry traits	**Berry weight (g)**	1.29 ± 0.05a	1.59 ± 0.07b	1.52 ± 0.03b	2.0 ± 0.19d	1.58 ± 0.01b	1.81 ± 0.14cd	1.55 ± 0.11b	1.66 ± 0.02bc
	Berry length (mm)	15.1 ± 0.75	16.19 ± 0.91	16.01 ± 0.72	16.85 ± 0.18	16.0 ± 0.12	15.2 ± 0.32	16.51 ± 0.05	16.78 ± 0.01
	Berry diameter (mm)	13.36 ± 0.47a	14.69 ± 0.74b	14.2 ± 0.72ab	15.36 ± 0.15b	15.1 ± 0.0b	16.53 ± 0.51c	14.52 ± 0.3b	15.12 ± 0.11b
	Berry shape	1.13 ± 0.01de	1.10 ± 0.01bcd	1.10 ± 0.01cde	1.1 ± 0.0bc	1.06 ± 0.01a	1.09 ± 0.01ab	1.14 ± 0.03e	1.11 ± 0.01b-e
	Berry mass	12005 ± 528	14025 ± 815	12837 ± 235	14034 ± 67	13301 ± 1095	13853 ± 413	13163 ± 344	13177 ± 2039
Seed traits	**Seed number**	2.4 ± 0.0abc	2.2 ± 0.28ab	2.18 ± 0.11a	2.4 ± 0.18abc	2.60 ± 0.07c	3.05 ± 0.14d	2.58 ± 0.11c	2.53 ± 0.18bc
	Seed weight (mg)	37.0 ± 0.9a	45.05 ± 0.73c	42.9 ± 0.54bc	40.0 ± 1.7ab	38.29 ± 1.11a	54.33 ± 0.30d	37.95 ± 1.50a	42.51 ± 2.33bc
	Seed nº/marc	8.31 ± 0.52d	6.4 ± 0.2abc	6.85 ± 0.13bc	5.71 ± 0.92a	6.88 ± 0.24bc	7.14 ± 0.09cd	5.94 ± 0.03ab	5.9 ± 0.35ab
	Seed mass	1370 ± 45	1630 ± 51	1300 ± 16	1468 ± 132	1353 ± 12	1345 ± 51	1387 ± 14	1555 ± 294
Berry extracts	**Ant mono. (mg / L)**	46.0 ± 10.0	56.15 ± 0.87	58.58 ± 1.87	46.24 ± 1.24	49.44 ± 5.34	54.91 ± 6.63	51.71 ± 1.55	54.73 ± 3.16
	Total anthocy (mg/L)	60.9 ± 8.7ab	65.51 ± 0.2b	72.22 ± 3.06c	57.83 ± 0.5a	62.77 ± 6.17ab	69.82 ± 5.85bc	72.1 ± 16.7ab	68.47 ± 3.9bc
	Colour intensity (UA)	0.34 ± 0.1	0.43 ± 0.01	0.43 ± 0.01	0.32 ± 0.02	0.36 ± 0.01	0.39 ± 0.04	0.37 ± 0.0	0.42 ± 0.0
	Hue	0.7 ± 0.03a	0.79 ± 0.04bc	0.85 ± 0.02de	0.88 ± 0.03ef	0.90 ± 0.06f	0.82 ± 0.03cd	0.76 ± 0.03b	0.81 ± 0.01c
	Total phenolics (UA)	5.56 ± 0.01a	7.75 ± 1.03b	9.55 ± 0.13c	7.91 ± 0.13b	10.21 ± 0.49c	9.64 ± 1.97bc	10.03 ± 0.8bc	8.35 ± 0.27b
Musts	**ºBrix**	19.9	21	19.5	18.7	20.5	19.8	19.7	19.9
	Total acidity (g / L)	6.15	6.54	5.89	5.93	7.06	7.37	6.46	11.3
	pH	3.38	3.34	3.3	3.47	3.34	3.29	3.37	3.1
	NH4+ (mg N / L)	93.18	110.24	149.45	78.35	99.73	88.22	78.11	101.02
	Glucose +Fructose (g/L)	199.42	211.94	192.12	184.93	210.93	198.00	199.56	198.51
	Total antochyanins (UA)	10.83	11.11	9.93	8.32	10.50	8.62	9.66	9.47
	Color intensity (UA)	1.77	1.66	2.82	1.86	2.27	1.28	2.23	1.55
	Hue	4.07	4.31	2.35	3.26	4.42	2.55	4.34	3.59

Continue

		PN_115	PN_667	PN777	PN_Abel	PN_Am	PN_Maria	PN_UCD5	PN_UCD6
Aminoacid compounds	**Serine (mg/L)**	591.7	743.7	1147	565.6	882	833.4	985.1	1184
	Histidine (mg/L)	93.6	118.5	221.2	97.37	157.5	179.1	201.1	162.7
	Glycine (mg/L)	49.55	80.23	121.5	47.34	79.09	71.74	62.08	78.74
	Threonine (mg/L)	816.8	1038	1460	793.6	1200	922.4	1186	1450
	Arginine (mg/L)	2447	2938	6212	2306	3551	3690	4311	4148
	Alanine (mg/L)	1403	2140	2833	1437	2681	2022	2383	2728
	Tyrosine (mg/L)	31.53	44.05	56.93	29.89	49.75	62.6	44.41	55.21
	Valine (mg/L)	222.9	273.5	356	204.9	321.9	208.3	267.8	331.6
	Methionine (mg/L)	48.66	58.35	71.93	41.85	65.89	66.88	47.99	51.95
	Phenylalanine (mg/L)	60.93	71.33	78.6	49.66	79	63.33	79.69	85.44
	Isoleucine (mg/L)	90.43	100.9	126.5	79.11	115.7	83.1	106.8	128.3
	Leucine (mg/L)	136.3	169.6	226.9	118.2	197.2	174.6	191.2	232.6
	Lysine (mg/L)	35.46	42.46	64.31	33.95	46.97	53.91	42.76	50.25
	Proline (mg/L)	1879	2466	2248	1281	2277	1810	2303	2389

** reflects statistical differences at 0.01 level, * at 0.05.

Supplementary material 4.3.2. Summary of nitrogen compounds results in must in Study 2.

	PN_115			PN_UCD5		
	Mean ± SD	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**
Serine	923.5 ± 54.2*A	861.3	960.8	1039.8 ± 56.8B	974.5	1077.0
Histadine	213.6 ± 38.3*A	179.1	254.8	433.1 ± 169.8b	277.8	614.4
Glycine	83.8 ± 7.8	74.9	89.0	92.9 ± 8.5	83.4	100.0
Threonine	1312 ± 57.3*a	1249.0	1360.0	1445.3 ± 59.0b	1379.0	1492.0
Arginine	5827 ± 532.7*a	5217.0	6197.0	6985.3 ± 495b	6467.0	7454.0
Alanine	3665 ± 347.8*a	3281.0	3959.0	4621 ± 245.1b	4342.0	4799.0
Tyrosine	83.2 ± 0.9**a	82.2	84.0	105.1 ± 5.8b	101.6	111.8
Cystine	178.2 ± 15.4	168.7	196.0	164.1 ± 13	151.9	177.8
Valine	349.2 ± 10.5*a	338.7	359.6	390.7 ± 23.6b	365.0	411.3
Methionine	97.5 ± 23.1	80.4	123.8	106.7 ± 22.5	87.0	131.2
Phenylalanine	151.5 ± 14.8	136.4	165.9	154.5 ± 7.7	145.7	159.9
Isoleucine	138.9 ± 6.5*A	134.0	146.3	151.4 ± 7.0B	143.5	156.7
Leucine	247.5 ± 6.4**a	241.9	254.4	279 ± 13.6b	266.9	293.8
Lysine	56.6 ± 3.6A	52.7	59.9	65.1 ± 4.4B	60.2	68.6
Proline	2546 ± 141**a	2423.0	2701.0	3728 ± 336.8b	3352.0	4002.0

** reflects statistical differences at 0.01 level, * at 0.05 and capital letters at 0.1.

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CHAPTER 5.
GENERAL CONCLUSIONS

4. General conclusions

1. The phenotypic segregation of 12 traits including berry, flower, and seed-related parameters was assessed in two wine-grape segregant populations with Tempranillo as common parent, consisting of 130 and 151 plants derived from a cross with Grenache and Graciano varieties, respectively. Fourteen traits related to must composition, productivity and phenology stages were also studied in four consecutive years and two different plots in G x T progeny. All the parameters presented transgressive segregation and continuous variation. Year effect resulted significant for all traits except berry weight, flower diameter and seed weight. Plot effect resulted significant for all the traits analyzed. Broad-sense heritability estimatation resulted higher in T x G progeny, being particularly high for flower traits. Significant correlations among traits were observed, and moderate associations between berry length and berry shape, and between berry shape and pistil shape were found in both genetic backgrounds. Eleven white and red genotypes were pre-selected in Grenache x Tempranillo progeny, based on ripening date, cluster weight, yield, acidity and Brix degree by cluster analysis, that will need to be further analyzed.

2. Female plants showed rounder flower shape, larger flower diameter, lower number of seeds, and a delay in flowering and start veraison dates compared with hermaphrodites in both genetic backgrounds. A QTL region in LG2 was detected for flower-morphology, seed, productivity traits, and phenological stages (flowering date, veraison), confirming the influence of flower sex in the genetic determinism of these characters. Effects of sex resulted particularly strong in flower morphology traits as ovary shape in both progenies.

3. Significant QTL regions were detected for berry size and productivity parameters in LG17 in Grenache x Tempranillo progeny. In Tempranillo x Graciano population, regions in LG3 and LG5 resulted associated mainly to berry size and seed traits. In Tempranillo x Graciano progeny, a QTL in LG5 for berry, seed and flower traits covered the region of *FERONIA* locus. and a QTL in LG18 for seed traits resulted associated to locus *SDI*. For flower morphology, QTL in LG8, LG11 and LG14 were identified, with QTL on LG11 showing the strongest and most stable effect over the two years. A candidate gene VIT_11s0016g03650 with a function associated to pollen morphology is proposed associated to the highly significant QTL detected in LG11 for flower traits in both progenies.

4. In Grenache x Tempranillo progeny, main QTL were detected in LG17 and LG18 for yield, fertility index, cluster number, and cluster weight. A QTL in LG1 was detected for sugar content, and for total acidity five stable QTLs were found in LG4, LG12, LG13, LG14 and LG17. Concerning phenological traits, QTL were detected in LG10 and LG14 for Sprouting, in LG7 and LG10 for flowering, while veraison showed significant associations with genomic regions in LG11 and LG17, being ripening date significantly associated to LG8, LG11 and LG13. A QTL region in LG17 was found

significantly associated to berry size, productivity traits, phenology stages, and on LG7 and LG13 QTL for flower morphology and flowering date suggesting close linkage or pleiotropic effects. In Tempranillo × Graciano progeny, co-localizations of QTL for flower morphology, seed traits and phenology events were detected in LG3 and LG11.

5. The research conducted in Grenache x Tempranillo hybrids confirmed that smaller berries showed a higher extractability of anthocyanins and phenolic compounds than larger berries, presenting deeper colour. In Grenache x Tempranillo hybrids, wines from small berry size genotypes resulted in sensorialanalysis more astringent and sweeter with higher fruity notes than those from larger berries, which were plain and more alcoholic. These wines achieved consistently higher quality scores than those derived from large berry-genotypes. In Pinot Noir variety, a clear relationship between berry size and the accumulation of anthocyanidins, phenolic compounds was not found, probable because plot and environmental conditions also triggered great differences between clones, especially in berry weight, total acidity and nitrogen compounds.

6. The sensory profiles and quality scores of wines derived from twelve Graciano × Tempranillo selections were obtained in two different years. Based on wine expert's perception, two high quality hybrids, TG8 and TG63 were shown to consistently improve Tempranillo and Graciano due to a higher anthocyanin content, colour intensity, acidity, and positive aroma related to red fruit. Another selection, TG129, appears to be an interesting alternative to Tempranillo and Graciano in the context of global warming due to its late-ripening cycle, high polyphenol content and fruity aroma. Other selections with herbal and dried fruit aroma notes appeared as potential cultivars suitable to satisfy distinct consumer demands.

7. The genetic study of hybrids between traditional wine-grape cultivars proved useful to understand the genetic control of key traits that are linked with wine quality and to select new premium genotypes better adapted to future climate scenarios.

Conclusiones

1. La segregación fenotípica de 12 caracteres de baya, flor y semilla, fueron evaluados en dos poblaciones segregantes de uva de vinificación con Tempranillo como parental común, en un total de 130 y 151 plantas derivadas de un cruce con las variedades Garnacha y Graciano, respectivamente. Catorce parámetros relacionados con la composición del mosto, la productividad y la fenología se estudiaron en cuatro años consecutivos y dos ambientes diferentes en la progenie Garnacha x Tempranillo. Todos los parámetros presentaron segregación transgresiva y variación continua. El efecto del año resultó significativo para todos los rasgos, excepto el peso de la baya, el diámetro de la flor y el peso de la semilla, mientras que el efecto parcela resultó significativo para todos los rasgos analizados. Las estimaciones de heredabilidad en sentido amplio resultaron más altas en la progenie Tempranillo x Graciano, especialmente en los parámetros de flor. Se observaron correlaciones significativas entre caracteres, siendo moderadas entre la longitud y la forma de la baya, y entre la forma de la baya y el pistilo en ambos fondos genéticos. Once genotipos de uva tinta y once de uva blanca fueron preseleccionados en la progenie de Garnacha x Tempranillo en función de la fecha de maduración, el peso del racimo, el rendimiento, la acidez y el grado, que deberán ser analizadas en profundidad en el futuro.

2. Las plantas de flores femeninas mostraron una forma más redondeada, mayor diámetro de flor, un menor número de semillas y un retraso en la floración y fecha de inicio de envero en comparación con las hermafroditas en ambas poblaciones. Se detectó una región QTL en GL2 para la morfología de la flor, parámetros de semilla, productividad y estadíos fenológicos (fecha de floración, envero), confirmando la influencia del sexo en la determinación genética de estos caracteres. El efecto del sexo resultó particularmente significativo en los caracteres de morfología de la flor como la forma de ovario en ambas progenies.

3. Se detectaron regiones QTL significativas para el tamaño de la baya y los parámetros de productividad en GL17 en la progenie de Garnacha x Tempranillo. En la población Tempranillo x Graciano, regiones en GL3 y GL5 resultaron asociadas principalmente al tamaño de la baya y caracteres de semilla. En la progenie Tempranillo x Graciano una región QTL en GL5 para parámetros de baya, semilla y flor cubrió la región del locus *FERONIA* y un QTL en GL18 para rasgos de semilla resultó asociado al locus SDI. Para la morfología de las flores, se identificaron QTL en GL8, GL11 y GL14, siendo el localizado en GL11 el más significativo y estable en los dos años y ambas progenies y proponiéndose un gen candidato VIT_11s0016g03650 con una función asociada a la morfología del polen asociado a dicho QTL.

4. Se detectaron QTL significativos en GL17 y GL18 para rendimiento, índice de fertilidad, número de racimos y peso del racimo. Se detectó un QTL en GL1 para el contenido de azúcar, y para la acidez total se encontraron cinco QTL estables y

altamente significativos en GL4, GL12, GL13, GL14 y GL17. En cuanto a los parámetros fenológicos, se detectaron QTL asociaciones significativas para la fecha de envero con regiones genómicas en GL11 y GL17, y en el GL8, GL11 y GL13 para la fecha de maduración. Una región QTL en GL17 resultó significativamente asociada con parámetros como tamaño de la baya, productividad y fenología, y en los GL7 y GL13 para la morfología de la flor y la fecha de floración, lo que sugiere una estrecha vinculación entre estos caracteres o efectos pleiotrópicos. En la progenie Tempranillo × Graciano, se detectaron co-localizaciones de QTL para morfología de la flor, parámetros de semilla y estadíos fenológicos en los GL3 y GL11.

5. La investigación realizada en híbridos de Garnacha x Tempranillo confirmó que las bayas más pequeñas presentan una mayor capacidad de extracción de antocianinas y compuestos fenólicos que las bayas más grandes, presentando los vinos obtenidos un color más intenso. Los vinos de genotipos de bayas pequeñas resultaron sensorialmente más dulces, astringentes con mayores notas frutales más altas que los procedentes de baya más grande, que eran más planos aromáticamente y alcohólicos. Por ello, consiguieron puntuaciones de calidad más altas por parte de los expertos que los vinos derivados de genotipos de bayas grandes en los dos años de estudio. En la variedad Pinot Noir, no hubo una relación consistente entre tamaño de baya y acumulación de antocianos y compuestos fenólicos, quizá debido a que las condiciones ambientales y de la parcela desencadenaron grandes diferencias entre los clones, especialmente en el peso de la baya, la acidez total y los compuestos aminoácidos.

6. Los perfiles sensoriales y las puntuaciones de calidad de los vinos derivados de doce selecciones de Graciano × Tempranillo se realizaron en dos años diferentes. Según la percepción de los expertos, dos híbridos poseen una alta calidad, TG8 y TG63, mejorando consistentemente a Tempranillo y Graciano debido a un mayor contenido en antocianos, intensidad de color, acidez y aroma relacionado con la fruta roja. Otra selección, TG129, parece ser una alternativa interesante a Tempranillo y Graciano en el contexto del calentamiento global debido a su maduración tardía, alto contenido en polifenoles y aroma afrutado. Otras selecciones con perfiles sensoriales diferentes asociados a aromas herbáceos o fruta seca se presentan como adecuados para satisfacer las distintas demandas de los consumidores.

7. El estudio genético de híbridos entre variedades tradicionales de uva de vinificación demostró ser útil para comprender el control genético de los caracteres clave vinculados con la calidad del vino y para seleccionar nuevos genotipos mejorados y mejor adaptados a nuevos escenarios climáticos.