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*Programa de Doctorado en Ingeniería Agraria, Alimentaria,
Forestal y de Desarrollo Rural Sostenible*

TESIS DOCTORAL

Mejora de olivo para adaptación a diferentes
condiciones ambientales y sistemas de cultivo.

*Olive tree breeding for adaptation to different
environmental conditions and growing systems.*

Autor

Jose Francisco Navas López

Director

Dr. Raúl de la Rosa Navarro

Codirector

Dr. Lorenzo León Moreno

Área de Mejora Vegetal



Instituto de Investigación y Formación Agraria y Pesquera
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CONDICIONES AMBIENTALES Y SISTEMAS DE CULTIVO*

AUTOR: *José Francisco Navas López*

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Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

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ucopress@uco.es



TÍTULO DE LA TESIS: Mejora de Olivo para Adaptación a Diferentes Condiciones Ambientales y Sistemas de Cultivo.

DOCTORANDO/A: Jose Francisco Navas López

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La presente Tesis se ha desarrollado en el marco de una serie de ensayos comparativos de olivo establecidos en distintas condiciones climáticas de Andalucía. La evaluación agronómica, realizada de manera eficiente por el Tesinando, ha permitido recabar información sobre la interacción entre genotipo y ambiente para diversos caracteres de gran interés en olivo. Estos caracteres incluyen la fenología de floración, el patrón de acumulación de aceite y características de fruto, además del análisis de la mayoría de los componentes principales del aceite. Para la determinación de los componentes del aceite se han utilizado técnicas analíticas de evaluación directa de la pulpa de la aceituna, sin necesidad de extraer el aceite. Estas técnicas han permitido el análisis rápido de un gran número de muestras.

Dicha información sobre interacción entre genotipo y ambiente, muy escasa en la literatura previa, será de gran interés para mejorar la eficiencia del programa de mejora de olivo del IFAPA, en la que se enmarca esta Tesis.

El Tesinando ha mostrado gran capacidad de trabajo en la toma de datos en campo y el análisis de muestras en laboratorio. También destaca su capacidad para aprender técnicas estadísticas que le han permitido analizar de una forma muy minuciosa todos los componentes de la interacción entre genotipo y ambiente.

Publicaciones en revistas ISI elaborados durante el periodo de tesis doctoral y directamente relacionados con el contenido de la tesis:

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Navas-Lopez, J. F., León, L., Trentacoste, E. R., & de la Rosa, R. (2019a). Multi-environment evaluation of oil accumulation pattern parameters in olive. *Plant Physiology and Biochemistry*, 139, 485-494.

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Aportaciones a congresos nacionales e internacionales

J.F. Navas-López (2016). Pautas de acumulación de aceite en variedades y selecciones de mejora de olivo en Andalucía. I Congreso Ibérico de Olivicultura (SECH). Badajoz, Spain. Participación oral.

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Por todo ello, se autoriza la presentación de la tesis doctoral.

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Firma del/de los director/es



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Resumen

1. Introducción

Desde hace décadas, el cultivo del olivo se ha visto expuesto a una expansión globalizada debido principalmente a la alta rentabilidad conseguida por los nuevos sistemas de cultivo y a la gran importancia nutricional y saludable descrita para el aceite de oliva virgen extra. Variedades tradicionales ha sido utilizadas para ser plantadas en estas nuevas zonas, tanto en países donde el olivo no existía (China o Australia) como en ambientes de países productores donde el olivo no era un cultivo muy extendido. Algunas de estas variedades tradicionales, al plantarlas fuera de su zona de origen, han mostrado serios problemas en alguna de sus etapas productivas. Esto ha hecho que el cultivo tenga pérdidas significativas de rendimiento debido a ausencia de floración (Argentina) o a defectos en el aceite debido a las condiciones ambientales (Australia). Todo ello se debe, en parte, a que no existen trabajos sistemáticos para determinar la influencia de distintas condiciones ambientales en las variedades de olivo más cultivadas. Por ello, la evaluación de la interacción genotipo x ambiente en dichos caracteres es esencial para responder a estas incógnitas y ayudará a predecir el comportamiento del cultivo ante los efectos del cambio climático. Además, esta evaluación es de vital importancia para definir las mejores estrategias de selección en programas de mejora de olivo.

2. Contenido de la investigación

Las observaciones y toma de datos de los caracteres evaluados en esta tesis doctoral fueron llevados a cabo durante dos años (2015 y 2016) usando un ensayo multi-ambiente plantado en el 2006 por el IFAPA. Este ensayo está plantado en cinco ambientes (Antequera, Baena, Gibralfaró, Tabernas y Úbeda) y, constituido por un grupo de variedades tradicionales ('Arbequina', 'Arbosana', 'Carrasqueño', 'Changlot real', 'Frantoio', 'Koroneiki', 'Ocal' y 'Picual') y de selecciones de mejora ('Selección 1' y 'Selección 2', del programa de mejora en olivo UCO-IFAPA). En cada ambiente, las variedades estaban distribuidas en un diseño en bloques al azar. El primer carácter observado fue la floración, donde fenología de floración y calidad de flor fueron evaluadas. Se concluyó que para la fenología de floración el genotipo y la interacción muestran poca contribución, siendo el factor determinante el ambiente. La calidad de flor, por el contrario, sí mostró una elevada influencia genética, destacando 'Arbequina' como una variedad con alta calidad de flor independiente de donde sea plantada. El siguiente

carácter evaluado fue el patrón de acumulación de aceite, medido en proporción al peso seco en del fruto. Se distinguieron tres parámetros: pendiente de acumulación, máximo aceite acumulado y fecha en la que ese máximo fue alcanzado. La pendiente de acumulación y el máximo aceite acumulado mostraron una contribución similar de la varianza del genotipo, el ambiente y de su interacción. Para ambos parámetros, la ‘Selección 2’ mostró alta estabilidad, la cual también alcanzó el mayor valor para el máximo aceite acumulado. Sin embargo, la fecha en la que el máximo es obtenido fue un parámetro totalmente influenciado por el ambiente, señalando que la fecha de llegada al máximo acumulado es similar en todas las variedades para un ambiente dado. Finalmente, el último carácter observado en esta tesis doctoral fue la calidad del aceite de las diferentes variedades evaluadas. Todos los parámetros observados (ácidos grasos, tocoferoles, fitosteroles y esqualeno) mostraron una significativa interacción genotipo x ambiente. Por tanto, cada variedad tuvo un comportamiento diferencial según el ambiente en el que estaba plantada. ‘Selección 2’ fue el genotipo que destacó con alta estabilidad en la mayoría de los componentes observados, siendo ‘Arbequina’ la más inestable con valores de ácidos grasos cercanos a los límites inferiores marcados por el Consejo Oleico Internacional en algunos ambientes.

3. Conclusión

Se observó que la mayoría de los caracteres de interés agronómico evaluados en esta tesis doctoral mostraron una significativa interacción genotipo x ambiente. Ello indica que el uso de ensayos multi-ambiente para evaluar la interacción genotipo x ambiente en el olivo es conveniente para seleccionar de manera más precisa las variedades (tanto tradicionales como generadas por programas de mejora) más adecuadas para un ambiente dado o estables entre ambientes. Además, este tipo de ensayos dará la oportunidad de identificar los mejores ambientes para el cultivo del olivo ante las posibles variaciones producidas por los efectos del cambio climático.

Summary

1. Introduction

In the last decades, olive growing has been expanded around the world due principally to the high profitability obtained by the new growing systems and to the nutritional and healthy characteristics showed by the extra virgin olive oil. Traditional cultivars have been used to be planted in these new cultivation areas. However, no previous analysis of the olive behavior on those new climates has been performed. In fact, some of these cultivars have showed significant anomalies on the productive behavior. For example, a great yield loss has been observed in Argentina due to lack of flowering and very low olive oil quality has been obtained in Australia due to the climatic conditions. All this is partly due to the fact that there are no previous reports on the influence of different climatic conditions on the behavior of the most popular cultivars. Therefore, the evaluation of the genotype x environment interaction is essential to determine the adaptation of the cultivars to different environments and to predict the olive tree behavior under the climate change effects. Besides this genotype x environment evaluation is of paramount importance to develop efficient selection strategies in olive breeding programs.

2. Research content

This PhD was based on a multi-environment trial planted in 2006 by IFAPA and that was evaluated for several agronomic traits for two years (2015 and 2016). This trial is planted in five different environments (Antequera, Baena, Gibralfaró, Tabernas y Úbeda), using traditional cultivars ('Arbequina', 'Arbosana', 'Carrasqueño', 'Changlot real', 'Frantoio', 'Koroneiki', 'Ocal' y 'Picual') as well as two breeding selections ('Selection 1' and 'Selection 2') from the olive breeding program UCO-IFAPA. The first character considered was flowering, in which flowering phenology and flower quality were evaluated. Flowering phenology showed little genotype and interaction contribution, being the environment only factor with significant influence. For flower quality, genotype and genotype x environment interaction were highly significant, pointing 'Arbequina' out as a cultivar with high flower quality across environments. The second character evaluated on the above mentioned multi-environment trial was the olive oil accumulation pattern in fruit dry weight. Three parameters were evaluated: rate of oil

accumulation, maximum oil content reached and date of maximum oil reached. Rate of oil accumulation and maximum oil content reached showed similar genotype, environment and interaction contribution. High stability for both parameters was showed for 'Selection 2', which also obtained the highest maximum oil accumulated. However, date of maximum oil reached only showed a significance influence of environment. Therefore, all genotypes reached their maximum oil content in each environment in similar dates. Finally, the last character described in this PhD thesis was the olive oil quality. All components observed (fatty acids, tocopherols, phytosterols and squalene) showed significant genotype x environment interaction. 'Selection 2' was the genotype with higher stability for the most of olive oil components observed, being 'Arbequina' the cultivar having the most unstable olive oil, with fatty acid levels close to the lower limit set by the International Olive Council in some the environments here evaluated.

3. Conclusion

Most of olive tree characters here evaluated showed significant genotype x environments interaction. Therefore, it is advisable to use multi-environment trials in order to evaluate this interaction and, therefore, to accurately select the best genotypes for a given environment or those stable across environments. Besides, this kind of trials will also offer the opportunity to identify the best environments considering the possible variations due to the climatic change effects.



Capítulo 1:

Introducción General

Capítulo 1. Introducción General

1.1. El olivo y su confinamiento geográfico

El olivo (*Olea europea L.*) es un cultivo tradicional de la cuenca del Mediterráneo, donde fue originado entre 3500 – 4000 a. C. al norte del mar Muerto. Su expansión de oriente a occidente, recorriendo todo el Mediterráneo, fue principalmente causada por el tránsito de las civilizaciones y el aprendizaje de la propagación vegetativa. Con esta técnica se pudo conseguir multiplicar rápidamente un genotipo deseado para fijar un fenotipo de interés (Kaniewski et al. 2012). Esto conllevó a una rápida aparición de variedades de olivo por todo el Mediterráneo, seleccionadas dependiendo de las características de interés para su propagación como tamaño del fruto, rendimiento graso o adaptación al ambiente que le rodea. Esta última característica junto a la longevidad del cultivo causó el confinamiento de las variedades a su zona de origen, siendo poco difundidas y reduciendo la transferencia de sus caracteres de interés a otras zonas geográficas a lo largo del tiempo.

El cultivo del olivo ocupa en España un total de 2,413,021 ha para aceituna de almazara y siendo el principal productor de aceite del mundo (Figura 1.1). La mayor extensión del cultivo se concentra en el sur de la península entre las provincias de Jaén (24.23%), Córdoba (14.61%), Granada (8.23%) y Sevilla (7.21%) de acuerdo con MAGRAMA (2018). En nuestro país se ha encontrado una amplia variabilidad genética, repartida por todo el territorio, constituida por 272 variedades tradicionales catalogadas hasta la fecha (Rallo et al. 2005). Solamente 24 de ellas se consideran variedades principales (Barranco 2008). A pesar de esta gran variabilidad genética, pocas variedades son las que copan la mayoría de la superficie cultivada, como es el caso de Picual y Hojiblanca (58.6% y 17.8% de Ha cultivadas en Andalucía, respectivamente, según el Plan Director del Olivar Andaluz). Estas variedades han mostrado un gran rendimiento y buena adaptación en su zona de origen. Aún así, hay muy poca información sobre su comportamiento en nuevas zonas de cultivo, ya que ambas también se encuentran hasta la fecha delimitadas en su zona de origen (Rallo et al. 2005).

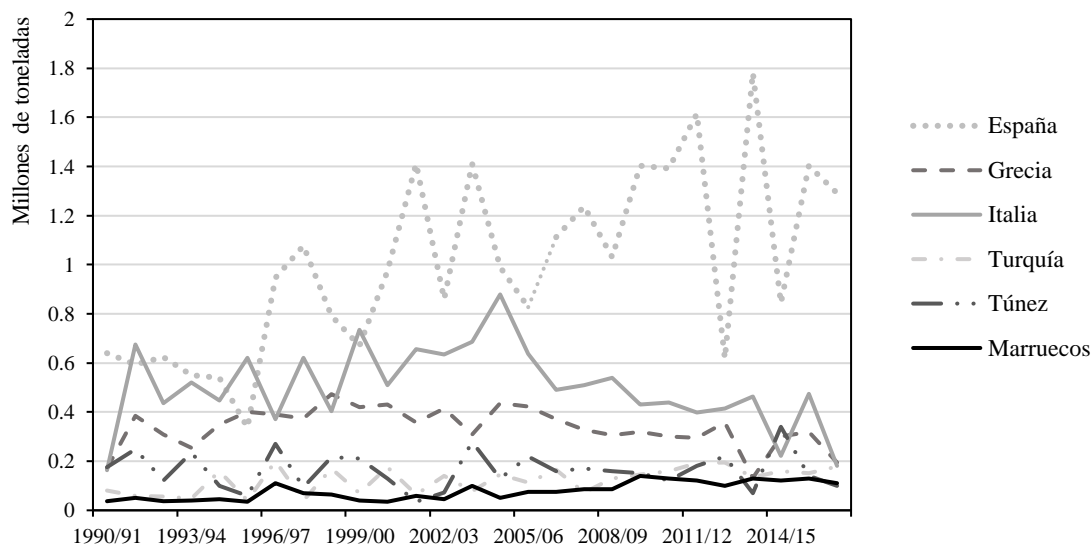


Figura 1.1. Evolución de la producción de aceituna de almazara en los principales países productores desde la campaña 1990/91 hasta 2017. Fuente: Consejo Oleico Internacional (COI).

1.2. Expansión del olivo y principales problemas

A principios del siglo XXI el olivar de almazara sufrió una gran revolución convirtiéndose en un cultivo altamente rentable. La transformación del cultivo ha supuesto pasar de un olivar tradicional en secano con 70-80 árboles/ha y rentabilidad muy baja (Duarte et al. 2008) a plantaciones más intensivas, llegando incluso a los 2000 árboles/ha, en el caso del olivar en seto (Tous et al. 2007). Esta intensificación hizo que bajara radicalmente el coste obtención de un litro de aceite (Rallo et al. 2013), por lo que el olivar intensivo podrá ser más competitivo en el mercado, aunque los precios del aceite caigan. Además, hay que añadirle el gran valor añadido que aporta el aceite de oliva virgen extra tanto a nivel nutricional como saludable (Estruch et al. 2018; Warleta et al. 2011), el cual ha hecho que aumente su consumo tanto en los países de la cuenca del Mediterráneo como fuera según el Consejo Oleico Internacional (COI 2018) (Figura 1.2). Este consumo de aceite en países fuera de la cuenca del Mediterráneo está incrementando de tal medida que se acercan al consumo de los países tradicionalmente consumidores. Entre los países que están incluyendo el aceite de oliva a su dieta se encuentran China, Japón, Australia o Brasil.

Por estas razones, el olivo está sufriendo una gran expansión alrededor el mundo. Durante los años 90 el cultivo del olivo empezó a expandirse en Argentina, USA y

Capítulo 1

México, usando variedades tradicionales de la cuenca del Mediterráneo como ‘Arbequina’, ‘Leccino’, ‘Frantoio’ o ‘Picual’. Más recientemente el cultivo fue llevado a países como Australia, China, Chile o Arabia Saudí (COI 2018). Todos ellos presentan climas muy diferentes al Mediterráneo donde el olivo nunca había sido cultivado. Incluso en España, el olivo está aumentando mucho su cultivo en zonas donde antes no tenía mucha extensión usando variedades tradicionales con diferente sitio de origen, por ejemplo, en zonas con influencia costera (Huelva) o desértica (Almería).

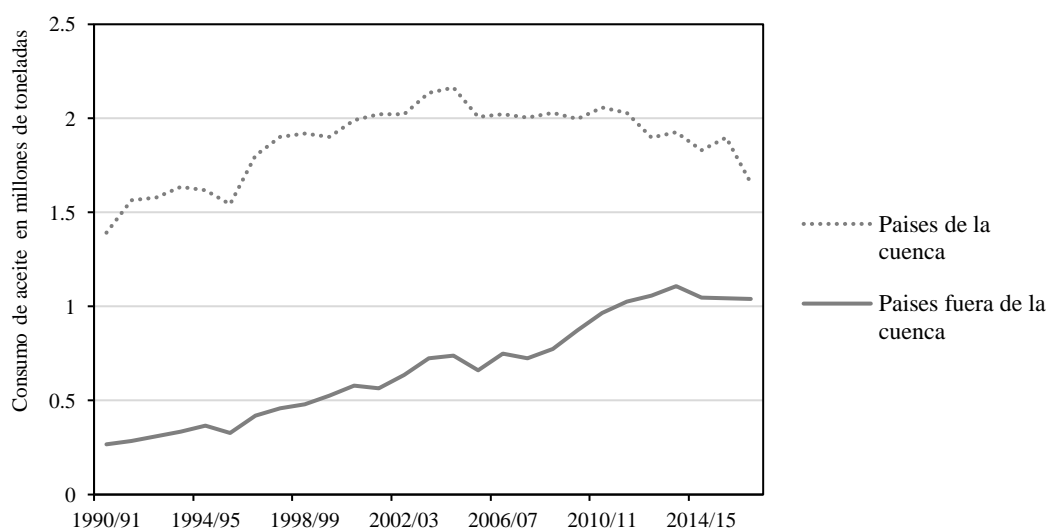


Figura 1.2. Evolución del consumo de aceite desde la campaña 1990/91 hasta 2016/17 en los países dentro y fuera de la cuenca del Mediterráneo. Fuente: el Consejo Oleico Internacional (COI).

En unas pocas de estas nuevas zonas de cultivo, investigaciones previas se han llevado a cabo para conocer el comportamiento de dichas variedades tradicionales a estos nuevos ambientes antes de realizar grandes plantaciones (Mailer et al. 2010; Wang et al. 2018). Pero en la mayoría, el cultivo se ha realizado sin estudios previos, provocando en algunos casos pérdidas de rendimiento a gran escala. Esto es debido a que el comportamiento mostrado por algunas de las variedades plantadas en estos nuevos ambientes no fue del todo la esperada, con reducciones totales o parciales del rendimiento al verse afectada alguna de las etapas de la fase productiva (Torres et al. 2017). Así, se han observado floraciones anómalas en Argentina, donde algunas variedades como ‘Leccino’ o ‘Frantoio’ mostraron floraciones anormales con reducida calidad en todos los años observados (Aybar et al. 2015). Otro parámetro de interés muy afectado es la calidad de aceite. Así, en Australia, se han descrito algunos ambientes específicos donde es

imposible obtener en Arbequina un aceite de oliva virgen extra, independiente del momento de su cosecha (Mailer et al. 2010).

Toda esta información sobre pérdidas de rendimiento y de calidad en algunas variedades de olivo plantadas en nuevas zonas geográficas pone de manifiesto la importancia del ambiente. Por lo tanto, variaciones en el clima tradicional del olivo, el Mediterráneo, causados por efectos del cambio climático también podría causar pérdidas en un futuro próximo. De hecho, Lionello et al. (2012) y Giorgi and Lionello et al. (2008) han descrito que el Mediterráneo será una de las más vulnerables regiones a los principales efectos extremos del cambio climático. Predicciones de un aumento de la temperatura de hasta 4°C en los meses cálidos (Giannakopoulos et al. 2009) o más frecuencia en la aparición de periodos muy secos y olas de calor (Giorgi and Lionello 2008; Tanasijevic et al. 2014) han sido descritas para esta región, con algunos efectos negativos sobre el olivo ya descritos (Benlloch-Gonzalez et al. 2018; Rapoport 2014).

Pero ¿Qué contribución tendrá el efecto ambiental en los principales parámetros de rendimiento y calidad en el olivo? ¿Todas las variedades se verán afectadas por igual? ¿Habrá alguna variedad con estabilidad en el comportamiento?

1.3. Interacción genotipo x ambiente, estabilidad y adaptabilidad.

El conocimiento de la interacción genotipo x ambiente (GxA) en la agricultura moderna es un requisito para predecir el comportamiento que tendrán los caracteres de interés en cada genotipo ante su globalización y los efectos que podrían causar el cambio climático. La GxA se muestra cuando un grupo de genotipos responden diferentemente a diversos ambientes (Figura 1.3) y se considera ineludible cuando el ranking de las genotipos se ve modificado dependiendo del ambiente (Yan and Kang 2002). Esto es debido a que presenta un impacto negativo sobre la heredabilidad, causando una gran dificultad en la mejora vía selección y reduciendo la correlación entre genotipo y fenotipo. Por lo tanto, el poder de predicción sobre el comportamiento de un carácter, con una alta GxA, será complicado.

Hay una serie herramientas estadísticas que estructuran y exploran la GxA según las estrategias que se estén buscando. Los más utilizados actualmente son los análisis

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multi-variantes usando biplots (Yan et al. 2007; Gauch et al. 2008). Estos métodos, son rápidos y claros de visualización para obtener conjuntamente resultados de estabilidad y adaptabilidad. Ambos parámetros son indispensables para la correcta interpretación de la GxA (Yan et al. 2007). La estabilidad genotípica se describe como el nivel de constancia de un comportamiento a través de los ambientes evaluados. En caracteres de cultivo con GxA significativa, la exploración de la estabilidad es útil para poder identificar posibles genotipos que mantenga un similar y alto comportamiento entre ambientes (Ebrahimi et al. 2016; Hassani et al. 2018). Por otro lado, la adaptabilidad se describe como el comportamiento superior de un genotipo a un ambiente específico. Por lo que un genotipo con poca estabilidad a través de los ambientes puede ser superior en un ambiente específico. En este caso, es importante explorarlo tanto para incrementar más el rendimiento en las zonas favorables de cultivo como para encontrar el genotipo más adecuado a ambientes muy desfavorables.

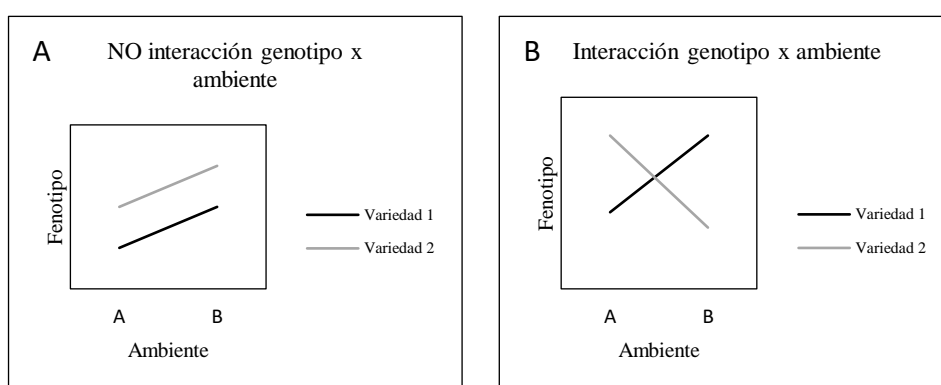


Figura 1.3. Representación gráfica de ausencia (A) o presencia (B) de interacción genotipo x ambiente

1.4. Programa de mejora y su etapa final de ensayos multi-ambiente

La incesante globalización del cultivo del olivo pone de manifiesto la necesidad de disponer de nuevas variedades, tanto genotipos obtenidos por cruzamiento y selección como variedades tradicionales con potenciales agronómicos. Todo ello para aportar gran variabilidad genética en cada carácter de interés como sistema de plantación, rendimiento graso, calidad del aceite o ambientes desfavorables, y reducir las tendencias al monocultivo.

El primer programa de mejora en España fue iniciado en Córdoba en el año 1990 fruto de la colaboración de la UCO y el IFAPA (Rallo 1995). Sus objetivos iniciales propuestos se centraron en acortar la fase juvenil, producción precoz, alto rendimiento graso y alto contenido en ácido oleico. Posteriormente se incluyeron dos nuevos objetivos, resistencia a *Verticilium dahliae* (Kleb.) y reducir el vigor de crecimiento vegetativo. Para conseguirlos, los primeros cruzamientos fueron realizados usando solamente tres genitores con características de mucho interés agronómico como son ‘Arbequina’ por su adaptación a super-intensivo, ‘Frantoio’ por alta tolerancia a *Verticilium dahliae* (Kleb.), y ‘Picual’ por su alta calidad de aceite. De ello, importantes éxitos han sido ya logrados como la obtención de una nueva variedad, ‘Sikitita’, adaptada al novedoso sistema de cultivo super-intensivo por su reducido vigor vegetativo (Rallo et al. 2008). Además, otras dos nuevas variedades ya han sido registradas (Sikitita 2 y Sikitita 3) desde el mismo programa de mejora, caracterizadas también por su adaptación al sistema super-intensivo, con alto rendimiento graso y gran calidad de aceite.

Los programas de mejora está constituidos por una serie de etapas consecutivas (Figura 1.4), desde el cruzamiento entre progenitores potenciales hasta ensayos multi-ambiente para observar el comportamiento de los genotipos considerados como avanzados (León 2014). Durante la progresión de las etapas, el número de genotipos se va reduciendo y el número de repeticiones va aumentando, siendo alrededor de 20 años hasta alcanzar la etapa final. Por lo que la búsqueda de métodos para reducir el tiempo en determinar los caracteres dentro del programa de mejora es esencial ante la rápida demanda del mercado. Algunos de estos caracteres han conseguido ser determinados y seleccionados en etapas tempranas del programa como la obtención de un corto periodo juvenil (de la Rosa et al. 2006), o en etapas intermedias como son algunos caracteres de productividad, vigor y calidad del aceite (Leon et al. 2015). En algunos casos esta evaluación precoz de caracteres ha aportado información bastante precisa para descartar y seleccionar genotipos en estas etapas tan tempranas debido a la gran variabilidad genética que la progenie obtenida por cruzamiento (Lavee 1990; Leon et al. 2004). Sin embargo, la mayoría de estos genotipos seleccionados siempre tienen que ser evaluados en las etapas finales del programa, ya que en etapas tempranas las repeticiones son muy bajas y su evaluación es poco representativa de la interacción con el ambiente (de la Rosa et al. 2008). Por ello, la utilización de ensayos comparativos y ensayos multi-ambiente,

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aun siendo las etapas menos usadas, siguen siendo determinantes para verificar lo seleccionado en etapas tempranas.

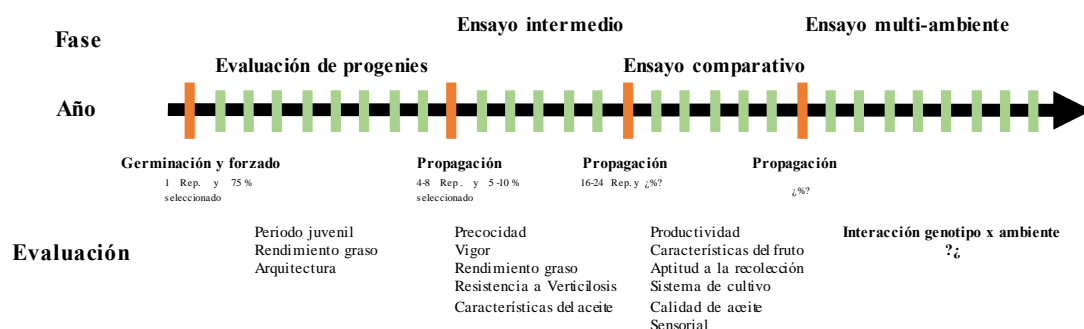


Figura 1.4. Etapas del programa de mejora de olivo (León 2014).

Los ensayos multi-ambiente en los programas de mejora en olivo han sido enmarcados como la última etapa y solamente son incluyen genotipos muy prometedores, que ya han sido bien seleccionados (León 2014), debido a su cotoso mantenimiento y a la laboriosa recogida de datos (Hardner et al. 2016). Aun así, este tipo de ensayos presentan más potencial que el asignado, ya que con su uso también se podrán elegir variedades o genotipos con alta estabilidad. Por lo tanto, puede permitir seleccionar nuevas variedades adaptadas a condiciones ambientales variadas y otras adaptadas específicamente a un ambiente (Yan et al. 2000). De hecho, este tipo de ensayos permite explorar la contribución genética, ambiental y de la GxA, aún desconocidos en el olivo. Sin embargo, a pesar de la importancia de la interacción GxA en los programas de mejora (Yan et al. 2000), existe muy poco conocimiento sobre la misma en olivo. Por lo tanto, la incorporación al programa de mejora de olivo de el Centro IFAPA “Alameda del Obispo” en Córdoba, en la cual gira la presente Tesis Doctoral, el estudio de la contribución GxA, en los objetivos principales de mejora, ayudará a escoger eficazmente los caracteres mejorables vía selección e incrementará la capacidad de selección del programa.

La etapa de ensayos multi-ambiente propuesto para este objetivo estuvo constituida tanto por dos selecciones de mejora (‘Selección 1’ y ‘Selección 2’) siguiendo el esquema general de mejora como por un grupo de variedades tradicionales (‘Arbequina’, ‘Arbosana’, ‘Carrasqueño’, ‘Changlot real’, ‘Frantoio’, ‘Koroneiki’, ‘Ocal’ y ‘Picual’) con características de interés agronómico para mejorar la variabilidad genética del cultivo. Estas últimas fueron previamente evaluadas en el Banco Mundial de

Germoplasma del Olivo (BMGO), ubicado en el IFAPA “Alameda del Obispo” de Córdoba. En el BMGO se conservan actualmente más de 900 variedades de 24 países (Belaj et al. 2013), por lo cual su principal función es conservar la biodiversidad genética del cultivo. Aunque también aporta gran cantidad de información que puede ser utilizada para la realización de nuevos cruces con alto potencial o directamente usar nuevas variedades tradicionales por su interés agronómico.

1.5. Hipótesis y Objetivos

El desarrollo de la presente Tesis Doctoral está enmarcado en la última etapa del programa de mejora de olivo, basada en ensayos comparativos en diversos ambientes. En estos ensayos se pretenden evaluar la importancia de la GxA en diversos caracteres agronómicos que se concretan en los siguientes objetivos específicos:

- Evaluar la contribución de la influencia genética, ambiental y de su interacción en la fenología de floración y calidad de flor en el olivo, además, estudiar la estabilidad genotípica para dichos caracteres (Capítulo 2).
- Evaluación multi-ambiente de los parámetros del patrón de acumulación de aceite en el olivo para observar la contribución genética, ambiental y de su interacción. Este último mediante análisis de estabilidad y adaptabilidad genotípica. Además, modelizar posibles factores ambientales con los parámetros de acumulación de aceite (Capítulo 3).
- Evaluar y estructurar la interacción genotipo x ambiente para los componentes de calidad de aceite en el olivo como son los principales ácidos grasos y los componentes menores y buscar posibles relaciones entre las diferentes rutas metabólicas (Capítulo 4).



Capítulo 2:

Genotype, Environment and
their Interaction Effects on

Olive Tree Flowering Phenology and Flower Quality

Authored by Jose F. Navas-López^a, Lorenzo León^a, Hava F. Rapoport^b, Inmaculada Moreno-Alías^b, Ignacio Lorite^a and Raúl de la Rosa^a.

^a IFAPA Centro “Alameda del Obispo”, Avda Menéndez Pidal s/n, 14004 Córdoba, Spain.

^b Institute for Sustainable Agriculture, (I.A.S.) -C.S.I.C. Avda Menéndez Pidal s/n, 14004 Córdoba, Spain.

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2.1. Summary

Flowering is one of the most critical processes in the reproductive phenology in fruit trees including olive. Under Mediterranean climate, olive flowering period normally occurs from early to late spring. Therefore, flowering time could be affected by environmental conditions such as water availability and temperature, thus influencing flower quality as well. Previous works have demonstrated the effect of genotype and environment on olive flowering, but those factors have never been compared in a single study nor their interaction evaluated. In the present work, we used a multi-environment trial established in four different agro-climatic conditions of Andalucía, Southern Spain, to test the genetic and environmental influences on flowering timing (flowering period, full bloom period, and full bloom date) and flower quality (flower number, perfect flower number and perfect flower percentage). Most of the variability found for flowering phenology parameters was due to environmental influence. On the contrary, for flowering quality parameters, most of the variability was of a genetic nature. In all cases, the genotype-environment interaction was significant. In this sense, the genotype by environment effects biplot model used to analyse the interaction showed that most of the genotypes evaluated have low stability for most of the parameters evaluated, except 'Picual' for flowering phenology parameters and 'Koroneiki' for quality. This emphasize the benefits of multi-environment trials on olive in order to select the best genotypes adapted to different environments and as a tool to face the future variability of environmental conditions caused by the climate-warming scenario. In conclusion, multi-environment trials allowed to efficiently quantify the effects of genetic and environmental factors and of their interaction on flowering phenology and on flower quality in olive.



Capítulo 3:

Multi-environment Evaluation of Oil Accumulation Pattern Parameters in Olive

Authored by Jose F. Navas-López^a, Lorenzo León^a, Eduardo R. Trentacoste^b and Raúl de la Rosaa.

^a IFAPA Centro “Alameda del Obispo”, Avda Menéndez Pidal s/n, 14004 Córdoba, Spain.

^b Estación Experimental Agropecuaria Junín (Instituto Nacional de Tecnología Agropecuaria), Mendoza, Argentina.

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3.1. Summary

The pattern of olive oil accumulation is of paramount importance in olive because its influence in determining final oil yield and optimal harvesting date. In this work, we have evaluated the genetic and environment influence on the olive oil accumulation pattern. For that purpose, a set of cultivars and breeding selections planted in a multi-environment trial was evaluated in two consecutive harvest seasons. Significant effect of the environment, genotype and their interaction were observed for the maximum oil content reached and the rate of oil accumulation. On the contrary, the date of maximum oil content seems to depend only on the environment. The two breeding selections evaluated showed, in general, high stability and adaptability in oil accumulation parameters. Among the potential environmental factors that could affect oil accumulation, PLS analysis suggests that temperature could play a determinant environmental effect in the oil accumulation parameters tested in this study. These results underline the relevance of using multi-environment trials for adequate characterization of genotypes showing either good behaviour in variable environments or only under specific environmental conditions.



Capítulo 4:

Genotype by Environment Interaction
for Oil Quality Components
in Olive Tree

Under revision in *European Journal of Agronomy*.

Capítulo 4. Genotype by Environment Interaction for Oil Quality Components in Olive Tree

4.1. Summary

Olive oil is highly valued by its nutritious and healthy function mainly due to its chemical composition. Oil chemical components are highly influenced by the cultivar and the environment, resulting in some cases in levels out of the limits set by the International Olive Council. In the present research, we used a multi-environment trial with five different agro-climatic conditions of Andalusia to evaluate how genetic and environmental factors and their interactions influence some of the most important olive quality components. Most of variation for olive components according to ANOVA was attributed to genetic effect, which was supported using principal component analysis and linear discriminant analysis. For the fatty acids, ‘Arbequina’ showed oils with the highest C16:0 and C18:2 values, and the lowest C18:1. Contrarily, an advanced breeding selection, ‘Selection 2’, and ‘Picual’ showed high C18:1 and low C16:0 and C18:2. The minor components varied depending of the genotype, underlining very high squalene values in ‘Carrasqueño’. Even so, the environment and the G x E interactions also resulted highly significant, with some genotype-environment combination unfavourable for oil composition as ‘Arbequina’-Gibraleón. The significant interactions were analysed separately by additive main effects and multiplication interaction to identify the most stable genotypes and adaptations to specific environments. For instance, ‘Arbequina’ showed low stability for C18:1 and C18:2, but very high stability for total tocopherol content and squalene content. These results underline the relevance of using multi-environment trials for adequate characterization of genotypes for oil components.

Keywords: oil olive components, genetic effect, environment effect, genotype by environment interaction, genotypic stability.

4.2. Introduction

Olive oil consumption is currently increasing around of world due to the classification of some of its constituents as functional nutrients (Paiva-Martins 2017),

with outstanding nutritional and healthy properties. Those included the importance of olive to fight against cardiovascular diseases (Estruch et al. 2018) or different types of cancer (Warleta et al. 2011; Escrich et al. 2006). Olive oil composition, characterized by high monounsaturated fatty acid concentration and active minor components such as tocopherols, squalene and sterols (Boskou 2009; Covas et al. 2006), is responsible for those medical properties. However, this olive oil composition and, hence, its healthy properties, is greatly influenced for both genetic (Uceda et al. 2008; Beltran et al. 2016) and environmental factors (Aguilera et al. 2005; Mailer et al. 2010). Also, the interaction genotype by environment could cause significant variation, which is currently unknown for the olive oil components.

High variability for oil content and composition has been reported in olive, both in traditional cultivars (Rondanini et al. 2011; Rotondi et al. 2010; Rondanini et al. 2014; Beltran et al. 2016; Uceda et al. 2008) and breeding selections (Leon et al. 2008; Leon et al. 2011; Ripa 2008) as well as in wild genotypes (Leon et al. 2018). Besides, a high heritability has been found for some olive oil quality components (de la Rosa et al. 2016; Leon et al. 2015), which underline the importance of the genotype. This could open up the possibility of efficiently perform breeding for improving health-related components of olive oil (Leon et al. 2018).

The environment has been suggested to have less influence on oil composition than the genotype (Velasco et al. 2014; Uceda et al. 2008), although significant variability among geographical locations has been reported (Aguilera et al. 2005; Borges et al. 2017). Even negative effects of certain climatic conditions on the olive oil quality have been observed, especially in locations outside the Mediterranean Basin (Mailer et al. 2010; Rondanini et al. 2011). Those negative effects have caused that some olive oil components can reach values outside of the regulatory ranges set by the International Olive Council (IOC) for olive oil.

Although genetic and environmental effects on olive oil quality have been largely explored, there is limited information on the relative contribution of each factor and their interactions (GEI) to the total variability. One useful option to efficiently quantify the contribution of each factor and GEI is the use of multi-environment cultivar trials (S.S. Miller et al. 2005a; Hardner et al. 2016). This is of great interest in olive, since high

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environmental and GEI effects are associated with low heritability (Yan 2002; Hardner et al. 2016) and, therefore, with low selection efficiency.

Stability and adaptability indexes are usually used to evaluate significant GEI and to determine genotypes well adapted to specific environments (Hassani et al. 2018; Ebrahimi et al. 2016). For that, the additive main effects and multiplicative interaction (AMMI) model is largely used, mainly for annual crops (Ebrahimi et al. 2016; Hassani et al. 2018), although it has also been used in olive to evaluate GEI for oil accumulation (Navas-Lopez et al. 2019b). To the best of our knowledge, no similar studies on stability and adaptability indexes have been conducted on olive oil quality traits. Accordingly, the objective of this study was to determine the relative contribution of genetic and environmental effects and GEI to the fatty acid profile of the oil and the total content and profile of other minor quality components in olives, using multi-environment cultivar trial. From these results, information about genotypic stability and adaptability was also extracted with a view to select superior genotypes for these olive oil components.

4.3. Materials and Methods

4.3.1. Experimental Sites and Plant Material

The experiment was carried out in a multi-environment trial (MET) established during 2008 in five different agro-climatic conditions of Andalusia, Southern Spain (Figure 4.1). Three orchard trials were located in traditional olive growing areas, Antequera and Baena under rainfed conditions, and Úbeda with drip irrigation. The other two orchards were established in new growing areas, where olive growing is currently expanding, Gibrleón with mild winter temperatures and Tabernas with desert climate, both with drip irrigation. All the trials included seven traditional cultivars, namely ‘Arbequina’, ‘Arbosana’, ‘Carrasqueño’, ‘Frantoio’, ‘Koroneiki’, ‘Ocal’ and ‘Picual’, and two advanced breeding selections from the cooperative olive breeding program of Córdoba (University of Córdoba and Andalusian Institute of Agricultural Research and Training - IFAPA), which were selected on the basis of their earliness bearing, high oil content and high oil quality (Leon et al. 2008; De la Rosa et al. 2006; Leon et al. 2011). The genotypes were arranged in each orchard trial in a randomized design with four blocks and four trees per elementary plot. Fruit samples of around 300 g were randomly

collected for each elementary plot-genotype-trial during 2015 and 2016 seasons on mid-October. Only trees with similar crop load were considered for sampling.

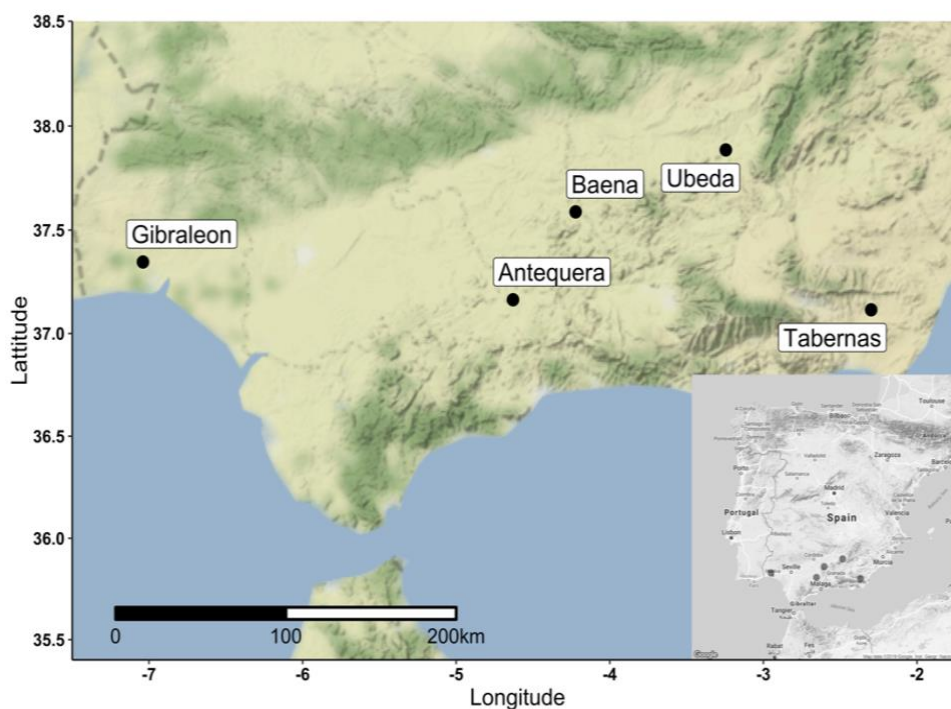


Figure 4.1. Geographical situation of the five locations used in this study situated in Andalusia, Southern Spain.

4.3.2. Olive oil quantification

For each fruit sample, three subsamples of 25 g were randomly selected to measure oil content as a percentage on dry weight basis. For that purpose, each subsample was dried in a forced-air oven at 105°C for 42 h (del Río and Romero 1999), then oil content was measured using an NMR fat analyser (Minispec MQone, Bruker Optik GmbH, Ettlingen, Germany).

4.3.3. Olive Oil Components Analysis

For oil quality analyses, a subsample of 20 randomly chosen fruits was stored shortly after harvest at -80 °C. After freeze drying, the stones were removed and the flesh milled in a laboratory ball mill. All the analyses were performed in duplicate following the methods reported by Velasco et al. (2014). In short, the fatty acid profile was

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analysed after simultaneous oil extraction and fatty acid methylation on a Perkin Elmer Clarus 600 Gas Chromatograph (Perkin Elmer, Inc., Waltham, MA) equipped with a BPX70 30 m x 0.25 mm internal diameter x 0.25 µm film thickness capillary column (SGE Analytical Science Pty Ltd., Ringwood, Australia). Individual fatty acid content was expressed as percentage of the total fatty acids. Tocopherols were extracted for 16 h with isooctane and analysed by high-performance liquid chromatography (HPLC) using iso-octane/tert-butylmethylether (94:6) as eluent at an isocratic flow rate of 0.8 ml/min, a LiChrospher 100 diol column (250 mm 9.2 mm I.D.) connected to a silica guard column (LiChrospher Si 60, 5 mm 9.4 mm I.D.) and a fluorescence detector (Waters 474) at 295-nm excitation and 330-nm emission. Rac-5,7-dimethyltocol (Matreya LLC, Pleasant Gap, PA, USA) was used as internal standard for tocopherol quantification. Total tocopherol content was expressed as mg kg⁻¹ dry fruit flesh. Individual tocopherols were expressed as percentage of total tocopherols. Phytosterols and squalene were analysed after unsaponifiable fraction extraction and silylation on a Perkin Elmer Clarus 600 Gas Chromatograph equipped with a ZB-5 30 m x 0.25 mm internal diameter x 0.10 µm film thickness capillary column (Phenomenex, Torrance, CA, USA). 5 α -cholestan-3 β -ol (Reference D6128, Sigma-Aldrich, St. Louis, MO, USA) and squalene (reference 442784, Supelco, Bellefonte, PA, USA,) were used as internal standards for phytosterol and squalene quantification, respectively. Total phytosterol and squalene contents were expressed as mg kg⁻¹ dry fruit flesh. Individual phytosterols were expressed as percentage of total phytosterols.

4.3.4. Data Analysis

In the present study, the factor environment was formed by a combination of location (Antequera, Baena, Gibrleón, Tabernas and Úbeda) and year (2015 and 2016). One of the combinations, Baena 2015, was excluded of the analysis because of insufficient production. Thus, nine environments were considered for the study. Variance analysis (ANOVA) to quantify the contribution of genotype, environment and their interaction (GEI) and mean separation using Honestly-significant-difference (HSD) were performed. HSD test was only performed for principals oil components (C16:0, C18:1, C18:2, total tocopherols, total phytosterols and squalene), and those with significant GEI were evaluated using stability and adaptability analysis through additive main effects and multiplication interaction (AMMI) model (Gauch and Zobel 1997).

Pearson correlation and principal component analysis (PCA) were performed to evaluate relationships between the olive oil components. PCA was also used to observe the relationships among oil components and the genotype-environment combinations performance. Then, discriminating among samples according to olive oil components was performed with linear discrimination analysis (LDA). The LDA model accuracy was tested using the predictive performances of repeated K-fold cross-validation (repeated K-fold-CV) technique. Repeated K-fold-CV parameterization used in this study followed (Rodrigues et al. 2018), with the formation of 4 K-folds with internal validation subsets of 25% initial data and 10 repetitions to stress the model.

Statistical analysis was performed using R software (R development Core Team, 2016) using ANOVA type III under Car package, AMMI model and HSD test from Agricolae package, PCA from Factoextra package and LDA from both Caret and MASS packages.

4.4. Results

4.4.1. Factors Affecting Olive Oil Quality Traits

4.4.1.1. Olive oil content on dry weight basis

Environment was the most important factor for oil content on dry weight (Table 4.1), retaining almost half of the variance contribution. Genotype and genotype by environment interaction (GEI) showed similar contribution for this oil trait, both significant.

4.4.1.2. Fatty acids composition

Anova analysis indicated that the genotype was the main contributor to the total variance for all fatty acids except for C18:0 (Table 4.1). The environment and the GEI effects were also highly significant.

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Table 4.1. Percentage of variance components and significance in the ANOVA for the olive oil compounds evaluated on mid-October. *** $P < 0.001$, ** $P < 0.01$.

	Fatty acids						Oil content
	C16:0 (%)	C16:1 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	
Genotype	64.3 ***	39.1 ***	25.1 ***	71.9 ***	71.9 ***	30.4 ***	19.1 ***
Environment	16.1 ***	24.7 ***	38.2 ***	10.6 ***	10.6 ***	24.3 ***	43.6 ***
GEI	8.2 ***	17.1 ***	17.1 ***	7.5 ***	7.5 ***	26.1 ***	17.6 ***
Residuals	10.5	19.2	19.7	9.7	9.7	18.9	19.54

C16:0 – palmitic acid; C16:1 – palmitoleic acid; C18:0 – stearic acid; C18:1 – oleic acid; C18:2 – linoleic acid; C18:3 – linolenic acid.

	Minor components					
	Total tocopherols (mg Kg ⁻¹)	α -tocopherol (%)	Total phytosterols (mg Kg ⁻¹)	β -sitosterol (%)	Δ 5-avenasterol (%)	Squalene (mg Kg ⁻¹)
Genotype	40.6 ***	64.8 ***	55.2 ***	34.6 ***	35.2 ***	75.9 ***
Environment	25.9 ***	6.2 ***	14.2 ***	11.2 ***	7.5 ***	6.8 ***
GEI	21.5 ***	17.5 ***	12.4 ***	11.4 ***	7.4 ***	5.8 ***
Residuals	11.8	11.3	18.1	42.6	49.7	11.4

Wide differences among genotypes were observed for C16:0 (Table 4.2), C18:1 (Table 4.3) and C18:2 (Table 4.4). ‘Selection 2’ showed the highest values for C18:1 and the lowest values for C16:0 and C18:2, with ‘Arbequina’ showing an opposite behaviour. Three groups of genotypes could be formed based on these three main fatty acids, characterized by i) high C16:0 and 18:2 and low C18:1 (‘Arbequina’, ‘Arbosana’ and ‘Frantoio’); ii) low C16:0 and 18:2 and high C18:1 (‘Selection 2’ and ‘Picual’); and iii) intermediate values of the three fatty acids: (‘Carrasqueño’, ‘Koroneiki’, ‘Ocal’ and ‘Selection 1’). Differences among environments were less evident, although with a trend to higher values for C16:1 and C18:2 and lower values for C18:1 for BA-16, GI-15 and GI-16 environments.

Table 4.2. Comparison of means among the nine genotypes tested at the nine environments (ENV) for olive oil C16:0 (%) (Palmitic acid) on mid-October. Coefficient of variation (CV) are also indicated. Different letters for genotype (Mean₁), environment (Mean₂) and genotype-environment interaction (3) represent significant difference at $p < 0.05$ level based on Honestly-significant-difference (HSD).

Genotype	C16:0 (%)									Mean ₁	CV(%)
	ENV										
	AN-15	AN-16	BA-16	GI-15	GI-16	TA-15	TA-16	UB-15	UB-16		
Arbequina	15.3 cdefgh ³	17.1 adcd	17.6 a	18.1 a	18.3 a	16.8 abcd	16.9 abcd	14.9 defghij	16.6 abcde	16.9 a	6.7
Arbosana	14.1 ghijkl	14.5 defghijk			17.5 ab	14.5 efghijk	14.9 defghijk	14.8 defghijk	16.4 abcdef	15.4 b	7.5
Carrasqueño	13.5 ghijkl	13.2 hijklm	15.1 defghi	14.6 defghijk	14.3 fghijk		13.6 ghijkl	13.2 ghijklmn	13.9 ghijkl	13.9 c	4.9
Frantoio	13.9 ghijkl	14.9 defghijk		14.8 defghijk	15.5 bcdefg			13.6 ghijkl		14.5 c	5.3
Koroneiki	12.4 klmn	12.4 klmn	13.6 ghijkl	13.1 ijklm	14.1 ghijk	13.6 ghijkl	13.1 ijklm	12.3 klmn	12.8 jklmn	13.1 d	4.8
Ocal	11.9 lmn	12.6 klmn		11.9 lmn	13.1 hijklm			12.3 klmn		12.3 e	4.1
Picual	10.6 n	12.3 klmn	13.9 ghijkl	14.5 efghijk	13.9 ghijkl		14.1 ghijk	12.3 klmn		13.1 d	10.6
Selection1	11.9 lmn	12.1 klmn	14.8 defghijk	13.4 ghijklm	13.8 ghijkl	11.9 lmn		11.3 mn	14.7 defghijk	13.1 d	10.4
Selection2	11.3 mn	12.5 klmn	13.1 hijklm	12.2 klmn	12.9 jklm	12.4 klmn		11.7 lmn	12.6 klmn	12.3 e	4.8
Mean ₂	12.7 f	13.3 de	14.8 a	14.1 bc	14.9 a	13.8 cd	14.5 abc	12.9 ef	14.6 ab		
CV(%)	11.9	12.5	10.8	13.8	12.8	14.1	10.2	9.9	11.8		

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016.

Considering GEI, average values of genotypes in individual environments ranged from 10.6% to 18.3% for C16:0 (Table 4.2) and from 1.8% to 15.7% for C18:2 (Table 4.4). The highest values for both fatty acids were found for ‘Arbequina’ in GI-16. There was also a wide range between the minimum and maximum values for C18:1 (Table 4.3), observed for ‘Arbequina’ in GI-15 (61.4%) and ‘Selection 2’ in UB-15 (82.9%).

The stability of the genotypes, determined by their contribution to total GEI, was analysed using additive main effects and multiplication interaction (AMMI) model (Figure 4.2). High stability for C16:0 was identified for ‘Carrasqueño’ and ‘Frantoio’, whereas stability was low for ‘Arbequina’, ‘Picual’ and ‘Selection 1’. In the case of C18:1, three groups of genotypes were identified having high (‘Frantoio’, ‘Picual’ and ‘Selection 1’), intermediate (‘Carrasqueño’, ‘Selection 2’ and ‘Ocal’) and low (‘Arbequina’, ‘Koroneiki’ and ‘Arbosana’) stability. Again, ‘Arbequina’ and ‘Arbosana’ showed very low stability for C18:2. AMMI could also be used to identify a specific adaptability of a given genotype to a given environment such as for instance particularly high values of C18:2 in ‘Arbequina’ under the conditions of GI-15.

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Table 4.3. Comparison of means among the nine genotypes tested at the nine environments (ENV) for olive oil C18:1 (%) (Oleic acid) on mid-October. Coefficient of variation (CV) are also indicated. Different letters for genotype (Mean₁), environment (Mean₂) and genotype-environment interaction (3) represent significant difference at $p < 0.05$ level based on Honestly-significant-difference (HSD).

C18:1 (%)											
Genotype	ENV									Mean ₁	CV(%)
	AN-15	AN-16	BA-16	GI-15	GI-16	TA-15	TA-16	UB-15	UB-16		
Arbequina	71.2 <i>klm</i> 3	69.4 <i>lm</i>	66.1 <i>mn</i>	61.4 <i>n</i>	66.1 <i>mn</i>	70.1 <i>lm</i>	68.5 <i>lm</i>	72.4 <i>jkl</i>	69.1 <i>lm</i>	68.2 <i>e</i>	4.8
Arbosana	77.7 <i>abcdefg</i> hij	77.1 <i>cdefg</i> hijk			68.8 <i>lm</i>	76.9 <i>defg</i> hijk	75.3 <i>ghijkl</i>	75.4 <i>ghijk</i>	72.2 <i>jkl</i>	74.4 <i>d</i>	4.3
Carrasqueño	77.8 <i>abcdefg</i> hi	78.7 <i>abcdefg</i> hi	76.1 <i>fg</i> hijk	74.2 <i>ijkl</i>	77.8 <i>abcdefg</i> hi		78.8 <i>abcdefg</i> hi	81.2 <i>abcdefg</i>	78.7 <i>abcdefg</i> hi	77.8 <i>c</i>	2.6
Frantoio	74.2 <i>ijkl</i>	73.6 <i>ijkl</i>		71.5 <i>ijklm</i>	69.1 <i>lm</i>			76.1 <i>ghijk</i>		72.9 <i>d</i>	3.6
Koroneiki	77.8 <i>abcdefg</i> hi	78.5 <i>abcdefg</i> hi	77.2 <i>cdefg</i> hij	78.4 <i>abcdefg</i> hi	77.7 <i>abcdefg</i> hij	77.8 <i>abcdefg</i> hi	79.1 <i>abcdefg</i> hi	80.2 <i>abcdefg</i> h	79.5 <i>abcdefg</i> hi	78.4 <i>c</i>	1.2
Ocal	78.3 <i>abcdefg</i> hi	77.5 <i>bcdefg</i> hij		78.1 <i>abcdefg</i> hi	76.1 <i>efg</i> hijk			79.1 <i>abcdefg</i> hi		77.9 <i>c</i>	1.4
Picual	82.5 <i>ab</i>	81.3 <i>abcdef</i>	79.1 <i>abcdefg</i> hi	76.1 <i>efg</i> hijk	79.4 <i>abcdefg</i> hi		79.7 <i>abcdefg</i> h	81.6 <i>abcde</i>		80.1 <i>ab</i>	2.6
Selection 1	81.1 <i>abcdefg</i>	80.6 <i>abcdefg</i>	74.8 <i>hijkl</i>	76.8 <i>efg</i> hijk	77.7 <i>abcdefg</i> hij	81.1 <i>abcdefg</i>		82.1 <i>abcd</i>	76.1 <i>fg</i> hijk	78.6 <i>bc</i>	3.5
Selection 2	82.3 <i>abc</i>	80.9 <i>abcdefg</i>	80.6 <i>abcdefg</i> h	81.4 <i>abcde</i>	79.9 <i>abcdefg</i> h	82.1 <i>abcd</i>		82.9 <i>a</i>	81.2 <i>abcdefg</i>	81.5 <i>a</i>	1.1
Mean ₂	78.1 <i>ab</i>	77.8 <i>ab</i>	75.3 <i>de</i>	74.8 <i>de</i>	74.6 <i>e</i>	77.6 <i>abc</i>	76.5 <i>bcd</i>	78.9 <i>a</i>	75.9 <i>cde</i>		
CV(%)	4.7	4.9	6.7	8.2	7.1	6.1	6.1	4.5	6.1		

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016.

Table 4.4. Comparison of means among the nine genotypes tested at the nine environments (ENV) for olive oil C18:2 (%) (Linoleic acid) on mid-October. Coefficient of variation (CV) are also indicated. Different letters for genotype (Mean₁), environment (Mean₂) and genotype-environment interaction (3) represent significant difference at $p < 0.05$ level based on Honestly-significant-difference (HSD).

C18:2 (%)											
Genotype	ENV									Mean ₁	CV(%)
	AN-15	AN-16	BA-16	GI-15	GI-16	TA-15	TA-16	UB-15	UB-16		
Arbequina	9.7 <i>bcd</i> 3	9.6 <i>bcde</i>	11.7 <i>b</i>	15.7 <i>a</i>	11.1 <i>b</i>	8.9 <i>bcdef</i>	9.9 <i>bcd</i>	8.8 <i>bcdef</i>	10.3 <i>bc</i>	10.7 <i>a</i>	19.8
Arbosana	4.1 <i>ijklm</i>	4.1 <i>ijklm</i>			8.6 <i>bcdefg</i>	4.5 <i>ijklm</i>	5.5 <i>fghijklm</i>	5.4 <i>ghijklm</i>	6.5 <i>defg</i> hij	5.7 <i>c</i>	28.2
Carrasqueño	3.3 <i>ijklm</i>	3.1 <i>klm</i>	3.9 <i>ijklm</i>	5.7 <i>fghijkl</i>	3.2 <i>ijklm</i>		3.1 <i>klm</i>	2.1 <i>m</i>	2.8 <i>klm</i>	3.4 <i>de</i>	31.1
Frantoio	7.7 <i>cdefgh</i>	7.4 <i>cdefg</i> hi		9.9 <i>bcd</i>	11.1 <i>b</i>			6.5 <i>defg</i> hij		8.4 <i>b</i>	22.7
Koroneiki	5.1 <i>hijklm</i>	4.6 <i>hijklm</i>	4.8 <i>hijklm</i>	4.1 <i>ijklm</i>	3.4 <i>ijklm</i>	4.3 <i>ijklm</i>	4.1 <i>ijklm</i>	3.1 <i>klm</i>	4.1 <i>ijklm</i>	4.1 <i>d</i>	15.4
Ocal	4.7 <i>hijklm</i>	5.1 <i>hijklm</i>		6.3 <i>efg</i> hij	6.7 <i>defg</i> hij			4.5 <i>hijklm</i>		5.4 <i>c</i>	18.2
Picual	2.2 <i>m</i>	2.2 <i>m</i>	2.8 <i>klm</i>	4.7 <i>hijklm</i>	2.4 <i>m</i>		2.3 <i>m</i>	1.9 <i>m</i>		2.5 <i>ef</i>	37.8
Selection 1	2.9 <i>klm</i>	3.1 <i>klm</i>	5.9 <i>fghijk</i>	4.6 <i>hijklm</i>	4.1 <i>ijklm</i>	2.6 <i>lm</i>		2.3 <i>m</i>	4.2 <i>ijklm</i>	3.7 <i>d</i>	34.9
Selection 2	1.8 <i>m</i>	2.1 <i>m</i>	2.1 <i>m</i>	2.2 <i>m</i>	3.1 <i>klm</i>	1.7 <i>m</i>		1.4 <i>m</i>	1.8 <i>m</i>	2.1 <i>f</i>	24.1
Mean ₂	4.7 <i>cde</i>	4.4 <i>de</i>	5.4 <i>bc</i>	6.6 <i>a</i>	5.9 <i>ab</i>	4.4 <i>cde</i>	4.8 <i>cde</i>	4.1 <i>e</i>	5.1 <i>bcd</i>		
CV(%)	55.4	51.2	64.1	64.7	62.4	63.1	62.4	60.9	60.1		

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016.

Table 4.5. Comparison of means among the nine genotypes tested at the nine environments (ENV) for olive oil total tocopherols (mg Kg⁻¹) on mid-October. Coefficient of variation (CV) are also indicated. Different letters for genotype (Mean₁), environment (Mean₂) and genotype-environment interaction (3) represent significant difference at p < 0.05 level based on Honestly-significant-difference (HSD).

Genotype	Total tocopherols (mg Kg ⁻¹)									Mean ₁	CV(%)
	ENV										
	AN-15	AN-16	BA-16	GI-15	GI-16	TA-15	TA-16	UB-15	UB-16		
Arbequina	141.4 <i>fghijklm</i> 3	145.1 <i>fghijklm</i>	161.1 <i>defghijk</i>	122.5 <i>ijklm</i>	175.8 <i>abcdefgh</i>	128.3 <i>hijklm</i>	91.3 <i>mn</i>	173.2 <i>bcdefghi</i>	187.1 <i>abcdef</i>	147.4 <i>bc</i>	20.6
Arbosana	207.1 <i>abcd</i>	169.9 <i>bcdefghij</i>			223.1 <i>a</i>	168.5 <i>cdefghij</i>	156.5 <i>defghijklm</i>	205.8 <i>abcd</i>	220.5 <i>ab</i>	197.9 <i>a</i>	13.8
Carrasqueño	147.3 <i>fghijklm</i>	117.1 <i>klmn</i>	143.8 <i>fghijklm</i>	119.3 <i>ijklmn</i>	122.2 <i>ijklm</i>		90.8 <i>mn</i>	156.1 <i>defghijklm</i>	126.3 <i>ijklm</i>	126.6 <i>d</i>	16.4
Frantoio	155.7 <i>defghijklm</i>	115.3 <i>ijklmn</i>		112.1 <i>klmn</i>	125.2 <i>ijklm</i>			182.3 <i>bcdefg</i>		140.1 <i>cd</i>	21.4
Koroneiki	180.4 <i>abcdefg</i>	150.7 <i>efghijklm</i>		141.7 <i>fghijklm</i>	197.1 <i>abcde</i>	125.2 <i>ijklm</i>	112.7 <i>klmn</i>	187.2 <i>abcdef</i>	105.3 <i>lmn</i>	153.3 <i>bc</i>	21.5
Ocal	72.1 <i>n</i>	91.7 <i>mn</i>		91.7 <i>mn</i>	89.9 <i>mn</i>			145.9 <i>fghijklm</i>		112.1 <i>e</i>	24.8
Picual	135.6 <i>ghijklm</i>	87.9 <i>mn</i>	109.9 <i>lmn</i>	106.7 <i>lmn</i>	102.9 <i>mn</i>		72.6 <i>n</i>	167.7 <i>cdefghijk</i>		99.6 <i>e</i>	31.5
Selection 1	158.7 <i>defghijkl</i>	130.1 <i>hijklm</i>	184.4 <i>abcdefg</i>	119.6 <i>ijklmn</i>	147.6 <i>efghijklm</i>	154.6 <i>defghijklm</i>		162.3 <i>defghij</i>	190.9 <i>abcdef</i>	155.9 <i>b</i>	15.5
Selection 2	128.7 <i>hijklm</i>	128.9 <i>hijklm</i>	218.9 <i>abc</i>	112.7 <i>klmn</i>	118.9 <i>ijklmn</i>	110.8 <i>lmn</i>		127.8 <i>hijklm</i>	155.1 <i>defghijklm</i>	134.2 <i>d</i>	26.5
Mean ₂	144.6 <i>c</i>	123.8 <i>de</i>	160.4 <i>ab</i>	116.85 <i>e</i>	146.8 <i>bc</i>	137.5 <i>cd</i>	96.8 <i>f</i>	168.1 <i>a</i>	167.7 <i>a</i>		
CV(%)	25.7	21.2	22.9	12.2	32.7	17.1	33.2	14.2	25.8		

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016.

4.4.1.3. Minor components

Similarly, to fatty acids, the genotype was the main contributor to the total variability for minor components (Table 4.1). The environment and GEI were also highly significant, particularly in the case of total tocopherol content, where the sum of environmental and GEI effects surpassed genotypic effects.

HSD analysis showed marked differences among genotypes for total tocopherol content (Table 4.5), total phytosterol content (Table 4.6) and squalene content (Table 4.7). Squalene exhibited the widest range of variation among genotypes, ranging from 896 mg Kg⁻¹ in ‘Arbequina’ to 4000 mg Kg⁻¹ in ‘Carrasqueño’. For the others two minor components, the range was quite lower, being ‘Arbosana’ the genotype with the highest average value for both components (197.9 mg Kg⁻¹ of total tocopherols and 1028 mg Kg⁻¹ of total phytosterols). On the contrary, the lowest value for total tocopherols was found in ‘Picual’ (99.6 mg Kg⁻¹; Table 4.5) and for total phytosterols in ‘Selection 2’ (571 mg Kg⁻¹; Table 4.6). Differences between environments were lower than those between genotypes, especially for squalene (from 2168 mg Kg⁻¹ in AN-15 to 3103 mg Kg⁻¹ in GI-15). GI-15 and GI-16 environments were characterized by high values for squalene and

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total phytosterol contents, whereas and UB-15 and UB-16 environments resulted in high total tocopherol content.

GEI combinations showed high ranges of variability for minor components. Total tocopherol (Table 4.5) and phytosterol contents (Table 4.6) showed higher values in ‘Arbosana’ (223 mg Kg⁻¹ in GI-16 and 1200 mg Kg⁻¹ in UB-15, respectively). On the contrary, the lowest values for both components were found in ‘Ocal’ and ‘Selection 2’, both in AN-15 environment (71.1 mg Kg⁻¹ and 303 mg Kg⁻¹, respectively). The maximum amplitude from GEI was showed for squalene (Table 4.7), where the values ranged from 601 mg Kg⁻¹ for ‘Arbequina’ in AN-16 to 5093 mg Kg⁻¹ for ‘Carrasqueño’ in GI-15.

Table 4.6. Comparison of means among the nine genotypes tested at the nine environments (ENV) for olive oil total phytosterols (mg Kg⁻¹) on mid-October. Coefficient of variation (CV) are also indicated. Different letters for genotype (Mean₁), environment (Mean₂) and genotype-environment interaction (3) represent significant difference at p < 0.05 level based on Honestly-significant-difference (HSD).

Total phytosterols (mg Kg⁻¹)										Mean ₁	CV(%)
Genotype	ENV										
	AN-15	AN-16	BA-16	GI-15	GI-16	TA-15	TA-16	UB-15	UB-16		
Arbequina	781 cdefghijkl 3	885 bcdefgh	850 cdefgh	1186 ab	1036 abcd	773 cdefghijkl	923 abcdef	1031 abcd	1053 abc	949 ab	14.6
Arbosana	828 cdefghij	889 bcdefgh			1121 abc	893 bcdefg	1177 abc	1200 a	1028 abcd	1020 a	14.8
Carrasqueño	714 efghijkl	894 bcdefg	937 abcdef	1190 ab	847 cdegh		856 cdefgh	825 cdefghijk	861 cdefgh	893 bc	15.3
Frantoio	994 abcde	951 abcdef		823 cdefghijk	1081 abc			1040 abcd		989 a	10.1
Koroneiki	608 ghijklm	545 ijklm	585 hijklm	716 efghijkl	659 fghijklm	491 klm	656 fghijklm	699 efghijkl	544 ijklm	614 e	12.4
Ocal	777 cdefghijkl	874 cdefgh		996 abcde	652 fghijklm			868 cdefgh		946 cd	13.5
Picual	610 ghijklm	750 defghijkl	841 cdefghi	1021 abcde	828 cdefghij		776 cdefghijkl	853 cdefgh		801 d	15.5
Selection 1	620 fghijklm	827 cdefghij	992 abcde	1006 abcde	806 cdefghijk	781 cdefghijkl		793 cdefghijk	849 cdefgh	843 cd	14.5
Selection 2	393 m	580 hijklm	644 fghijklm	663 fghijklm	711 efghijkl	485 lm		531 jklm	586 hijklm	571 e	18.1
Mean 2	701 d	792 c	818 bc	953 a	868 bc	685 d	831 bc	873 b	836 bc		
CV(%)	24.3	34.4	19.7	20.8	20.6	27.1	23.4	22.7	43.8		

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibrleón-2015; GI-16: Gibrleón-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016.

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Table 4.7. Comparison of means among the nine genotypes tested at the nine environments (ENV) for olive oil squalene (mg Kg⁻¹) on mid-October. Coefficient of variation (CV) are also indicated. Different letters for genotype (Mean₁), environment (Mean₂) and genotype-environment interaction (3) represent significant difference at p < 0.05 level based on Honestly-significant-difference (HSD).

Genotype	Squalene (mg Kg ⁻¹)									Mean ₁	CV(%)
	ENV										
	AN-15	AN-16	BA-16	GI-15	GI-16	TA-15	TA-16	UB-15	UB-16		
Arbequina	683 <i>no</i> 3	601 <i>no</i>	549 <i>o</i>	982 <i>nno</i>	993 <i>nno</i>	1139 <i>lnno</i>	1124 <i>lnno</i>	945 <i>mno</i>	950 <i>mno</i>	896 <i>d</i>	24.4
Arbosana	1485 <i>ijklmno</i>	1540 <i>ijklmno</i>			1190 <i>lnno</i>	1600 <i>ijklmno</i>	1376 <i>ijklmno</i>	1011 <i>mno</i>	1101 <i>lnno</i>	1302 <i>d</i>	17.6
Carrasqueño	3489 <i>abcdefg</i>	2388 <i>efghijklmn</i>	3538 <i>abcdefg</i>	5093 <i>a</i>	5055 <i>ab</i>		4416 <i>abc</i>	4174 <i>abcde</i>	3904 <i>abcdef</i>	4000 <i>a</i>	22.3
Frantoio	1246 <i>lnno</i>	1100 <i>lnno</i>		1330 <i>ijklmno</i>	1103 <i>lnno</i>			1322 <i>klmno</i>		1212 <i>d</i>	9.3
Koroneiki	1535 <i>ijklmno</i>	1745 <i>hijklmno</i>	1481 <i>ijklmno</i>	2634 <i>efghijkl</i>	2474 <i>efghijklmn</i>	2878 <i>cdefghijk</i>	2192 <i>ghijklmn</i>	2322 <i>fghijklmn</i>	2389 <i>efghijklmn</i>	2175 <i>c</i>	22.6
Ocal	2332 <i>fghijklmn</i>	3334 <i>cdefgh</i>		3883 <i>abcdef</i>	4629 <i>abc</i>			3445 <i>bcdefg</i>		3446 <i>b</i>	24.3
Picual	2532 <i>efghijklm</i>	3880 <i>abcdef</i>	3086 <i>cdefghij</i>	3613 <i>abcdefg</i>	4418 <i>abc</i>		3657 <i>abcdefg</i>	3682 <i>abcdefg</i>		3550 <i>ab</i>	16.8
Selection 1	3833 <i>abcdefg</i>	3075 <i>cdefghij</i>	2368 <i>efghijklmn</i>	3340 <i>cdefgh</i>	2980 <i>cdefghij</i>	4327 <i>abc</i>		3033 <i>cdefghij</i>	3220 <i>cdefghi</i>	3248 <i>b</i>	18.1
Selection 2	2704 <i>defghijkl</i>	3396 <i>cdefg</i>	2477 <i>efghijklmn</i>	3525 <i>abcdefg</i>	3988 <i>abcde</i>	4186 <i>abcd</i>		3609 <i>abcdefg</i>	3468 <i>bcdefg</i>	3460 <i>b</i>	16.7
Mean ₂	2168 <i>e</i>	2441 <i>cde</i>	2237 <i>de</i>	3103 <i>a</i>	2918 <i>ab</i>	2826 <i>abc</i>	2734 <i>abcd</i>	2556 <i>bcde</i>	2512 <i>bcde</i>		
CV(%)	48.5	55.1	48.6	43.7	55.4	51.5	52.5	48.9	49.7		

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibrleón-2015; GI-16: Gibrleón-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016.

The stability of the cultivars and breeding selections was very different for each minor component evaluated using AMMI models (Figure 4.3). For instance, ‘Arbequina’ was the most stable genotype for total tocopherol and squalene contents, while ‘Selection 2’ was the most stable for total phytosterol content. The lowest stability was observed for ‘Koroneiki’ (tocopherol content), ‘Arbosana’ (squalene content) and ‘Carrasqueño’ (phytosterol content). Again, some genotypes were specifically adapted to some environments according to the AMMI model, such as for instance ‘Selection 2’ showing high values for tocopherol content under BA-16 conditions.

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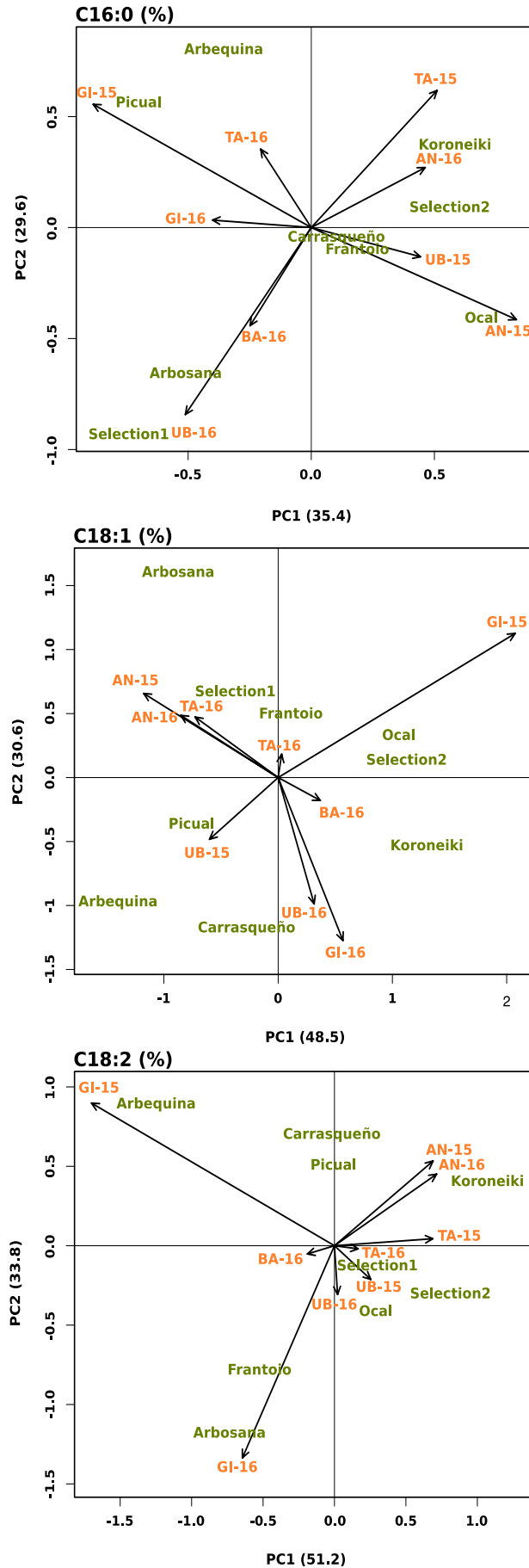


Figure 4.2. AMMI model biplot for IPC1 vs IPC2 of main three olive oil fatty acids (C16:0, C18:1 and C18:2) for nine olive genotypes evaluated in nine environments (AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016) on mid-October.

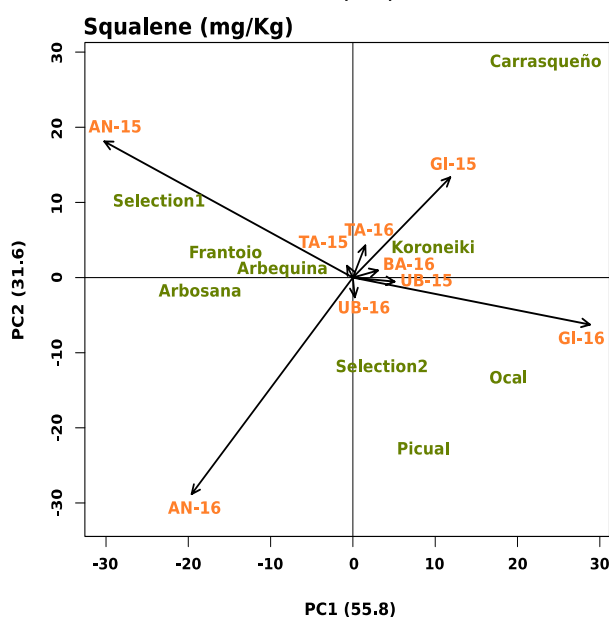
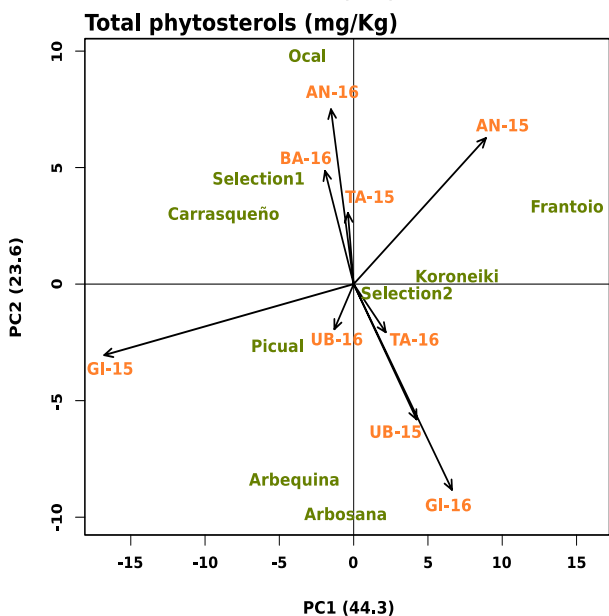
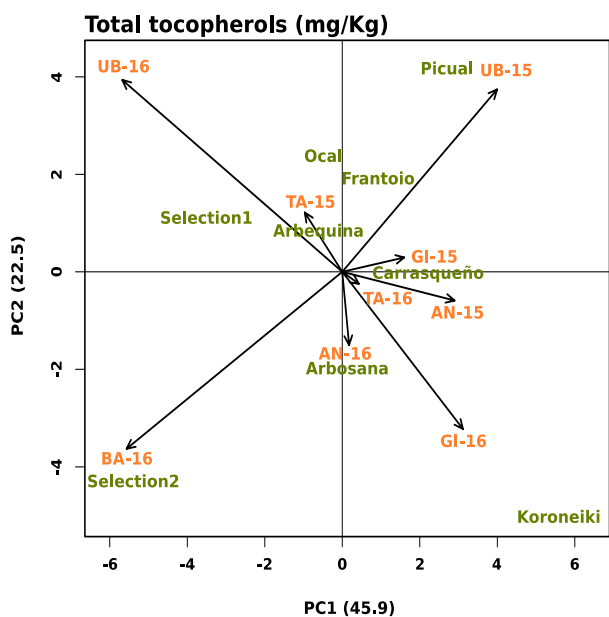


Figure 4.3. AMMI model biplot for IPC1 vs IPC2 of total tocopherols, total phytosterols and squalene in olive oil for nine olive genotypes evaluated in nine environments (AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016) on mid-October.

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Quality Components in Olive Tree*

*4.4.2 Relationship between Olive Oil Components and Discrimination between
Monovarietal Olive Oil.*

Pearson correlation coefficients were calculated between all the olive oil components under study (Table 4.8), including oil content, the percentage of individual fatty acids in the oil (C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3), minor components (total tocopherol content, percentage of α -tocopherol, total phytosterol content, percentages of β -sitosterol and Δ^5 -avenasterol, and squalene content). Highly significant correlations were found among olive oil components both of the same and different chemical family. Among components of the same chemical family, negative correlations of C18:1 with C16:0 and with C18:2 fatty acids were found, as well as the positive one between C16:0 and C18:2. Also, a negative correlation between C16:1 with 18:1 was showed. For tocopherols, a positive significant correlation between total tocopherol content and α -tocopherol was observed. Among phytosterols, the only high correlation was found between β -sitosterol and Δ -avenasterol being negative. When components of different chemical families were considered, the highest correlation coefficients were observed between C18:1 and both squalene (positive) and total phytosterol content (negative). Both C16:0 and C18:2 were negatively correlated with squalene and positively correlated with phytosterol content. A negative correlation between α -tocopherol and squalene, a negative correlation between oil content and both total tocopherol content and proportion of α -tocopherol, and a positive correlation between oil and squalene contents were also observed.

Table 4.8. Correlations among main olive oil components evaluated for genotype/environment on mid-October.

	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total tocopherols	α -tocopherol	Total phytosterols	β -sitosterol	Δ 5-avenasterol	Squalene	Oil content
C16:0	X	0.62	-0.38	-0.93	0.81	-0.02	0.24	0.44	0.61	-0.16	0.31	-0.59	-0.05
C16:1	<0.0001	X	-0.48	-0.52	0.38	-0.17	0.11	0.22	0.41	-0.03	0.14	-0.06	0.22
C18:0	0.0003	<0.0001	X	0.29	-0.32	0.21	-0.12	-0.21	-0.24	0.01	-0.08	0.15	-0.11
C18:1	<0.0001	<0.0001	0.0051	X	-0.97	-0.04	-0.21	-0.35	-0.62	0.16	-0.31	0.67	0.01
C18:2	<0.0001	0.0035	0.0045	<0.0001	X	0.05	0.13	0.28	0.56	-0.17	0.28	-0.69	0.02
C18:3	0.7973	0.1757	0.1237	0.7305	0.6126	X	0.42	0.15	0.01	-0.09	0.08	-0.16	-0.36
Total tocopherols	0.0504	0.3861	0.3429	0.1821	0.4437	0.0008	X	0.57	0.19	-0.22	0.23	-0.42	-0.51
α -tocopherol	0.0002	0.0362	0.0982	0.0037	0.0215	0.2365	<0.0001	X	0.14	-0.38	0.36	-0.51	-0.43
Total phytosterol	<0.0001	0.0002	0.0301	<0.0001	<0.0001	0.9869	0.1333	0.2265	X	0.04	0.20	0.0142	0.17
β -sitosterol	0.0636	0.6441	0.9366	0.1044	0.1635	0.1621	0.0069	0.0001	0.5056	X	-0.91	<0.0001	0.14
Δ 5-avenasterol	0.0007	0.2704	0.5159	0.0019	0.0069	0.2244	0.0068	0.0002	0.1037	<0.0001	X	<0.0001	-0.05
Squalene	<0.0001	0.7327	0.0829	<0.0001	<0.0001	0.1660	0.0003	0.0003	-0.27	0.36	-0.42	X	0.47
Oil content	0.7135	0.0822	0.3963	0.9861	0.8667	0.0561	<0.0001	<0.0001	0.0587	0.0452	0.2381	<0.0001	X

Pearson correlation coefficients and signification levels are indicated above and below the diagonal, respectively.

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The two first PCA components accounted for 60.4 % of total variability (Figure 4.4). The negative relationship of C16:0, C18:2 and total phytosterols with C18:1 was evident, as they were located in opposite quadrants. The loadings of the remaining oil components evaluated were located in the other quadrants of the plot defined by the first two PCA components: oil content, squalene and β -sitosterol in the first quadrant while total tocopherols, α -tocopherol and C18:3 were in the third quadrant. PCA plot also showed some grouping of the samples according to the different genotypes, although no clear separation by genotype at 0.95 significance was observed.

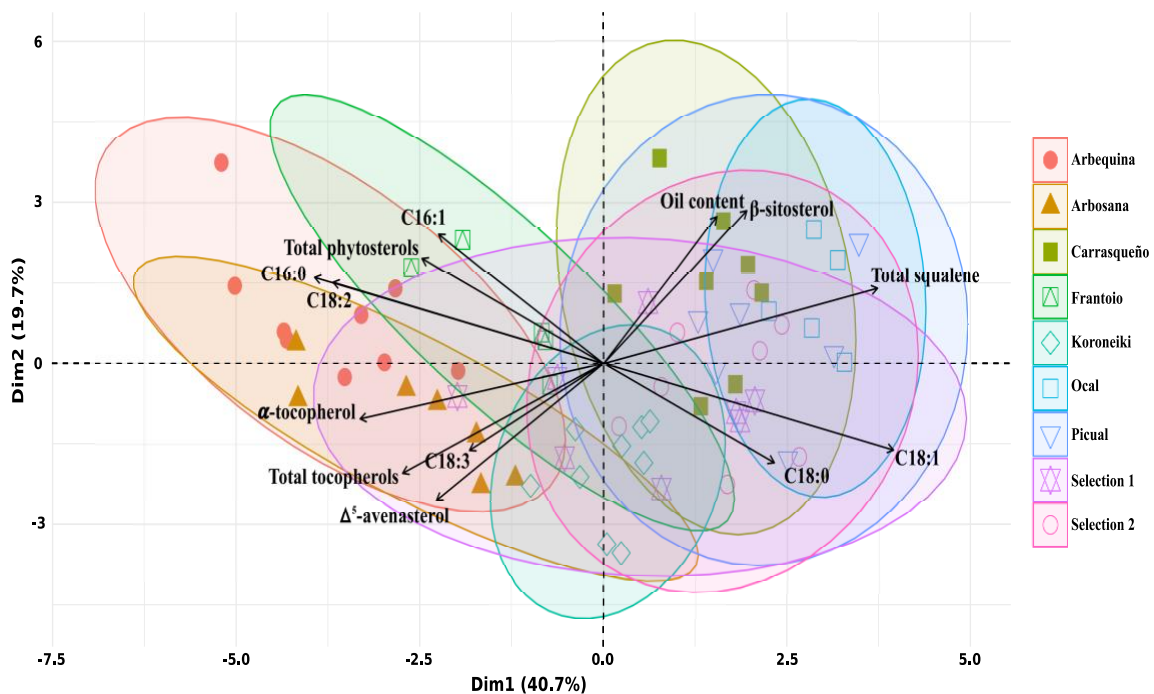


Figure 4.4. Principal components analysis (PCA) for fifteen olive oil components on nine genotypes evaluated in nine environments on mid-October. Confidence ellipses were performed around midpoint of each genotype.

Finally, linear discriminant analysis (LDA) was used to provide a clearer separation by genotype (Figure 4.5). The model showed a correct genotypic classification of 87.2 % the original grouped data and 85.6% for the repeated K-fold-CV (4 folds x 10 repeats leading to 40 randomly runs, with sensitivities ranging from 86% to 100%). The data of each genotype recorded on the different environments was clearly located in a specific region of the space described by the three linear discriminant functions using

these olive oil components. ‘Arbosana’, ‘Carrasqueño’, ‘Koroneiki’, ‘Picual’, ‘Selection 1’ and ‘Selection 2’ showed some data points mixed as having a similar oil composition. Contrarily, the data of ‘Ocal’, ‘Arbequina’ and ‘Frantoio’ coming from the different environments were closely plotted by cultivar and far from rest of genotypes.

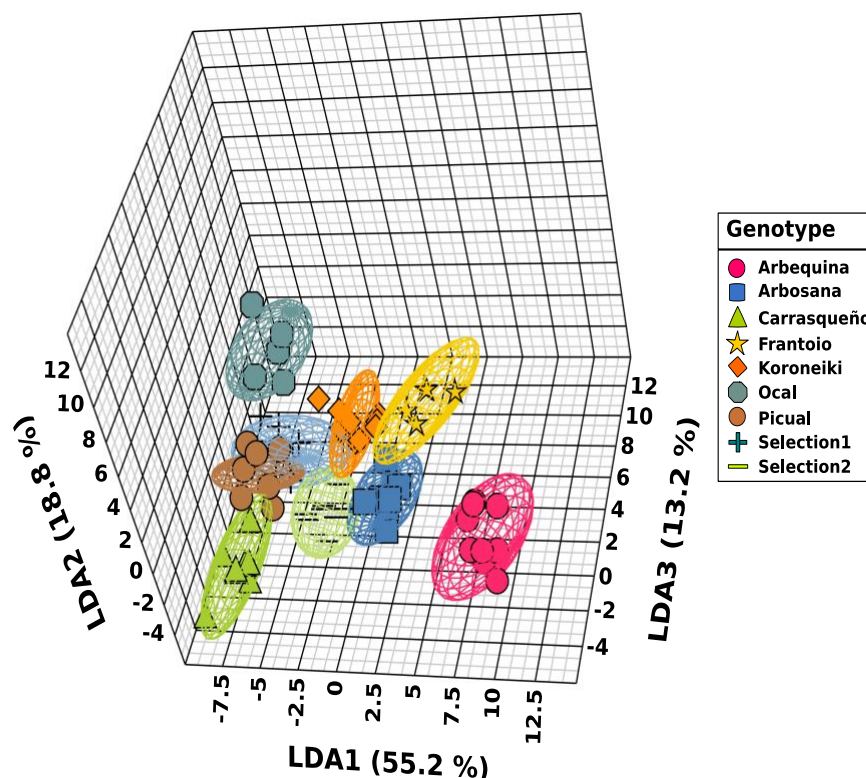


Figure 4.5. Linear discriminant analysis (LDA) using 3 linear discriminant functions (87.2 % of the variability explained) for the olive oil discrimination of nine genotypes, based on 13 olive oil components in nine environments on mid-October.

4.5. Discussion

Many works have described the healthy and nutritional properties of the extra virgin olive oil (EVOO), suggesting that it is one the main Mediterranean diet components contributing to increase life expectancy (Guo et al. 2018). These benefits are highly related to its particular oil composition (Perez-Jimenez et al. 2007; Estruch et al. 2018), mainly formed by monounsaturated fatty acid and a significant proportion of phenols, sterols, tocopherols and squalene. However, low-quality olive oil composition have been

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reported for some specific cultivar-environment combinations, some of them even being out of the limits set by International Olive Oil Council regulation for extra virgin olive oil (Mailer et al. 2010; Rondanini et al. 2011). In this sense, multi-environment cultivar trials, as the one here reported, offer the opportunity to test the relative influence of genotype, environment and their interaction on olive oil components, scarcely studied until now in olive. They can be also used to determine the stability and adaptability for specific genotype.

4.5.1. Variability Factors for Olive Oil Quality

Considering a multi-environment genotype data set, the genetic effect was the main source of variation for all the oil components considered in this study. This result revealed a consistent and strong genotype influence on oil components, which is in agreement with previous studies based on single-location trials (Salazar-García et al. 2018; Leon et al. 2008; Uceda et al. 2008; Rotondi et al. 2010; Beltran et al. 2016). In particular, the three main olive fatty acids (C18:1, C18:2 and C16:0), the percentage of α -tocopherol and the squalene content showed very high genetic variance. The evaluation here performed confirmed, independently of the cultivated environment, the high percentage of C18:1 and squalene content and low percentage of C16:0 and 18:2 of ‘Picual’ and the opposite behaviour of ‘Arbequina’, well known from previous studies (Leon et al. 2011; Fernandez-Cuesta et al. 2013). Interestingly, the two breeding selections tested showed quite similar fatty acid composition than ‘Picual’, that is very convenient for the oil stability and healthy properties (Aparicio et al. 1999). In particular, ‘Selection 1’ also showed high content in squalene and total tocopherols, being a breeding selection with high nutritional values. For total phytosterols, ‘Arbosana’, ‘Frantoio’ and ‘Arbequina’ were the cultivars with higher values. Contrarily to these oil components, oil content on dry weight showed its main variance source in the environment, indicating that the oil accumulation depends mostly of its surrounding. Similar result was showed by Navas et al. (2019) on the date of maximum oil content reached, where was suggested that the cultivars cultivated in a given environment could be harvested at same optimal time.

The significant genetic influence on oil components was also consistently supported using principal components analysis (PCA) and linear discriminant analysis (LDA). Both analyses have already been used in single-location trials to discriminate commercial cultivars (Salazar-García et al. 2018; Wang et al. 2018) and advanced breeding genotypes (Leon et al. 2011) from oil components obtaining a significant separation between them. In our case, some overlapping among genotypes was observed from these analyses, due to the variability caused by the different environments tested. Only ‘Arbequina’ and ‘Frantoio’ were largely distinguished from LDA, probably due to their high C16:0, C18:2 and total phytosterols values, and low C18:1. Both monovarietal oils were already grouped together by presenting similar oil composition (Leon et al. 2011), with low C18:1 and high C18:2.

Even so, the environment and genotype by environment interaction (GEI) also were highly significant in all components evaluated. Environmental influence of olive oil components has been reported for single cultivars grown in different locations as the case of ‘Arbequina’ (Mailer et al. 2010; Borges et al. 2017) and ‘Arauco’ (Garcia-Inza et al. 2014). This influence has been also found at gene expression level (Hernandez et al. 2019). In this sense, the predicted climate warming, that could specially affect the Mediterranean climate (Lionello 2012), could greatly affect the olive oil composition and, therefore, its quality.

The significant GEI identified in this research for olive oil quality traits suggested a differential performance of cultivars under different environments, as previously reported for flowering and fruit traits (Navas-Lopez et al. 2019b; Navas-Lopez et al. 2019a). This emphasizes the necessity of local evaluation of olive cultivars to determine those better adapted to a given environmental conditions. Moreover, extrapolation of results from a set of cultivars in a given environment to a different one should be avoided. In fact, for some genotype-environment combinations here reported, the content of some oil components are close to the limits established by market regulations: for instance, low values of C18:1 for ‘Arbequina’ under GI-15 environmental conditions (61 %) are close to the minimum values (55 %) imposed by International Olive Council for Olive Oil.

Into this significant GEI, some cultivars showed higher stability across environments than others. Environmental stability could be evaluated by AMMI models,

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previously used for olive studies (Hassani et al. 2018; Ebrahimi et al. 2016). In our case, AMMI model showed that ‘Arbequina’ has low stability for most of the oil components except for tocopherols and squalene. On the contrary, ‘Selection 2’ showed intermediate-high stability for all the oil components except for total tocopherols. This is very interesting since, in breeding programs, high stability among environments of the characters under selection is crucial for the new selections to be considered as new cultivars (Janick and Moore. 1996)

4.5.2. Relationships between Olive Oil Components

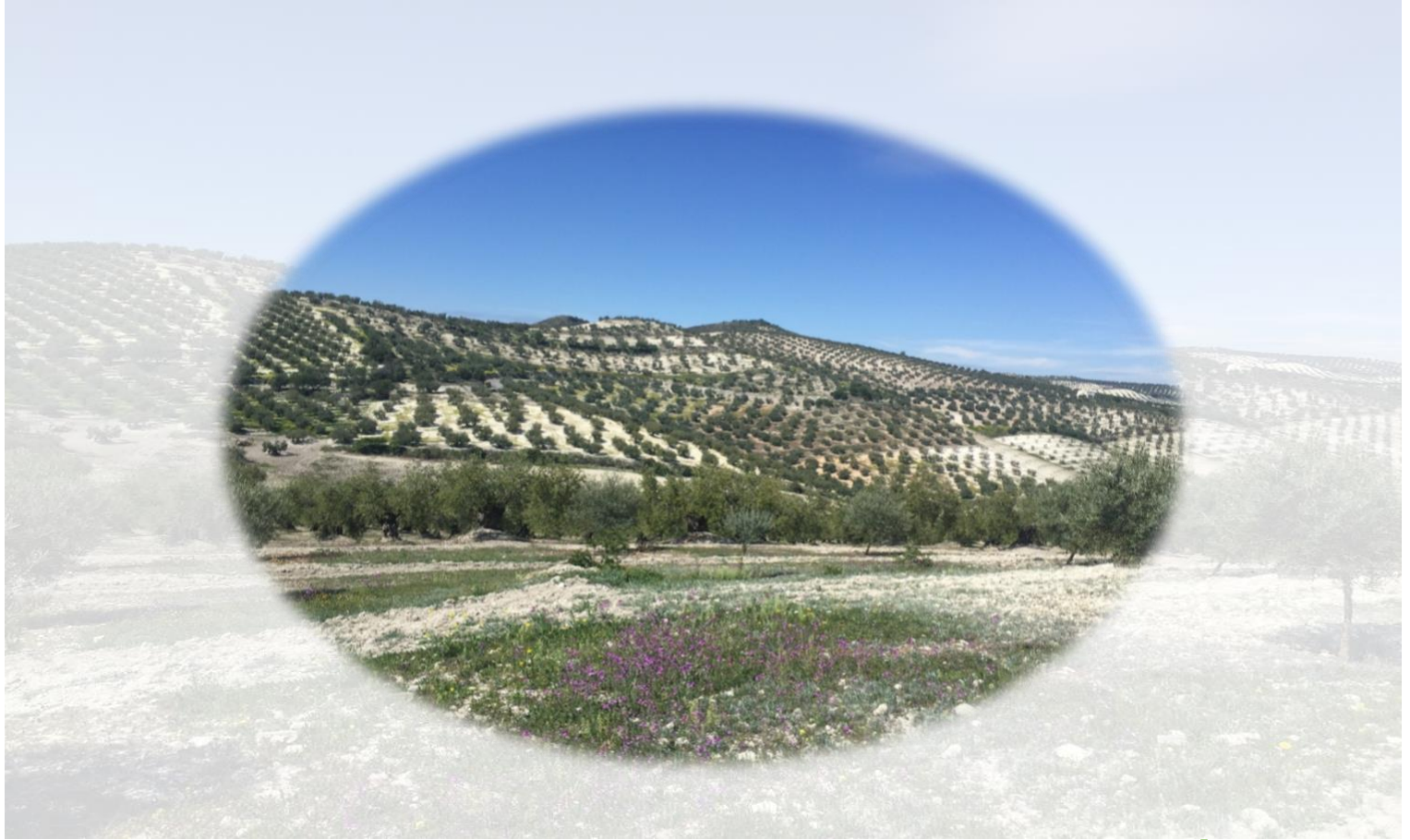
As expected, significant negative correlation between components from the same chemical family were showed for C16:0 and C18:1, C18:1 and C18:2 or β -sitosterol and Δ 5-avenasterol. Such correlations were already reported for olive (de la Rosa et al. 2016; Fernandez-Cuesta et al. 2012) remarking the existing interrelationships between these components of the same biosynthetic pathway. Besides, significant correlations were found among some fatty acids with squalene and total phytosterols. Correlation between different chemical families could suggest a genetic link between biosynthetic pathways for olive oil as it was already proposed in some specific vegetable oils (KamalEldin and Andersson 1997). Also, oil content was found to be correlated with some minor oil constituents such as squalene and total tocopherols. This last correlation, between oil content and total tocopherols, was previously observed in sunflower (Nolasco et al. 2004) with reduction of total tocopherols and increase of oil content. However, those correlations between different chemical family components on olive oil have not been found in previous reports using single locations (de la Rosa et al. 2016; Leon et al. 2011).

4.6. Conclusions

Genetic effect was the main variance contributor both the main fatty acids (C16:0, C18:1 and C18:2) and the principal minor components (total tocopherols, total phytosterols and squalene). Even so, the significant contribution found for environment and genotype by environment interaction indicates that the use of multi-environment cultivar trials is very convenient to select the optimal cultivars for a given environment and even better, cultivars with stable behaviour across environments.

Acknowledgements

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Capítulo 5:

Conclusiones Generales

1. El uso de ensayos multi-ambiente y multi-genotipo han mostrado una gran utilidad para cuantificar eficazmente la contribución genética, ambiental y su interacción en los principales caracteres de interés del cultivo del olivo (Capítulo 2 al capítulo 4).
2. La fenología de floración ha estado influenciada principalmente por el ambiente, que mostró un adelanto considerable de la floración en ambientes con inviernos suaves. La limitada variabilidad entre genotipos indica una gran dificultad para obtener nuevas variedades con floración precoz desde los programas de mejora (Capítulo 2).
3. Todos los parámetros de calidad de flor, excepto número de flores por inflorescencia, mostraron una alta contribución del genotipo. Entre los genotipos evaluados, 'Arbequina' fue la variedad que obtuvo mejores caracteres de calidad de flor en todos los ambientes (Capítulo 2).
4. Los tres factores analizados (genotipo, ambiente y su interacción) contribuyeron de manera similar en la varianza para contenido máximo de aceite en peso seco. Esto indica un riesgo potencial de reducción para este carácter asociado tanto a las plantaciones en nuevas áreas de cultivo como a los efectos ambientales potenciales que pueda ocasionar el cambio climático en las áreas tradicionales de cultivo (Capítulo 3).
5. El ambiente mostró la mayor contribución sobre la pendiente de acumulación de aceite y fue el único factor significativo para la fecha de obtención del máximo aceite acumulado en peso seco del fruto. Por tanto, considerando el momento de máxima acumulación de aceite, el grupo de variedades y genotipos aquí evaluados podrían cosecharse en épocas similares, variables en función del ambiente cultivado (Capítulo 3).
6. Se observó una alta correlación negativa del máximo aceite acumulado con la temperatura del aire durante los meses verano, inicio de la lipogénesis. Sin embargo, esta influencia no ocurre por igual en todos los genotipos, siendo, por ejemplo, más fuerte en 'Koroneiki' que en 'Arbequina' (Capítulo 3).
7. El genotipo fue la principal fuente de variación tanto en los principales ácidos grasos como en los componentes menores del aceite. No obstante, la interacción genotipo x ambiente también fue significativa, por lo que parte de la variabilidad explicada

dependerá de donde esté plantada una variedad. Ello debe ser considerado a la hora de la elección varietal ya que podría tener incluso importantes connotaciones comerciales (Capítulo 4).

8. Se han observado las correlaciones esperadas entre componentes de la misma ruta bioquímica como ácido oleico y ácido linoleico o ácido palmítico y ácido linoleico, con una relación negativa y positiva, respectivamente. Además, también se han encontrado otras correlaciones significativas entre componentes de diferentes rutas bioquímicas como fue el caso del ácido oleico con la cantidad de escualeno y fitosteroles totales en el aceite. Estas relaciones encontradas podrían facilitar los criterios de selección de variedades con una composición específica en el aceite, uno de los factores principales a tener en cuenta en el mercado mundial del aceite de oliva virgen extra (Capítulo 4).

9. La influencia de la interacción genotipo x ambiente mostrada en los principales caracteres agronómicos del olivo debe ser considerada en el diseño del protocolo de actuación en las etapas intermedias y finales de los programas de mejora. Es decir, además de seleccionar los genotipos por presentar caracteres óptimos, habrá también que considerar su estabilidad entre ambientes. El empleo de ensayos multi-ambiente en etapas intermedias del programa de mejora se debe evaluar en futuros estudios (Capítulo 2 al capítulo 4).

10. Los resultados que derivan de esta Tesis Doctoral ponen de manifiesto la importancia de la investigación a nivel regional y local del comportamiento agronómico para cada variedad, ya que se verán afectadas de manera diferencial dependiendo del ambiente. Por lo tanto, la continuación de futuros trabajos utilizando ensayos multi-ambiente con un grupo más amplio de variedades continúa siendo de vital importancia para una correcta recomendación varietal, en particular considerando los nuevos sistemas de cultivo que se están desarrollando (Capítulo 2 al capítulo 4).

Analysis of multi-environment trials (MET) in the sugarcane breeding program of Réunion Island

Saïa Guilly · Thomas Dumont · Audrey Thang-Chan · Laurent Hervé · Jean-Yves Huerne

Saïa Guilly · Thomas Dumont · Audrey Thang-Chan · Laurent Hervé · Jean-Yves Huerne

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Abstract The sugarcane variety development program in Réunion Island is conducted in multi-environment nurseries (MNs) distributed across the island. The objective of this study was to assess the impact of the multi-environment trials (MET) on the selection of superior genotypes. The analysis of MET was conducted using a mixed-effects model (MEM) and an economic index (EI). The results showed that the best genotypes were selected in the MET compared to the MN. The MEM and EI allowed the selection of superior genotypes with high yield and low input requirements.

Keywords Sugarcane · Multi-environment trials (MET) · Genotype × location (GL) interaction · Genotype main effect plus genotype × environment (GGE) model

Introduction Sugarcane (*Saccharum spp.*) is the world's most important biomass-producing crop. It is a tall, perennial, evergreen grass that is cultivated in tropical and subtropical regions. The breeding program for the cane industry aims to select superior genotypes with high yield and low input requirements. Multi-environment trials (MET) are conducted to evaluate the performance of genotypes across different environments. The analysis of MET is essential for the selection of superior genotypes that perform well in all environments.

The Influence of Growing Region on the Diversity of Australian Cattle

Robert J. Miller · James John · Kerrie Cookson · Steve Capstick · Emma Hume

Robert J. Miller · James John · Kerrie Cookson · Steve Capstick · Emma Hume

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Abstract The quality and diversity of cattle are important for the Australian beef and dairy industries. The objective of this study was to assess the impact of the growing region on the diversity of Australian cattle. The analysis was conducted using a mixed-effects model (MEM) and an economic index (EI). The results showed that the best genotypes were selected in the MET compared to the MN. The MEM and EI allowed the selection of superior genotypes with high yield and low input requirements.

Keywords Cattle · Genotype × environment (GE) interaction · Genotype main effect plus genotype × environment (GGE) model

Optimal spatial and temporal replications for reducing environmental variation for oil content components and fruit morphology traits in olive breeding

Lorena León · María José Ceballos · Raúl de la Haza · José María Martínez · Enrique Ceballos

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Abstract The need for new improved cultivars adapted to modern cultivation techniques and changing climatic conditions requires precise studies to provide a better understanding of the genotype × environment interaction. In general, the design of the experiment is crucial for the success of the study. The objective of this study was to assess the impact of the growing region on the diversity of olive breeding. The analysis was conducted using a mixed-effects model (MEM) and an economic index (EI). The results showed that the best genotypes were selected in the MET compared to the MN. The MEM and EI allowed the selection of superior genotypes with high yield and low input requirements.

Keywords Olive · Genotype × environment (GE) interaction · Genotype main effect plus genotype × environment (GGE) model

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