Departamento de Biotecnología

Prospección, recuperación, selección y pre-domesticación de plantas autóctonas con alto potencial funcional

Tesis doctoral

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Valencia, octubre de 2019

Resumen

Las sociedades modernas, conscientes de la relación que hay entre dieta y salud, han aumentado en las últimas décadas la demanda de alimentos funcionales. En estas sociedades, además, se ha promovido la alimentación como una experiencia gastronómica. Surge así una oportunidad de revalorizar el uso de plantas silvestres que habían caído en desuso, mediante la domesticación y adaptación a cultivo. Algunas de estas especies, además de incorporar aromas y sabores distintos a los productos actualmente comercializados, son percibidas como beneficiosas para la salud, factor que a nivel científico puede corresponder con la acumulación de compuestos bioactivos.

La presente Tesis se desarrolla como un trabajo de evaluación y pre-domesticación de dos especies de amplia distribución en nuestra región, destacadas por un alto potencial funcional y calidad organoléptica. El objetivo es, por un lado, profundizar en el conocimiento de estos dos componentes de la calidad; y por otra parte, establecer una base para los programas actuales y futuros de domesticación y adaptación a cultivo. En este sentido, el uso de material autóctono puede suponer una ventaja, considerando el proceso de selección natural que han experimentado estos materiales como consecuencia de su desarrollo bajo condiciones climáticas determinadas.

La primera parte de esta Tesis Doctoral se centra en el potencial de la berraza para constituir un nuevo cultivo. Hasta la fecha se había descrito el alto potencial antioxidante de la berraza en términos de contenido en fenoles totales y capacidad reductora de radicales libres, a partir de dos poblaciones silvestres. Nuestros resultados ampliando el número de muestras no sólo confirman dicho potencial, sino que establecen además una clara correlación entre ambos caracteres. Se sugiere así que la capacidad reductora de la berraza está principalmente definida por la acumulación de compuestos fenólicos, especialmente derivados de la quercetina tal y como revela el estudio del perfil fenólico. Por otro lado, el perfil volátil revela una prevalencia de compuestos terpenoides y fenilpropanoides. Este perfil resulta único en comparación con diversas especies cultivadas tomadas como

referencia; se identifican, sin embargo, ciertas similitudes que podrían explicar la relación de aroma y sabor que se puede establecer entre dichas especies. Pese a su interés funcional y aromático, la adaptación a cultivo convencional resulta poco prometedora. Queda no obstante la vía abierta a nuevos trabajos de adaptación a cultivo hidropónico como alternativa prometedora.

La segunda parte de la Tesis está orientada al estudio de la rabaniza. Debido a la latencia secundaria determinada en su semilla, se hace necesario en primer lugar establecer un tratamiento efectivo que permita obtener una germinación elevada y uniforme. A partir de los resultados se sugiere un uso combinado de hipoclorito de sodio como escarificante y ácido giberélico. Este tratamiento es adecuado para facilitar los programas de mejora, y puede ser además adaptado para su aplicación comercial a gran escala.

Los resultados de calidad funcional destacan especialmente la acumulación de vitamina C, encontrada principalmente en su forma reducida que es además la responsable del poder antioxidante de este compuesto. En su perfil de glucosinolatos destaca la sinigrina, de potencial funcional y responsable además en gran medida de su aroma; los resultados sugieren, no obstante, la síntesis de otros glucosinolatos. Es, por el contrario, acumuladora de nitratos como compuesto antinutriente, factor que va a ser determinante en las prácticas que se empleen para su futuro cultivo.

Resulta necesaria además la caracterización de los materiales disponibles de rabaniza para identificar rasgos de interés a nivel morfológico y/o agronómico. Se ha registrado una moderada variabilidad morfológica. Esto tiene claras implicaciones para los programas de mejora actuales y futuros, limitando el número de variedades comerciales a desarrollar. Por el contrario, el estudio de aceptación por consumidores potenciales sugiere que se pueden desarrollar distintos productos comerciales, desde germinados a brotes tiernos, llegando a distintos nichos de mercado y aumentando, en definitiva, la oferta.

Finalmente, la adaptación a cultivo se ha hecho mediante la evaluación de dos sistemas modelo, campo e invernadero, evaluando además el comportamiento en diversos ciclos. La producción en campo aumenta

tanto la calidad visual, principalmente en términos de morfología de hoja y coloración, como la calidad funcional, incrementando el contenido en vitamina C y compuestos fenólicos y reduciendo la acumulación de nitratos. Sugerimos pues la producción comercial futura bajo estas condiciones. No obstante, el efecto excesivo de condiciones ambientales desfavorables en los meses más fríos abre la vía a nuevos estudios para mejorar la calidad durante esos periodos.

En definitiva, los trabajos realizados en esta Tesis aumentan, por un lado, el conocimiento de las plantas silvestres utilizadas desde un punto de vista morfológico, agronómico, nutracéutico y de perfil volátil. Suponen además una base para el futuro establecimiento de estas especies como nuevos cultivos adaptados a las regiones mediterráneas, identificando puntos clave para su domesticación y adaptación a sistemas agrícolas.

Resum

Les societats modernes, conscients de la relació existent entre dieta i salut, han augmentat en les últimes dècades la demanda d'aliments funcionals. En aquestes societats, a més, s'ha promogut l'alimentació com una experiència gastronòmica. Sorgeix així una oportunitat de revaloritzar l'ús de plantes silvestres que havien caigut en desús, mitjançant la domesticació i adaptació a cultiu. Algunes d'aquestes espècies, a més d'incorporar aromes i sabors diferents als productes actualment comercialitzats, són percebudes com a beneficioses per a la salut, factor que a nivell científic pot correspondre amb l'acumulació de compostos bioactius.

La present Tesi es desenvolupa com un treball d'avaluació i pre-domesticació de dues espècies d'àmplia distribució a la nostra regió, destacades per un alt potencial funcional i qualitat organolèptica. L'objectiu és, d'una banda, aprofundir en el coneixement d'aquests dos components de la qualitat; i d'altra banda, establir una base per als programes actuals i futurs de domesticació i adaptació a cultiu. En aquest sentit, l'ús de material autòcton pot suposar un avantatge, considerant el procés de selecció natural que han experimentat aquests materials com a conseqüència del seu desenvolupament sota condicions climàtiques determinades.

La primera part d'aquesta Tesi Doctoral se centra en el potencial del creixen (de sèquia) per a constituir un nou cultiu. Fins hui s'havia descrit l'alt potencial antioxidant del creixen en termes de contingut en fenols totals i capacitat reductora de radicals lliures, a partir de dues poblacions silvestres. Els nostres resultats ampliant el nombre de mostres no solament confirmen aquest potencial, sinó que estableixen a més una clara correlació entre els dos caràcters. Se suggereix així que la capacitat reductora del creixen està principalment definida per l'acumulació de compostos fenòlics, especialment derivats de la quercetina tal com revela l'estudi del perfil fenòlic. D'altra banda, el perfil volàtil revela una prevalença de compostos terpenoids i fenilpropanoids. Aquest perfil resulta únic en comparació amb diverses espècies cultivades utilitzades com a referència; s'identifiquen, no obstant això, certes similituds que podrien explicar la relació d'aroma i sabor que es pot establir entre aquestes espècies. Malgrat el seu interés funcional i

aromàtic, l'adaptació a cultiu convencional és poc prometedora. No obstant això, queda oberta la via a nous treballs d'adaptació a cultiu hidropònic com a alternativa prometedora.

La segona part de la Tesi està orientada a l'estudi de la ravenissa. A causa de la latència secundària determinada en la seua llavor, es fa necessari en primer lloc establir un tractament efectiu que permeta obtindre una germinació elevada i uniforme. A partir dels resultats se suggereix un ús combinat d'hipoclorit de sodi com escarificant i àcid giberèlic. Aquest tractament és adequat per a facilitar els programes de millora, i pot ser a més adaptat per a la seua aplicació comercial a gran escala.

Els resultats de qualitat funcional destaquen especialment l'acumulació de vitamina C, trobada principalment en la seua forma reduïda que és a més la responsable del poder antioxidant d'aquest compost. En el seu perfil de glucosinolats destaca la sinigrina, de potencial funcional i responsable a més en gran manera de la seua aroma; els resultats suggereixen, no obstant això, la síntesi d'uns altres glucosinolats. És, per contra, acumuladora de nitrats com a compost antinutrient, factor que serà determinant en les pràctiques que s'empren per al seu futur cultiu.

Resulta necessària a més la caracterització dels materials disponibles de ravenissa per a identificar caràcters d'interés a nivell morfològic i/o agronòmic. S'ha registrat una moderada variabilitat morfològica. Això té clares implicacions per als programes de millora actuals i futurs, limitant el nombre de varietats comercials a desenvolupar. Per contra, l'estudi d'acceptació per consumidors potencials suggereix que es poden desenvolupar diferents productes comercials, des de germinats a brots tendres, arribant a diferents mercats i augmentant, en definitiva, l'oferta.

Finalment, l'adaptació a cultiu ha requerit l'avaluació de dos sistemes model, camp i hivernacle, així com el comportament en diversos cicles. La producció en camp augmenta tant la qualitat visual, principalment en termes de morfologia de fulla i coloració, com la qualitat funcional, augmentant el contingut en vitamina C i compostos fenòlics i reduint l'acumulació de nitrats. Suggerim doncs la producció comercial futura sota aquestes condicions. No obstant això, l'efecte excessiu de condicions ambientals

desfavorables en els mesos més freds obri la via a nous estudis per a millorar la qualitat durant aqueixos períodes.

En definitiva, els treballs realitzats en aquesta Tesi augmenten, d'una banda, el coneixement de les plantes silvestres utilitzades des d'un punt de vista morfològic, agronòmic, nutricèutic i de perfil volàtil. Suposen a més una base per al futur establiment d'aquestes espècies com a nous cultius adaptats a les regions mediterrànies, identificant punts clau per a la seua domesticació i adaptació a sistemes agrícoles.

Abstract

Modern societies are greatly conscious of the relationship between diet and health. Thus, demands on functional foods have increased during the last decades. In addition, considering food as a gastronomic experience is also promoted in these societies. These two key points can encourage the revalorization of wild plants by means of the domestication and adaptation to crop systems. Some of these species, apart from providing new aromas and tastes that are not currently found in markets, are also perceived as beneficial for health. The perceived benefits of wild plants can scientifically correspond to the accumulation of bioactive compounds.

The current Doctoral Thesis is conceived as a work for the evaluation and pre-domestication of two wild species broadly found in our region. These species highlight for their functional potential and their organoleptic quality. The aim of this Thesis is, on one hand, to increase the knowledge on these two components of quality; and, on the other hand, to establish a basis for the current and future domestication and crop adaptation programs. In this sense, the use of indigenous material can be advantageous, especially considering the natural selection process that has occurred in these materials as consequence of the development under specific climatic conditions.

The first part of this Doctoral Thesis is focused on analyzing the potential of water celery as new crop. Previous authors have described high antioxidant properties for this species in terms of content in total phenolics and free radical scavenging capacity, but using only two populations. Our work with a larger quantity of materials confirms this potential, and also establishes a clear correlation between both traits. According to these results, we suggest that the free radical scavenging capacity is mainly due to the accumulation of phenolic compounds, mainly quercetin derived compounds as found in the analysis of the phenolic profile. On the other hand, results show the importance of terpenoid compounds and phenylpropanoids in describing the volatile profile of the species. Such profile is unique compared to other cultivated species that have been evaluated as reference. However, there are some similarities that may be responsible of the resemblances among those species. Despite the functional and aromatic

interest of water celery, its adaptation into conventional crop systems is not greatly promising. On the contrary, we suggest that future works could consider the cultivation under hydroponic systems as a promising alternative.

The second part of this Thesis is focused on the study of wall rocket. Mature seeds in this species display secondary dormancy. Thus, in a first step, it is required to establish an adequate treatment that allows a high and uniform germination. According to our results, we suggest the synergistic use of sodium hypochlorite as scarification agent together with gibberellic acid. This treatment can be successfully in breeding programs, and may be also adapted for commercial application in a productive scale.

Results of the functional quality on this species highlight the accumulation of vitamin C, mainly found in its reduced form. This form is in fact responsible of the antioxidant power of vitamin C. Regarding the glucosinolates profile, sinigrin is found as the main compound. Sinigrin has high functional potential and is in addition great responsible of the aroma in wall rocket. Results suggest, however, the biosynthesis of other glucosinolates. On the other hand, wall rocket accumulates high levels of nitrates as antinutrient compounds. This trait is a critical determinant for the cultivation practices that may be used for future commercial production.

In addition, it is necessary to characterize the available materials of wall rocket with the aim of identifying morphologic and/or agronomic interesting traits. We have found a moderate morphological variability among materials. These results have clear implications for the current and future breeding programs, and may restrict the quantity of commercial cultivars that could be obtained. On the contrary, the study of consumers' acceptance suggests that several products of wall rocket may be commercially exploited, including microgreens and baby-leaf crops. The development of different commercial products can increase the market opportunities.

Finally, adaptation into cultivation systems has required the evaluation of two model systems, field and greenhouse, as well as the behavior of plants under different growing cycles. The field production increases the visual

quality mainly in terms of morphology and color of leaves. This system also increases the functional quality by enhancement of vitamin C and total phenolic contents and decreasing at the same time the accumulation of nitrates. Thus, we suggest that commercial production of wall rocket should be performed under field conditions. Nevertheless, increasing the quality during the coldest months, where highly unfavorable conditions can be registered, is still an area of study.

In summary, the works performed during this Doctoral Thesis increase the knowledge on the wild plants used from a morphologic, agronomic, nutraceutical and volatile point of view. In addition, these works are a basis for the future establishment of these species as new crops adapted to the Mediterranean regions, with the identification of key points for domestication and crop adaptation.

Índice

Introducción

1. Uso de las plantas silvestres en la alimentación: necesidad y tradición

La domesticación de plantas y animales es uno de los procesos más importantes que el ser humano ha llevado a cabo durante su Historia. A la domesticación inicial ha seguido, además, un proceso de mejora genética, más o menos continuo, de aquellas especies de mayor importancia para su adaptación a nuevos requerimientos. En el caso de las especies vegetales, como consecuencia de esta domesticación y mejora selectivas se ha establecido un número limitado de cultivos para alimentación. Así, menos de 200 especies vegetales son explotadas actualmente a nivel comercial con producción significativa, de los cuales únicamente nueve (arroz, maíz, trigo, patata, soja, caña de azúcar, remolacha azucarera, palma de aceite y yuca) suman el 66% de la producción agrícola global (FAO, 2019). Ésta es, sin embargo, una pequeña porción del total de especies comestibles que se pueden considerar como parte de la alimentación humana (Fig. 1). De hecho, se estima que de entre las aproximadamente 300.000–400.000 especies de plantas vasculares que existen en el mundo, más de 30.000 serían comestibles (Garn y Leonard, 1989; Bacchetta *et al*., 2016). Y de éstas, en torno a 6.000–7.000 especies se han utilizado en algún momento de la historia del hombre como alimento (Thakur *et al*., 2017; FAO, 2019).

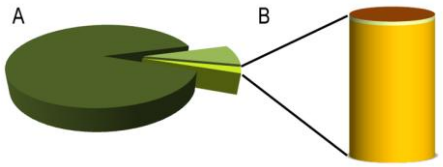

Fig.1. Representación del estado del mundo vegetal en la alimentación. A) Porcentaje de especies utilizadas como alimento en la historia de la humanidad ( ) y otras especies que se estiman adecuadas para el consumo ( ). B) Porcentaje de especies establecidas en la actualidad como cultivo con producción significativa ( ) y cultivos de mayor interés (> 60% de la producción agrícola,). Referencias: Bacchetta *et al*., 2016; Thakur *et al*., 2017; FAO, 2019.

Un dato clave de entre los referidos anteriormente es el número de especies que el ser humano ha utilizado en algún momento de su Historia con fines alimentarios. Este grupo incluye, además de los cultivos actuales (modernos y tradicionales), aquéllos infrautilizados o abandonados, así como plantas silvestres comestibles. Con el término 'plantas silvestres comestibles' o 'WEPs' (del inglés 'wild edible plants') se hace referencia de forma general a aquéllas que crecen de forma espontánea en ambientes naturales o semi-naturales, que son adecuadas para la alimentación humana y se recolectan directamente de estos ambientes para su consumo (Carvalho y Barata, 2017). El término WEP engloba desde plantas silvestres en sentido estricto hasta aquéllas arvenses y ruderales –crecen en ambientes altamente modificados pero no requieren de la actuación del hombre para su supervivencia–, e incluso plantas semi-domesticadas que, siendo silvestres, se han manejado como cultivo (Tabuti, 2007; FAO, 2019).

La recolección y consumo de WEPs es una actividad que el ser humano ha desarrollado durante toda su Historia, desde su etapa como cazador-recolector hasta la era contemporánea. Así lo recogen, por ejemplo, Torija-Isasa y Matallana-González (2016) en su revisión centrada en el área mediterránea. Aunque ha habido una clara decadencia en esta práctica, la FAO estima que en torno a mil millones de personas en todo el mundo seguiría recurriendo al consumo de plantas silvestres como complemento a la dieta en las últimas décadas (Bharucha y Pretty, 2010), especialmente entre comunidades rurales e indígenas (Grivetti y Ogle, 2000; Carvalho y Barata, 2017). En el caso específico de la Unión Europea, este organismo estima que alrededor del 20% de la población seguiría consumiendo WEPs (Bacchetta *et al*., 2016). Su uso en la alimentación humana atiende a tres motivos principales: a) como complemento a la dieta en dietas con carencias; b) como alimentos funcionales y remedios naturales en la medicina tradicional; y c) para enriquecer dietas aportando sabores distintos.

No resulta fácil hacer una separación clara de los motivos que motivan el consumo de una planta en concreto, puesto que el interés en las diversas especies puede atender a una o varias razones. Por ejemplo, la malva (*Malva sylvestris* L.) es un alimento que aporta micronutrientes como diversas vitaminas y ácido α-linoleico, es rica en compuestos antioxidantes –ha sido

considera durante generaciones como una planta de gran poder medicinal– y cuyas semillas dulces se usan en la elaboración de pan y queso (Guarrera y Savo, 2013, 2016). No obstante, el análisis del uso de las WEPs desde las distintas perspectivas permite entender mejor su importancia tradicional y actual.

1.1. Complemento a la dieta en situaciones de carencia

Las WEPs se consideran importantes desde un punto de vista nutricional por el contenido en micronutrientes que acumulan sus órganos comestibles (Grivetti y Ogle, 2000; Salvatore *et al*., 2005; FAO, 2017; Pinela *et al*., 2017a; Shin *et al*., 2018), contenidos que pueden superar incluso los niveles en cultivos establecidos (Flyman y Afolayan, 2006; Bacchetta *et al*., 2016). En este sentido, su uso puede contribuir a la alimentación doméstica en dietas empobrecidas, salvaguardando la seguridad alimentaria cuando ésta no se puede satisfacer con los cultivos establecidos. En particular, el aporte extra de micronutrientes obtenido de plantas silvestres ayudaría a limitar el riesgo de 'hambre encubierta' ('hidden hunger'), ello es, dietas con deficiencias en micronutrientes como minerales y vitaminas esenciales que conllevan a diversos problemas de salud (Kennedy *et al.*, 2003). Por ejemplo, el consumo de WEPs de hoja con colores muy intensos y oscuros se ha utilizado como fuente de provitamina A, especialmente por mujeres y niños (Zeitlin *et al.*, 1992; Grivetti y Ogle, 2000). Este conocimiento etnobotánico se ha comprobado además por diversos autores que han estudiado durante décadas el contenido en nutrientes de especies silvestres comestibles (Flyman y Afolayan, 2006). Ya en 1963, Cowan *et al.* señalan diversas WEPs consumidas en Líbano como fuente de provitamina A. Entre los trabajos más actuales, la publicación dirigida por Sánchez-Mata y Tardío (2016) hace una compilación exhaustiva del valor nutricional de diversas especies.

Así pues, la recolección de plantas silvestres ha sido en el pasado un punto clave para cubrir las necesidades nutricionales de comunidades con escasez de recursos alimentarios. Dicha escasez podía derivar de diversos factores que incluirían, entre otros, factores económicos (Tabuti, 2007; Geraci *et al*., 2018); agronómicos por fallos en la cosecha, debido a ataques

severos de plagas y enfermedades o a condiciones ambientales adversas como sequías o inundaciones (Łuczaj *et al*., 2012); o incluso socio-políticos como serían los conflictos bélicos (Łuczaj y Pieroni, 2016). Especialmente los dos primeros casos se verían agravados además en áreas rurales con una agricultura de subsistencia basada en minifundios (Carvalho y Barata, 2017). Con el uso complementario de plantas silvestres en la dieta se conseguiría, pues, superar tales limitaciones nutricionales, con la ventaja añadida de que son alimentos de fácil acceso para toda la población y libres de cargo económico (Tabuti, 2007). Es más, su recolección puede tener incluso una repercursión económica directa si se venden en mercados locales.

En la actualidad, sin embargo, su consumo con fines estrictamente nutricionales está más limitado, aunque seguiría siendo importante en regiones en vías de desarrollo (Ceccanti *et al.*, 2018). En Europa, su uso fue importante hasta el siglo XX, especialmente en épocas de guerra y post-guerra (Reyes-García *et al*., 2015; Łuczaj y Pieroni, 2016). Geraci *et al.* (2018) subrayan por ejemplo la recolección de WEPs como una práctica habitual en Sicilia, tanto en comunidades rurales como urbanas, derivada de la situación de pobreza severa que se vivía en la isla; mientras que esta actividad es prácticamente nula en la actualidad entre la gente joven. Una de las causas principales que habría motivado el abandono de las plantas silvestres como complemento a la dieta es el establecimiento de una producción agrícola a gran escala en detrimento de una agricultura familiar de subsistencia, y la mayor accesibilidad a los productos cultivados durante todo el año. Otro factor que ha podido resultar determinante es la asociación de esta práctica con situaciones de pobreza y subsistencia precaria (Pieroni *et al.*, 2005). Dicha asociación ha provocado el rechazo de su consumo en mayor o menor grado, en el contexto de una sociedad con mejores recursos económicos. El rechazo difiere, además, entre especies. Łuczaj *et al.* (2012) recopilan el caso extremo de ejemplos de plantas de gran importancia en Europa hasta el siglo XX, ahora prácticamente olvidadas.

1.2. Uso de las plantas silvestres en el mantenimiento de la salud

Las plantas han sido un recurso utilizado en medicina durante milenios (Abbasi *et al*., 2013), cuyas propiedades, uso y forma de

preparación forman parte de la tradición etnobotánica de muchas culturas. Así, estudios etnobotánicos y epidemiológicos señalan el valor cultural de las plantas silvestres como remedio para diversas afecciones tales como desórdenes alimentarios, gastrointestinales y úlceras; problemas dermatológicos, respiratorios y cardiovasculares; inflamaciones y problemas reumáticos; o producción de leche y desórdenes menstruales (Nebel *et al*., 2006; Abbasi *et al*., 2013; Shin *et al*., 2018).

En la actualidad, el uso de plantas silvestres para tratar trastornos concretos está cayendo en desuso, al menos entre las comunidades de países desarrollados. Por el contrario, cada vez existen más evidencias científicas que relacionan una dieta de calidad, como la dieta mediterránea, con un menor riesgo de padecer ciertas enfermedades cardiovasculares y degenerativas (e.g., Sofi *et al*., 2010; Casas *et al*., 2014; Schwingshackl *et al*., 2017). A consecuencia de ello, las sociedades modernas, que cuentan con recursos adecuados para cubrir las necesidades nutricionales básicas, buscan obtener además un beneficio complementario sobre la salud mediante la alimentación. En este contexto surge el término de 'alimento funcional' como aquél que, en cantidades normales de consumo, demuestra un efecto beneficioso sobre alguna función del organismo, derivando así en una mejora del estado general de salud y/o reduciendo el riesgo de enfermedad (Nutrition Society, 1999). El término 'alimento nutracéutico' se usa también como sinónimo de 'alimento funcional' (e.g., Bacchetta *et al*., 2016), más allá de la referencia que hace al compuesto o ingrediente en cuestión que ejerce la actividad beneficiosa.

La consideración de muchas plantas silvestres como potenciales alimentos funcionales ha atraído la atención de la comunidad científica. En general, sus niveles en compuestos bioactivios son superiores a los determinados en especies cultivadas (Trichopoulou *et al*., 2000; Nebel *et al*., 2006; Licata *et al*., 2016), donde factores como un ambiente más controlado o la erosión genética asociada a la domesticación podrían haber afectado negativamente dicho potencial. De hecho, las especies silvestres están expuestas a condiciones más o menos cambiantes y diversos estreses de tipo abiótico y biótico, factores que pueden desencadenar un aumento en la acumulación de compuestos bioactivos. Así, existen muchos trabajos

centrados en el potencial funcional de las plantas silvestres, especialmente derivados de plantas consumidas en el área mediterránea (e.g., Trichopoulou *et al*., 2000; Morales *et al*., 2012; García-Herrera *et al*., 2013; Morales *et al*., 2014; Skotti *et al*., 2014; Petropoulos *et al*., 2019). Estos estudios, que constatan el valor funcional de las plantas silvestres, abren la puerta a un nicho de mercado en auge: la comercialización de productos con alto valor funcional añadido (Olayanju, 2018).

1.3. Enriquecimiento gastronómico

Además de su importancia nutricional y nutracéutica, la utilización de plantas silvestres atiende también a un intento por enriquecer el sabor de dietas monótonas (Łuczaj *et al*., 2012). Así, las WEPs han formado parte de las tradiciones culinarias de diversas culturas durante generaciones, entendiéndose como tal una cocina que incorpora ingredientes localmente cultivados, producidos o recolectados, utilizados en la elaboración de especialidades locales (Nebel *et al*., 2006). En este sentido, plantas silvestres utilizadas como condimento y especias han tenido un gran valor en la gastronomía local (Tardío *et al.*, 2006; Pinela *et al*., 2017b; Shikov *et al*., 2017).

En sociedades modernas de países desarrollados, las plantas silvestres han perdido importancia para cubrir necesidades nutricionales y para el mantenimiento de la salud. Así pues, el factor culinario parece determinante para la supervivencia del conocimiento etnobotánico a través de generaciones hasta la actualidad. De hecho, los trabajos de Serrasolses *et al.* (2016) y Thakur *et al.* (2017) señalan el motivo sociocultural como principal factor que determina el uso actual de WEPs (Fig. 2), con especial influencia del aroma y sabor que aportan (motivación principala para el 40–50% de respuestas). Otras motivaciones incluirían, aunque en mucha menor consideración, la asociación con tradiciones y elaboración de productos locales, así como su recolección aprovechando otras actividades (e.g., senderismo, recolección de setas). Por el contrario, estos estudios reflejan que los motivos económicos y nutricionales se tendrían en poca consideración, si bien diversos informantes coinciden en el valor añadido de su calidad como alimento funcional.

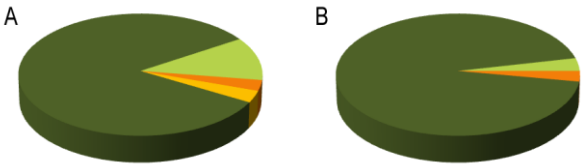

Fig.2. Importancia relativa (%) de los motivos dados por los informantes que condicionan el uso (o desuso) actual de las plantas silvestres en la alimentación: motivos socioculturales ( ), ambientales ( ), económicos ( ) y otros (incluyen cambios en prácticas agrícolas y modificación del paisaje que afectan a la disponibilidad de WEPs, así como consumo por otros animales,). De acuerdo al estudio de A) Thakur *et al.*, 2017 o B) Serrasolses *et al.*, 2016.

Existen discrepancias en cuanto a la importancia actual de las especies silvestres. Por un lado, diversos autores hablan de un interés creciente en el uso de WEPs, al querer recuperar la tradición e identidad gastronómica local (e.g., Schunko *et al*., 2015; Carvalho y Barata, 2017; Pinela *et al*., 2017b). Este interés habría promovido incluso el sector económico. Así, en la actualidad existe la posibilidad de adquirir plantas silvestres en mercados locales de distintas regiones (Łuczaj *et al*., 2013; Geraci *et al*., 2018; Pieroni y Cattero, 2019) e incluso a través de compañías establecidas (Evans y Irving, 2018); así como productos tradicionales elaborados utilizando plantas silvestres, tales como mermeladas, postres, licores o siropes (Pardo-de-Santayana *et al*., 2007; Łuczaj y Pieroni, 2016). Es posible incluso hablar de chefs de renombre y restaurantes que han incorporado las WEPs en su cocina (Łuczaj y Pieroni, 2016). Por el contrario, otros autores han documentado una decadencia en la recolección y consumo de WEPs por el público general (Reyes-García *et al*., 2015; Serrasolses *et al*., 2016; Thakur *et al*., 2017). Esta decadencia vendría motivada por una suma de factores (e.g., urbanización de la sociedad y pérdida del contacto con la naturaleza; producción agrícola a gran escala; alta disponibilidad de alimentos; o cambios en el estilo de vida) que conducen a una pérdida del conocimiento etnobotánico y de interés entre las nuevas generaciones.

Pese a tales discrepancias, resulta innegable que las plantas silvestres constituyen una oportunidad para enriquecer la gastronomía. Su inclusión como ingrediente de la dieta doméstica aportaría sabores y texturas diversas,

elevando la necesidad de alimentarse a una mayor experiencia culinaria. En este sentido, la alternativa a la recolección incontrolada pasaría nuevamente por la revalorización de estas especies como nuevos cultivos introducidos en el mercado.

1.4. Peligrosidad y toxicidad asociada al consumo de WEPs

Pese al uso tradicional de las WEPs en la alimentación y su interés funcional y gastronómico, no puede ignorarse la peligrosidad asociada a éstas. De acuerdo a Łuczaj *et al.* (2012), uno de los principales riesgos de toxicidad por el consumo de plantas silvestres deriva de una mala identificación de la especie recolectada. Por ejemplo, se han identificado casos de intoxicación debido a la confusión de plantas tóxicas de la familia *Apiaceae* con otras comestibles morfológicamente parecidas (Irving, 2009; Łuczaj *et al*., 2012). La incorrecta identificación se puede ver agravada en las generaciones jóvenes por la pérdida de contacto con la naturaleza y la inexperiencia en recolectar este tipo de alimentos.

Por el contrario, la inocuidad las plantas tradicionalmente utilizadas está en principio garantizada por la transmisión del conocimiento etnobotánico entre generaciones. El hombre ha aprendido a seleccionar durante generaciones aquellas especies aptas para el consumo mediante el conocimiento empírico, por ejemplo, imitando el comportamiento de los animales o evitando plantas que hayan provocado toxicidad en otros individuos (Morales *et al.*, 2017). Más aún, ha aprendido a diferenciar entre los órganos comestibles y los que pueden resultar tóxicos; a identificar el momento adecuado para su recolección; e incluso a utilizar preparaciones adecuadas para inactivar los compuestos tóxicos. Así, la cocina tradicional de diversas regiones utiliza hojas jóvenes y brotes tiernos de *Bryonia dioica* Jacq. (nueza blanca) recolectados antes de la floración, usando la cocción para inactivar los principios tóxicos; por el contrario, evita el consumo de sus frutos y raíces por la acumulación de cucurbitacinas en estos órganos (Morales *et al*., 2012; Pinela *et al*., 2017b). Del mismo modo, las semillas de especies de *Brassicaceae* como *Eruca vesicaria* (L.) Cav. (oruga) son evitadas por sus niveles en ácido erúcico, si bien el consumo de brotes tiernos es frecuente (Guil-Guerrero y Torija-Isasa, 2016).

Otro factor a tener en cuenta es la presencia de antinutrientes, compuestos que pueden acumularse en cantidades importantes en plantas consumidas por sus hojas. Dos ejemplos muy citados son la acumulación de ácido oxálico y nitratos. El ácido oxálico es un compuesto con capacidad de formar sales insolubles de oxalato de calcio; por lo tanto, reduce la biodisponibilidad de este mineral, aumentando el riesgo de osteoporosis y otras enfermedades (Amalraj y Pius, 2015). Además, la acumulación de esta sal puede provocar cálculos renales, especialmente en individuos sensibles (Ceccanti *et al*., 2018). Por lo tanto, especies con un alto contenido en ácido oxálico, o un ratio oxálico/calcio superior a 2,5, como por ejemplo *Montia fontana* L. (coruja), *Silybum marianum* (L.) Gaertn (cardo mariano) o *Chenopodium ambrosioides* L. (hierba hormiguera) deberían ser consumidas con moderación (Pinela *et al*., 2017b). Por su parte, los nitratos no se consideran tóxicos en sí mismos; no obstante, su reducción en otros compuestos tras la ingesta se ha relacionado con enfermedades como metahemoglobinemia y aumento del riesgo de cáncer (Bondonno *et al*., 2018; Lundberg *et al*., 2018). Así pues, WEPs con gran acumulación de nitratos deberían consumirse con moderación, e incluso evitarse en grupos como niños o adultos con sensibilidad a su consumo. Ejemplos de especies silvestres que deberían considerarse en este sentido son *Beta maritima* L. (acelga silvestre), *Capsella bursa-pastoris* (L.) Medik. (zurrón de pastor) o *Eruca vesicaria* (L.) Cav. (oruga) (Tardío *et al*., 2016).

2. Perspectivas futuras en el uso de las WEPs: de la recolección a la comercialización

La preocupación de la sociedad por la importancia de la dieta en el mantenimiento de la salud, conjuntamente con la demanda de una mayor riqueza organoléptica de los platos que consumen, ha provocado un interés creciente entre científicos y productores asociado al estudio de las WEPs y su revalorización.

Más allá de la recolección tradicional de este tipo de alimentos vegetales, una alternativa sugerida por diversos autores es su explotación

comercial como nuevos cultivos (Tabuti, 2007; Pinela *et al*., 2017b). La adaptación a cultivo puede suponer un aumento en la popularización de estas especies, al volverse más accesibles para toda la comunidad sin necesidad de recurrir a la recolección directa. Esta conversión conlleva además una serie de ventajas que no se pueden obtener de otra manera. Entre ellas, destacan aquéllas relacionadas con la seguridad y salud de los consumidores. Por un lado, la adquisición de cultivares comercializados elimina el riesgo de intoxicación que puede ocurrir como consecuencia de una mala identificación si las plantas son directamente recolectadas por el consumidor. Por otra parte, el proceso de domesticación puede ser utilizado para reducir e incluso eliminar los compuestos de mayor o menor toxicidad. Un ejemplo claro de de eliminación de compuestos tóxicos lo constituye el género *Cucurbita*, cuyas especies cultivadas pueden ser consumidas sin riesgo gracias a la eliminación de cucurbitacinas a través de la domesticación y mejora de estas especies (Guil-Guerrero, 2014).

Otras ventajas de la domesticación actual y adaptación a cultivo de plantas silvestres incluyen: 1) una mayor disponibilidad de producto y uniformidad del mismo; y 2) una mejor explotación de sus caracteres más deseables si se busca un producto de alto valor añadido. Por un lado, el cultivo comercial favorece una disponibilidad menos dependiente del ciclo natural de la especie y menos ligada a factores ambientales adversos. Por otra parte, durante el programa de domesticación y mejora se puede incluir como objetivo el mantenimiento o mejora de la calidad funcional y/o organoléptica. Es más, la selección y manejo adecuado de las condiciones de cultivo puede asimismo incrementar el valor del producto final, dada la influencia del ambiente sobre la acumulación de diversos compuestos de interés y antinutrientes (Galieni *et al*., 2015; Bonasia *et al*., 2017; Schiattone *et al*., 2018).

Hoy en día es posible encontrar ejemplos de la revalorización de especies silvestres a cultivos introducidos, tales como la producción comercial de rúcula (*Diplotaxis tenuifolia* L.) y canónigo (*Valerianella locusta* L.) (Ramos-Bueno *et al*., 2016). Hay además otras especies de interés agrícola creciente como alimento o cultivo industrial, como son la borraja (*Borago officinalis* L.), verdolaga (*Portulaca oleracea* L) o

lechuguilla dulce (*Reichardia picroides* (L.) Roth), especies silvestres sobre las que se habrían iniciado trabajos de adaptación a cultivo (Bacchetta *et al.*, 2016).

3. Riqueza mediterránea y consumo de plantas silvestres

La dieta mediterránea se considera un modelo de alimentación saludable, y se ha relacionado con una reducción en el riesgo de enfermedades cardiovasculares y cáncer (e.g., Casas *et al*., 2014; Schwingshackl *et al*., 2017; Bravi *et al*., 2018; Estruch *et al*., 2018). Se basa, entre otros aspectos, en un consumo abundante de frutas, verduras, legumbres y cereales (Fundación Dieta Mediterránea, 2004). Si bien los productos vegetales derivan generalmente de cultivos establecidos, las plantas silvestres se han identificado además como un componente auxiliar dentro de la dieta mediterránea tradicional (Geraci *et al*., 2018). En su revisión, Carvalho y Barata (2017) señalan la gran riqueza etnobotánica en el uso de WEPs en los países europeos del área mediterránea, con gran apreciación por las hortalizas silvestres de hoja (Fig. 3); por contraposición, los países del norte de Europa tienen menor tradición, restringida principalmente a frutos y semillas.

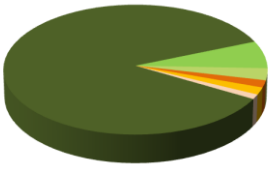

Fig. 3. Órganos consumidos y frecuencia relativa de uso (%), en plantas silvestres de uso tradicional en la isla de Sicilia, Italia (n=253 especies; para algunas especies, más de un órgano es consumido): hojas y brotes tiernos ( ), flores/inflorescencias ( ), bulbos ( ), jugo de tallos o flores ( ), raíces y tubérculos ( ) y frutos ( ). Adaptado de Geraci *et al.*, 2018.

La relación entre dieta mediterránea, plantas silvestres y salud puede haber motivado el interés científico sobre estas especies. Es posible encontrar de hecho numerosos trabajos en los que se pretende: 1) recoger y conservar el conocimiento tradicional, tanto de consumo como uso en medicina tradicional; o, 2) estudiar la base de las propiedades bioactivas de estos alimentos silvestres (e.g., Morales *et al*., 2012; Sánchez-Mata *et al*., 2012; Guarrera y Savo, 2016; Geraci *et al*., 2018).

Si bien el consumo de plantas silvestres ha sido más o menos generalizado en las regiones mediterráneas, existen diferencias significativas en la elección de especies según la región o país (Geraci *et al*., 2018). Estas diferencias atienden a diversos motivos que podrían incluir, entre otros, la abundancia en la región, la apreciación organoléptica por la población, la consideración como planta de uso medicinal e incluso la creencia de toxicidad asociada. Tanto es así que en el estudio etnobotánico llevado a cabo por Leonti *et al*. (2006) sólo se identifican 18 especies (incluyendo especies semi-cultivadas) utilizadas en los tres países analizados, de un total de 318 citadas. Por otro lado, la estacionalidad ha marcado el uso de las especies y definido las preparaciones culinarias, recetas que se iban modificando para adaptarlas a las plantas disponibles en cada momento. Por ejemplo, la 'Minestra delle 18 erbe' italiana se podía preparar con diferentes plantas según la disponibilidad (Guarrera y Savo, 2016). Finalmente, en la actualidad se ha reducido el consumo generalizado de plantas silvestres, si bien todavía se puede identificar un consumo regular de distintas especies en regiones rurales mediterráneas (Nebel *et al*., 2006; Ghirardini *et al*., 2007; Łuczaj *et al*., 2013; Pieroni y Cattero, 2019). En la Tabla 1 se recoge una muestra de estas especies.

En el litoral valenciano ha tenido lugar una modificación importante del paisaje para su adaptación a huertos hortícolas y leñosos. Dicha modificación ha condicionado el desarrollo de plantas silvestres, favoreciendo el establecimiento de aquéllas adaptadas como arvenses a los agrosistemas creados. Por otra parte, el sistema de riego por acequias establecido por los árabes y mantenido hasta la actualidad ofrece un hábitat adecuado para el desarrollo de plantas que habitan en cursos de agua. Así pues, especies como la berraza, rabaniza, verdolaga, colleja, mostaza

silvestre o diente de león son fácilmente identificadas en esta región y pueden ser recolectadas para su consumo o, en el marco de una alternativa más novedosa, como material inicial para la obtención de nuevos cultivos.

Tabla 1. Selección de especies vegetales silvestres consumidas actualmente en Grecia, Italia y/o Croacia.

Especie	Nombre común	Parte consumida	Uso tradicional
Apium nodiflorum (L.) Lag.	Berraza	V	Fresco/cocinado
Asparagus acutifolius L.	Espárrago silvestre	V	Cocinado
Borago officinaleis L.	Borraja	V	Fresco/cocinado
Capsella bursa-pastoris (L.) Medik.	Zurrón de pastor	V	Cocinado
Chenopodium album L.	Cenizo	V	Cocinado
Cichorium intybus L.	Achicoria	V	Fresco/cocinado
Cynara cardunculus L.	Cardo lechero	Fl	Cocinado
Diplotaxis spp.	Rúcula	V	Fresco
Lactuca serriola L.	Lechuga borde	V	Fresco/cocinado
Malva sylvestris L.	Malva	V, Fr	Fresco/cocinado
Papaver rhoeas	Amapola	V	Cocinado
Portulaca oleracea L.	Verdolaga	V	Fresco
Reichardia picroides (L.) Roth.	Lechuguilla dulce	V	Fresco/cocinado
Scolymus hispanicus L.	Cardillo	V	Cocinado
Silene vulgaris (Moench) Garcke	Colleja	V	Cocinado
Sonchus spp.	Diente de león	V	Cocinado
Urtica dioica L.	Ortiga	V	Cocinado

Fl: Flores, inflorescencias o botones florales. Fr: Frutos. V: Parte aérea vegetativa, incluyendo hojas y/o brotes tiernos. Referencias: Nebel *et al*., 2006; Ghirardini *et al*., 2007; Łuczaj *et al*., 2013; Pieroni y Cattero, 2019.

3.1. Descripción e interés de *Apium nodiflorum* (L.) Lag.

3.1.1. Descripción botánica de la especie

La familia *Apiaceae* (≡ *Umbelliferae*) es una amplia familia que incluye entre 2.500-3.700 especies englobadas en 300-450 géneros –la clasificación de algunas especies y géneros resulta complicada puesto que las delimitaciones resultan un tanto dudosas– (Nieto *et al*., 2003). A esta familia pertenecen numerosas especies de interés económico como alimento, especias y/o hierbas aromáticas y obtención de aceites esenciales. Así, incluye cultivos tales como apio (*Apium graveolens* L.), perejil (*Petroselinum crispum* (Mill.) Fuss), zanahoria (*Daucus carota* L.), hinojo (*Foeniculum vulgare* Mill.), cilantro (*Coriandrum sativum* L.), comino (*Cuminum cyminum* L.) o chirivía (*Pastinaca sativa* L.). Por el contrario, esta familia incluye también especies de toxicidad moderada a grave, incluso letal, como por ejemplo cicuta (*Conium maculatum* L.), angélica (*Angelica sylvestris* L.) o nabo del diablo (*Oenanthe crocata* L.).

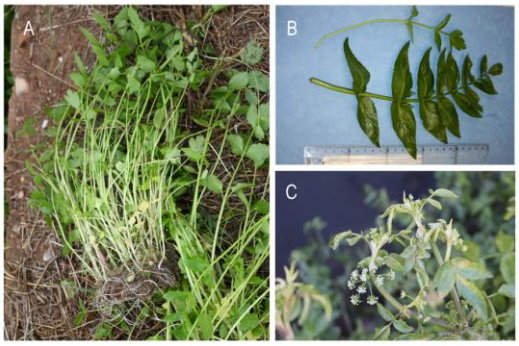

Fig. 4. Planta de berraza. A) Detalle de tallos jóvenes y base estolonífera. B) Comparación entre hoja joven (comestible) y hoja completamente desarrollada. C) Umbela de berraza.

Apium nodiflorum (L.) Lag. (≡ *Helosciadium nodiflorum* (L.) W.D.J.) es una especie silvestre comúnmente conocida como berraza, berrera, apio borde, apio bastardo o berra; en valenciano, api de sèquia, creixen de sèquia,

creixen bord o agret; y en inglés, ‘fool's watercress’ o, más recientemente, ‘water celery’. Se trata de una planta herbácea perenne. El tallo, fistuloso y finamente asurcado puede alcanzar los 100 cm de longitud, desarrollándose prostrado en sus nudos inferiores y erecto en el resto. Los nudos inferiores son, además, enraizantes. Desarrolla hojas compuestas imparipinnadas, con 3 a 13 foliolos de de 1,5-6 cm de longitud y forma lanceolada u ovada. Los foliolos son sésiles y de margen serrado o crenado, algo lobulados en hojas jóvenes. Las flores crecen en umbelas compuestas, de 3 a 15 radios de 1-2 cm de longitud. En el extremo apical de los radios se desarrollan las umbélulas, con radios de 1-2 mm que terminan en flor. Las umbelas no desarrollan por lo general brácteas en su base, pero sí se identifican de 4 a 7 bractéolas en la base de las umbélulas. Las flores se componen de un cáliz compuesto de cinco sépalos soldados, aunque a veces ausente, y una corola formada por cinco pétalos de color verde blanquecino, libres y agudos. Presentan gineceo ínfero bicarpelar con dos estilos. El fruto es un esquizocarpo dividido en dos mericarpos ovoides, con una longitud de 2-2,5 mm y cinco costillas gruesas y prominentes (Fig. 4) (Knees, 2003). Florece entre abril y octubre, y tiene alta capacidad de reproducción vegetativa por estolones.

3.1.2. Hábitat y distribución

La berraza es una especie helofítica que crece en suelos encharcados poco profundos, donde los tallos pueden rebasar la superficie de modo que las hojas y flores se desarrollan en el medio aéreo. Adicionalmente, puede crecer en suelos con humedad muy elevada o prácticamente saturante, aunque no se encuentren inundados. Por lo tanto, su desarrollo es común en márgenes de aguas someras móviles, como fuentes, torrentes o remansos. Crece también fácilmente en acequias agrícolas de tierra u obra que acumulan sedimentos en el fondo que permitan su supervivencia (Fig. 5). Puesto que forma grandes masas en los hábitats donde se establece, puede llegar a suponer un problema para el paso del agua en acequias, por lo que es común ver a los agricultores haciendo labores de limpieza para eliminar las plantas de berraza.

Esta especie se distribuye comúnmente en el centro y sur de Europa (especialmente en el suroeste), centro y oeste de Asia y norte de África, entre los 0–1.200 m de altitud. En España, se desarrolla en buena parte de la Península Ibérica y en las Islas Baleares; así como en diversas regiones de Portugal (Knees, 2003). En el litoral valenciano, su distribución se ha visto altamente favorecida por el sistema de acequias agrícolas que cubren toda la parte llana del litoral. El uso de las mismas asegura un flujo más o menos continuo de agua, dándose así unas condiciones adecuadas para el crecimiento de la berraza. No obstante, la adopción creciente de sistemas de riego localizado está provocando el abandono de acequias y reduciendo así el hábitat para el desarrollo de esta especie.

Fig. 5. Población de berraza creciendo en acequia.

3.1.3. Valor gastronómico y nutricional

El consumo tradicional de la berraza se ha documentado en diferentes países de la región mediterránea como España (Parada *et al*., 2011), Italia (Nebel *et al*., 2006; Licata *et al*., 2016; Geraci *et al*., 2018), Portugal (Pinela *et al*., 2017b) o Túnez (Lentini y Venza, 2007). La parte comestible de esta especie corresponde a las hojas y tallos tiernos, y es consumida principalmente en primavera y verano, antes de la floración (Licata *et al*., 2016). Su consumo más tradicional es en fresco como ingrediente de ensaladas de primavera, si bien puede utilizarse también hervida como condimento para aromatizar sopas, frita con pimientos o incluso en conserva

(Lentini y Venza, 2007; Menendez-Baceta *et al*., 2012; Tardío *et al*., 2016). El sabor de la berraza se ha descrito como picante (Guarrera y Savo, 2016), con un aroma con connotaciones de apio, zanahoria o una mezcla de ambos (Nebel *et al*., 2006; Heshmati Afshar *et al*., 2017).

Tabla 2. Principales compuestos nutricionales y bioactivos descritos en la berraza.

	Contenido		**Contenido**
Humedad (%)	89,56–94,0		
Proteína[a]	1,1-2,1	Fibra[a]	1,9–3,4
K[b]	105–219	Na[b]	137–379
Ca[b]	64–246	Mg[b]	16,4–44,9
Vitamina C[b]	9,51–33,35	Ácido ascórbico[b]	4,53–14,44
Tocoferoles totales[b]	0,27	Folatos totales[b]	0,097–0,138
Ácido α-linolénico (%)	43,46	Ácido linoleico (%)	24,60
Fenoles totales[c]	143,29	Flavonoides totales[d]	80,99
Ácido oxálico[a]	0,11–0,92		

Unidades: [a]g $100g^{-1}$ MF. [b]mg $100g^{-1}$ MF. [c]Expresados como GAE (equivalentes de ácido gálico). [d]Expresados como CE (equivalentes de catequina). Referencias: Morales, 2011; García Herrera, 2014.

La especie está considerada como diurética y se ha usado tradicionalmente como tónico para la presión sanguínea, así como de estimulante para el apetito dado su potente aroma (Guarrera y Savo, 2013; Licata *et al*., 2016). Existen pocos trabajos en referencia a la composición nutricional de la berraza (Morales, 2011; Morales *et al*., 2012; García-Herrera, 2014; Tardío *et al*., 2016). La Tabla 2 recoge los aspectos nutricionales más importantes. De estos estudios cabe destacar su contenido en compuestos fenólicos, pero también los altos contenidos en sodio y ácido oxálico. Puesto que el ácido oxálico reduce la biodisponibilidad de calcio y aumenta el riesgo de cálculos renales y otras enfermedades (Amalraj y Pius, 2015), el consumo de berraza debe ser moderado, como complemento a la

dieta pero no una hortaliza principal. Su uso debe ser especialmente controlado o evitado por grupos sensibles que requieren de dietas pobres en sodio.

Por otra parte, su perfil aromático como alimento no ha sido descrito por otros autores. Sin embargo, varios trabajos analizan el contenido en compuestos orgánicos volátiles de los aceites esenciales extraídos de poblaciones de berraza. Los principales resultados de estos análisis están resumidos en la Tabla 3 (Menghini *et al*., 2010; Maxia *et al*., 2012; Benelli *et al*., 2017; Heshmati Afshar *et al*., 2017; Koutsaviti *et al*., 2017). Estos trabajos han estudiado, además, el uso de su aceite como antifúngico frente a patógenos humanos, antibacteriano frente a *Helicobacter pylori* o como plaguicida de pre-cosecha y post-cosecha.

Tabla 3. Descripción centesimal (porcentaje, %) del aceite esencial de esta especie. Están indicados los principales grupos de compuestos volátiles y aquellos compuestos individuales para los que se ha determinado un mayor contenido relativo.

Compuesto	Contenido relativo
Monoterpenos hidrocarburos	11,5–91,4
Limoneno	nd[a]–40,6
Terpinoleno	0,2–58,4
γ-Terpineno	0,6–14,0
Sesquiterpenos hidrocarburos	2,0–6,8
Fenilpropanoides	nd–70,8
Miristicina	nd–49,1
Dilapiol	nd–70,8

[a]nd: no detectado. Referencias: Maxia *et al*., 2012; Benelli *et al*., 2017; Heshmati Afshar *et al*., 2017; Koutsaviti *et al*., 2017.

Hasta donde sabemos, no se han realizado acciones de domesticación o adaptación a cultivo en esta especie. Sin embargo, sí se ha documentado su explotación comercial como hortaliza silvestre. En concreto, la empresa 'Forager' establecida en Reino Unido, dedicada a la recolección de WEPs y

comercialización a restaurantes y consumidores particulares, incluye la berraza en sus 'bolsas verdes' (Evans y Irving, 2018).

3.2. Descripción e interés de *Diplotaxis erucoides* (L.) DC.

3.2.1. Descripción botánica de la especie

La familia *Brassicaceae* (≡ *Cruciferae*) es una gran familia que engloba aproximadamente 3.500 especies en 350 géneros, distribuida principalmente por las regiones templadas del hemisferio norte (Nieto, 1990). Incluye numerosas especies provechosas para la alimentación humana y animal, la obtención de aceites, producción de plaguicidas e incluso especies ornamentales, por lo que la familia ha adquirido gran importancia económica. Pertenecen a ella hortalizas como col, coliflor o brócoli (*Brassica oleracea* L.), nabo (*B. napus* L. subsp. *rapifera* Metzg), colza (*B. napus* L. subsp. *rapa* L.), mostaza negra (*B. nigra* (L.) Koch), rábano (*Raphanus sativus* L.) o mostaza blanca (*Sinapis alba* L.). También pertenece a esta familia *Arabidopsis thaliana* (L.) Heynh, extensamente utilizada como organismo modelo y primera planta de la que se secuenció el genoma entero.

Diplotaxis erucoides (L.) DC subsp. *erucoides* –en la bibliografía suele identificarse únicamente como *D. erucoides*, probablemente dada su gran popularidad– es una planta silvestre de la familia tradicionalmente consumida en diversas culturas. Se conoce comúnmente como rabaniza, rabaniza blanca, oruga silvestre o roqueta; en valenciano, ravenissa blanca o ravenell blanc; y en inglés, como 'wall rocket' o, menos común, 'white wall rocket', 'purple wall rocket' o 'purplish rocket'. Está relacionada taxonómicamente con la rúcula (*D. tenuifolia* y, en menor medida, *Eruca vesicaria* subsp. *sativa* o simplemente *E. sativa*), por lo que es común su comparación en estudios científicos (Bennett *et al*., 2006; D'Antuono *et al*., 2008, 2009; Di Gioia *et al*., 2018).

Es una planta herbácea anual, poco pilosa. Generalmente forma una roseta inicial, elongándose después al iniciarse el desarrollo floral; los tallos pueden alcanzar entonces más de 80 cm de altura. Las hojas inferiores presentan un limbo de 5-18 cm de longitud, y 1,5-8 cm de anchura. Son de

pinnatífidas a profundamente pinnatipartidas, algo liradas y con un peciolo más o menos alargado. Presentan de dos a cinco pares de lóbulos laterales con forma ovada, elíptica u oblonga, y un segmento terminal mayor de ápice agudo a redondeado. Las primeras hojas que se desarrollan, por el contrario, son enteras a poco lobuladas. Una vez alcanzada la madurez reproductiva, desarrolla además hojas sésiles en la parte superior de los tallos. Las flores crecen en racimos. La corola está compuesta de cuatro pétalos, de 8-10 mm x 4-6 mm, de limbo obovado. Los pétalos son blancos y con nerviación poco visible; si bien al secarse se tiñen frecuentemente de un tono más o menos violeta. Tiene androceo tetradínamo y ginoceo con estigma bilobado y ovario con 20 a 40 primordios por lóculo. El fruto es una silicua de 25 a 35 mm de longitud, dehiscente. Las semillas, ovoides a elipsoidales, de 1-1,2 x 0,6-0,9 mm, se disponen en dos filas por lóculo (Fig. 6) (Martínez-Laborde, 1990). Puede desarrollarse y florecer durante todo el año, si bien su crecimiento está más limitado durante los meses cálidos (junio a agosto), al menos en el litoral mediterráneo español.

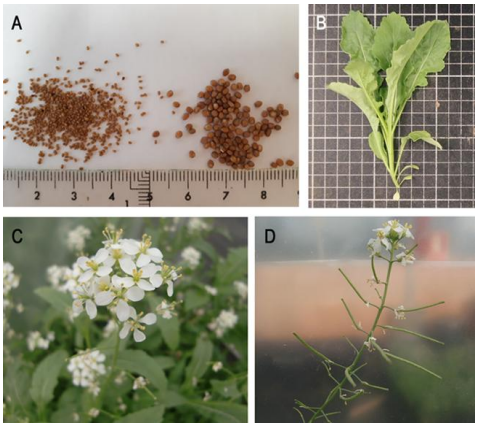

Fig. 6. Planta de rabaniza. A) Semillas de rabaniza (izquierda) y de rúcula (*E. sativa*; derecha) como referencia. B) Planta en crecimiento vegetativo. C) Racimo floral. D) Silicuas formándose.

Dentro de esta especie, existe además una segunda subespecie, subsp. *longisiliqua* (Cosson) Gómez-Campo. Inicialmente clasificada como *D. virgata* (Cav.) DC. var *longisiliqua* (Cosson), fue posteriormente

reclasificada a nivel taxonómico como *D. erucoides* (César Gómez-Campo, 1981). Es endémica del noreste de Algeria y significativamente diferente de la subsp. *erucoides* por caracteres morfológicos como color de la flor (amarilla y no blanca) o forma y textura de la hoja (Pignone y Martínez-Laborde, 2011). Hasta donde sabemos, no se ha documentado el uso como WEP de la subsp. *longisiliqua*.

3.2.2. Hábitat y distribución

La rabaniza es una planta ruderal y arvense, frecuente en cultivos leñosos como viñedos y olivares así como en campos de hortícolas. Su condición arvense está favorecida en gran medida por tres aspectos, de carácter a) morfológico y reproductivo; b) fisiológico; y c) ecológico. Por un lado, es una especie con una alta capacidad reproductiva dado el gran número de semillas que produce por silicua así como la cantidad de silicuas que un solo individuo es capaz de formar (Fig. 7). De este modo, una población establecida en un ecosistema concreto incorpora una gran cantidad de semillas al 'banco de semillas del suelo' en cada ciclo reproductivo, acción favorecida, además, por la dehiscencia de la silicua. Un segundo aspecto que favorece su carácter arvense se corresponde con el desarrollo de latencia secundaria (Martínez-Laborde *et al*., 2007), la cual condiciona la germinación de semillas maduras aun en condiciones adecuadas para ello. Como consecuencia, se produce una germinación escalonada en el tiempo, situación que favorece la competencia interespecífica con el cultivo y reduce la eficacia de las acciones de control. Finalmente, la rabaniza es una especie de bajas exigencias ecológicas que crece en diversos substratos (Martínez-Laborde, 1990), y fácilmente adaptada tanto a condiciones de secano como de mayor regadío.

La rabaniza crece entre los 0-1.500 m de altitud. Está ampliamente distribuida a lo largo del área mediterránea de Europa y África, en Europa central y el oeste de Asia; se puede encontrar, además, naturalizada en el continente americano (Pignone y Martínez-Laborde, 2011). Dentro de la Península Ibérica, su crecimiento es frecuente en prácticamente todas las regiones, excepto los Pirineos, el cuadrante noroeste de España y Portugal,

donde su crecimiento es raro (Martínez-Laborde, 1990). Es frecuente, también, en las Islas Baleares.

Fig. 7. Campo de cultivo abandonado donde se ha establecido una población de rabaniza como especie dominante.

3.2.3. Valor gastronómico y nutricional

La rabaniza ha sido tradicionalmente utilizada en países de la región mediterránea como España (Parada *et al*., 2011), Francia (Couplan, 2015) y comúnmente en Italia (Salvatore *et al*., 2005; Guarrera y Savo, 2016; Licata *et al*., 2016; Disciglio *et al*., 2017). Principalmente se ha consumido por sus hojas jóvenes y brotes tiernos, cosechados comúnmente de otoño a primavera, antes de la floración. Tienen un sabor y aroma picante, algo amargo, diferenciado de la rúcula común; por el contrario, varios informantes han descrito connotaciones similares a mostaza, wasabi y hojas de rábano. Se puede consumir en fresco para añadir sabor en ensaladas y platos de pasta; así como hervida en sopas o frita en tortillas (Couplan, 2015; Guarrera y Savo, 2016). Disciglio *et al.* (2017) señalan también su uso en pasteles rústicos de vegetales silvestres. Por otra parte, Bianco *et al.* (1998) refieren el uso de las flores, elemento decorativo comestible de sabor similar a las hojas pero menor intensidad. Finalmente, es una planta melífera muy apreciada por las abejas, por lo que se pueden usar sus flores para la obtención de miel.

Pese a la popularidad de esta hortaliza silvestre, existen pocos estudios de su perfil nutricional; en la Tabla 4 se resumen los aspectos nutricionales más relevantes. El conocimiento tradicional identifica la rabaniza como laxativo (Guarrera y Savo, 2013). Por otra parte, hasta donde sabemos, no se ha detallado su perfil aromático –sí se ha descrito su perfil en glucosinolatos, precursores de compuestos volátiles determinantes del aroma y sabor–. La principal característica de esta hortaliza silvestre es posiblemente la acumulación de sinigrina, responsable de su característico aroma (D'Antuono *et al*., 2009).

Tabla 4. Principales compuestos nutricionales y bioactivos descritos en la rabaniza.

	Contenido		**Contenido**
Humedad (%)	86,4–89,6		
Proteína[a]	3,5[*]		
K[a]	400[*]–511	Na[a]	13–40[*]
Ca[a]	344–350[*]	Mg[a]	26–37,5[*]
SO_4^{2-}[a]	18[*]–320	NO_3^-[a]	202,4–244,1
PO_4^{3-}[a]	14[*]–102		
Vitamina C[a]	14,52[‡]	Ácido ascórbico[a]	13,91[‡]
Fenoles totales[a]	205[†*]–292,54[‡]	Flavonoides totales[a]	287,67[‡]
Glucosinolatos[b]	8,36–13,36		
Carotenoides[a]	15,13[‡]	Clorofilas[a]	76,79[‡]
Actividad antioxidante[a]	392,0[^]		

[a]mg $100g^{-1}$ MF. [b]mg g^{-1} MS. [†]Equivalentes de ácido gálico. [^]Equivalentes de Trolox. [‡]Analizado sobre material hervido. [*]Valor obtenido por interpretación gráfica. Referencias: Bianco *et al.*, 1998; Salvatore *et al.*, 2005; D'Antuono *et al.*, 2008; Disciglio *et al.*, 2017; Di Gioia *et al.*, 2018.

La empresa Shamrock Seed Company, Inc. (actualmente Vilmorin North America; Salinas, CA, EEUU) ha desarrollado un cultivar comercial de rabaniza identificado como cv. 'Wasabi' (Shamrock Seed Company, Inc.), posiblemente dada la similitud de sabor con la especie *Wasabia*

japonica Matsum. Bennett *et al*. (2006) hacen referencia además a otros dos materiales comerciales de rabaniza. Sin embargo, estos autores no identificaron sinigrina en el perfil de glucosinolatos descrito. Los resultados de dicho estudio sugieren pues que podría tratarse de una subespecie distinta o incluso de una hibridación interespecífica. En este sentido, sería de gran interés un estudio comparativo de los distintos materiales a nivel genético, morfológico y analítico.

4. Componentes de la calidad funcional en berraza y rabaniza

Cuando se habla de calidad funcional de los alimentos vegetales, uno de los puntos mayoritariamente referidos es su capacidad protectora frente a radicales libres dada su composición en antioxidantes. Los radicales libres son moléculas que presentan electrones desapareados; esta condición los hace altamente reactivos, con capacidad de dañar macromoléculas como ácidos nucleicos, proteínas o lípidos (Ashor *et al*., 2016). Entre los radicales libres destacan los compuestos derivados del oxígeno (conocidos como 'especies reactivas de oxígenos'), tales como radicales superóxido, hidroxilo y derivados, peróxido de hidrógeno u oxígeno reactivo. Pero también diversas moléculas derivadas del nitrógeno ('especies reactivas de nitrógeno'), como radicales nitroxilo y peroxinitrito, tienen importancia por su actividad pro-oxidante (Craft *et al*., 2012). Los radicales libres están naturalmente presentes en los organismos como consecuencia de diversos procesos metabólicos, como la respiración celular (Zaluski *et al*., 2015). Sin embargo, un desequilibrio favorable a la producción y acumulación de radicales libres, de modo que se excede la capacidad del organismo para neutralizarlos, produce una situación de estrés oxidativo. En el hombre, el estrés oxidativo se ha relacionado con el proceso de envejecimiento y un aumento del riesgo de padecer diversas enfermedades como enfermedades cardiovasculares, neurodegenerativas o desarrollo de células tumorosas (Institute of Medicine, 2000; Zaluski *et al*., 2015; Ashor *et al*., 2016; Prasad *et al*., 2017).

Por su parte, un antioxidante es toda molécula que, aun presente en baja concentración comparado con un substrato susceptible de ser oxidado,

es capaz de inhibir o reducir significativamente dicha oxidación (Carr y Frei, 1999). Los antioxidantes se pueden agrupar de acuerdo a criterios como el modo de actuación o el origen de los mismos. Según el modo de actuación, se puede hablar de antioxidantes directos e indirectos. El modo general de actuación de los primeros es la neutralización de los radicales libres por cesión de iones H^+ o electrones, inactivando así la capacidad de dañar otras moléculas (Craft *et al.*, 2012). Por su parte, los antioxidantes indirectos actúan sobre otros compuestos relacionados con la formación o inhibición de radicales libres; por ejemplo, pueden ser quelantes de elementos envueltos en su producción, o restaurar la capacidad antioxidante de enzimas y vitaminas (Hassimotto *et al*., 2005). Hay antioxidantes que pueden ejercer a la vez un efecto directo e indirecto; mientras que otros tienen una actuación principalmente indirecta. En cuanto a su origen, los antioxidantes se pueden clasificar como endógenos, tales como ácido úrico, glutatión o diversas enzimas naturalmente presentes en el cuerpo (Ashor *et al.*, 2016). Sin embargo, el hombre puede además incorporar otros mediante la dieta (antioxidantes exógenos o alimenticios); destacan en este sentido la vitamina C y la familia de los polifenoles dada su relevancia y su distribución generalizada en el reino vegetal.

En el caso específico de la berraza, su calidad funcional está particularmente relacionada con la alta capacidad antioxidante dado su contenido en compuestos fenólicos (Morales *et al*., 2012). Por su parte, la rabaniza presenta también cantidades relevantes de polifenoles (Disciglio *et al*., 2017) y, presumiblemente, de vitamina C tal y como se ha determinado para la rúcula cultivada (Jin *et al*., 2009). La calidad funcional de esta especie está relacionada, además, con el contenido en glucosinolatos de sus órganos comestibles (Di Gioia *et al*., 2018). Finalmente, la rabaniza es una planta bioacumuladora de nitratos (Disciglio *et al*., 2017).

4.1. Vitamina C y ácido ascórbico

La vitamina C es una molécula hidrosoluble de seis carbonos aislada por primera vez en 1928 por A. Szent-György. El isómero *L*- de esta molécula es la forma biológicamente activa (Ashor *et al*., 2016), y se puede presentar en dos formas interconvertibles: ácido *L*-ascórbico o forma

reducida, y ácido *L*-dehidroascórbico o forma oxidada (Fig. 8). El ácido dehidroascórbico puede regenerarse *in vivo* por la acción de reductasas dependientes del glutatión, NADH o NADPH (Institute of Medicine, 2000). Alternativamente, la forma oxidada puede ser hidrolizada irreversiblemente a ácido 2,3-diquetogulónico, el cual deriva posteriormente en otros compuestos tales como ácido oxálico y ácido treónico (Adikwu y Deo, 2013).

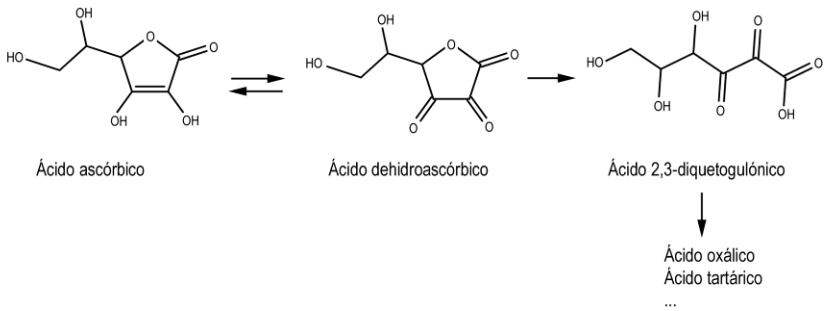

Fig. 8. Estructura química del ácido ascórbico y sus derivados.

La vitamina C es un micronutriente necesario para el funcionamiento metabólico correcto del organismo, siendo el ácido ascórbico la forma primaria y funcional *in vivo* (Institute of Medicine, 2000). Dado su bajo potencial de reducción es capaz de reaccionar y reducir una gran cantidad de moléculas (Carr y Frei, 1999); esta característica le da por lo tanto un alto poder como antioxidante. Así, el ácido ascórbico funciona como cofactor de diversas enzimas que se requieren para la biosíntesis de colágeno, carnitina y hormonas y aminoácidos de tipo neurotransmisor (Carr y Frei, 1999; Mandl *et al*., 2009; Smirnoff, 2018), probablemente mediante reducción de iones metálicos (Fe, Cu) presentes en la estructura de dichas enzimas o actuando como cosubstrato (Institute of Medicine, 2000). El ácido ascórbico es necesario, además, para mantener la homeostasis redox en orgánulos como mitocondria y retículo endoplasmático (Mandl *et al*., 2009). Finalmente, actúa como compuesto bioactivo por su capacidad de inhibir o retardar el estrés oxidativo causado por especies reactivas de oxígeno y nitrógeno

(Adikwu y Deo, 2013), reduciendo el daño sobre otras macromoléculas; y es también considerado un antioxidante indirecto por su capacidad de regenerar otras moléculas antioxidantes como la vitamina E (Ashor *et al*., 2019).

La mayoría de eucariotas sintetizan vitamina C, si bien algunos grupos de animales como los primates han perdido dicha capacidad (Smirnoff, 2018). Por lo tanto, la vitamina C se convierte en un elemento esencial que el ser humano debe incorporar con la dieta, especialmente mediante la ingesta de frutas y hortalizas de hoja. Destacan en este sentido alimentos como guayava, kiwi, litchi, calabaza, fresa, brócoli, berza, espinaca, kale y los cítricos (Barba *et al*., 2014; Vincente *et al*., 2014).

La ingesta de vitamina C es necesaria para evitar los síntomas del escorbuto, enfermedad común en el pasado entre marineros y soldados asociada a la ausencia de frutas y verduras en la dieta (Mandl *et al*., 2009). En este sentido, un aporte diario de 10 mg es suficiente para prevenir los efectos adversos por carencia (Carr y Frei, 1999), siendo una cantidad fácilmente obtenida con una dieta actual equilibrada. Sin embargo, la cantidad diaria recomendada es de siete a diez veces superior, dada la función protectora que tiene como compuesto bioactivo. Así pues, el Departamento de Salud y Servicios Humanos de Estados Unidos recomienda una cantidad diaria en hombres adultos igual a 90 mg; y 75 mg para mujeres, aumentando a 85 mg durante el embarazo y a 120 mg durante la lactancia (Institute of Medicine, 2000). Con estas cantidades diarias recomendadas se pretende una incidencia positiva sobre la salud, dada la relación entre su ingesta como parte de los alimentos y una reducción del riesgo de enfermedades cardiovasculares (Ye y Song, 2008; Ellingsen *et al*., 2009; Ashor *et al*., 2019).

Por otro lado, no hay evidencias científicas que demuestren un efecto tóxico asociado a la ingesta excesiva de vitamina C como parte de la dieta (Adikwu y Deo, 2013). Sin embargo, se ha asociado el aporte extra con un riesgo aumentado de padecer cálculos renales, dada la conversión metabólica de la vitamina C en ácido oxálico (Urivetzky *et al*., 1992); así como problemas de absorción excesiva de hierro (Mandl *et al*., 2009). Por ello, se considera adecuado un consumo máximo de 2 g día^{-1} de vitamina C (Institute of Medicine, 2000; Ashor *et al*., 2016). Pese a ello, tratamientos vía

inyección con dosis elevadas de esta vitamina, en forma de ácido *L*-ascórbico, pueden utilizarse como terapia contra el cáncer, dado el efecto citotóxico selectivo de esta molécula frente a células tumorosas (Du *et al*., 2012; Pires *et al*., 2016).

4.2. Compuestos fenólicos y actividad antioxidante

Los compuestos fenólicos son un grupo heterogéneo de moléculas derivadas del metabolismo secundario de las plantas. Este grupo incluye más de 8.000 estructuras conocidas, derivadas principalmente de la fenilalanina por la ruta del ácido siquímico (Cartea *et al*., 2011). Se definen por la presencia de al menos un anillo aromático hidroxilado. De acuerdo a la estructura química de su esqueleto, estos compuestos se pueden clasificar en distintos grupos y subgrupos (Fig. 9), incluyendo ácidos fenólicos (incluye ácidos hidroxicinámicos e hidroxibenzoicos), flavonoides (a su vez dividido en flavanoles, flavonoles, flavonas, flavanonas, antocianinas), isoflavonoides (isoflavonas) y otros grupos tales como lignanos, estilbenos, cumarinas o polímeros fenólicos (proantocianinas y taninos) (Barba *et al*., 2014).

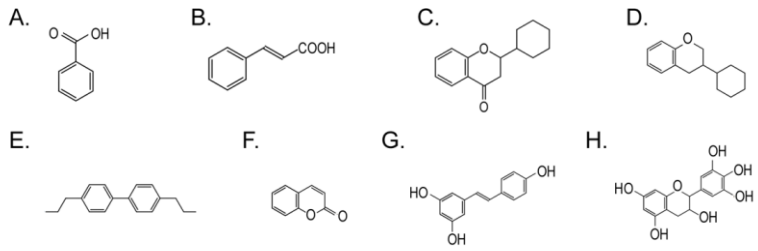

Fig 9. Estructura química de los principales grupos de compuestos fenólicos. A) Ácidos fenólicos hidroxibenzoicos. B) Ácidos fenólicos hidroxicinámicos. C) Flavonoides. D) Isoflavonoides. E) Lignanos. F) Cumarinas. G) Estilbenos. H) Polímeros fenólicos. Adaptado de Barba *et al*., 2014.

Estos compuestos se encuentran generalmente conjugados con otras moléculas. La asociación más común se da con azúcares –principalmente glucosa, pero también galactosa, xilosa, ramnosa o arabinosa– mediante

enlaces *O*-glicosídico; aunque pueden estar asociados además a otras moléculas como ácidos, aminas, lípidos o incluso otros fenoles (Bravo, 1998). Estas conjugaciones permiten aumentar la solubilidad de los compuestos fenólicos, facilitando su compartimentalización en vacuolas (Justesen *et al*., 1998). Sin embargo, esto puede dificultar el análisis por técnicas cromatográficas. Por ello, y dada la naturaleza ácido-lábil del enlace, es común realizar una hidrólisis ácida previa, especialmente cuando se analizan flavonoides (Justesen y Knuthsen, 2001; Bae *et al*., 2012). El término 'aglicona' se utiliza entonces para designar la parte de naturaleza flavonoide obtenida tras la hidrólisis.

Los compuestos fenólicos son importantes como metabolitos secundarios de las plantas. Están envueltos, por ejemplo, en la protección frente al estrés fotooxidativo por su capacidad de absorber radiación UV (Kyriacou y Rouphael, 2018); las antocianinas, además, contribuyen a la pigmentación de flores y frutas, responsables de aportar coloración naranja, roja, azul, violeta o morada (Barba *et al*., 2014). Por otro lado, están envueltos en mecanismos de defensa frente a patógenos y herbívoros, actuando como fitoalexinas (Bravo, 1998) o bien aumentando el sabor amargo y astringencia de sus órganos, incluyendo frutos inmaduros –destacan en este sentido los taninos, conocidos por el hombre por aportar matices en bebidas como vino y té verde– (Barba *et al*., 2014; Vincente *et al*., 2014; Brandt, 2016). Otras funciones de los compuestos fenólicos en las plantas incluyen, por ejemplo, el control fitohormonal y la liberación de señales de atracción para insectos polinizadores (Cartea *et al*., 2011; Khanam *et al*., 2012).

Por otro lado, muchos de estos compuestos han sido estudiados durante décadas por su potencial funcional. Destacan en este sentido los flavonoides, y en menor medida los ácidos fenólicos hidroxicinámicos, dada su extensa distribución entre frutas y verduras (Fig. 10, Tabla 5). La capacidad antioxidante de estos compuestos viene definida por características específicas como el número y posición de los grupos hidroxilo (–OH) que tiene la molécula, o la conjugación con otras moléculas –flavonoides con un residuo de azúcar tienen menor capacidad antioxidante que la correspondiente aglicona– (Cartea *et al*., 2011; Zaluski *et al*., 2015).

Dada la estructura que posee la quercetina, este flavonol es uno de los compuestos de mayor capacidad antioxidante (Bravo, 1998).

Tabla 5. Clasificación de los principales ácidos fenólicos y flavonoides estudiados en los alimentos por su potencial bioactivo, y ejemplos de alimentos en los que destacan.

Grupo	Compuestos representativos	Alimentos
Flavonoides		
Flavonoles	Kaempferol, miricetina, quercetina	Brócoli, cebolla, frutas del bosque, grelo, hinojo, kale, manzana, repollo, rúcula, té
Flavonas	Apigenina, luteolina, rutina	Apio, naranja (piel), perejil
Flavanoles	Catequina, epicatequina, epigalocatequina	Cacao, manzana, pera, té, uva, vino
Flavanonas	Hesperidina, naringenina	Cítricos
Antocianidinas	Cianidina, delfinidina, pelargonidina	Cereza, col roja, coliflor morada, ciruela, frutas del bosque, granada, uva
Ácidos fenólicos		
Ácidos hidroxicinámicos	Ácido cafeico, ácido clorogénico, ácido ferúlico, ácido sinápico	Café, cereales, frutas del bosque, grelo, kale, repollo
Ácidos hidroxibenzoicos	Ácido elágico, ácido gálico	Chocolate, nuez, té verde, uva, vino

Referencias: Cartea *et al*., 2011; Vincente *et al*., 2014; Zhou *et al*., 2016.

Al igual que el ácido ascórbico, estos compuestos pueden actuar: 1) como antioxidante directo sobre especies reactivas; y 2) como antioxidante indirecto ejerciendo su actuación sobre otras enzimas, especialmente sobre enzimas de fase II, o regenerando otras moléculas como la vitamina E (Procházková *et al*., 2011). Así, el consumo de dietas ricas en flavonoides y otros compuestos fenólicos se ha relacionado con una reducción de procesos inflamatorios agudos y crónicos (Ambriz-Pérez *et al*., 2016), del riesgo de

enfermedades cardiovasculares (Lovegrove *et al*., 2017), y del desarrollo de ciertos tipos de cáncer (Zhou *et al*., 2016).

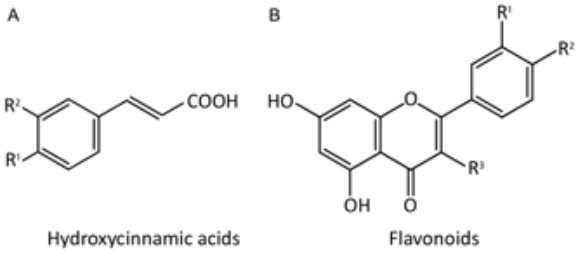

Fig 10. Estructura química de los principales compuestos fenólicos detectados en hortalizas de hoja con importancia antioxidante. A) Estructura general de los ácidos hidroxicinámicos. Ácido cafeico (R1 = R2 = OH). Ácido clorogénico (esterificación de ácido cafeico (R1 = R2 = OH) con ácido quínico). Ácido *p*-cumárico (R1 = OH, R2 = H). Ácido ferúlico (R1 = OH, R2 = CH3O). B) Estructura general de los flavonoides. Apigenina (R1 = OH, R2 = R3 = H). Luteolina (R1 = R2 = OH, R3 = H). Quercetina (R1 = R2 = R3 = OH). Kaempferol (R1 = R3 = OH, R2 = H). Adaptado de Justesen *et al*., 1998, y Kaushik *et al*., 2015.

4.3. Glucosinolatos

Los glucosinolatos son metabolitos secundarios propios del orden *Brassicales*, incluyendo la familia *Brassicaceae*, de gran importancia económica, pero también otras como las familias *Capparaceae* o *Moringaceae* (Romeo *et al*., 2018). Químicamente, los glucosinolatos se componen de un esqueleto formado por una molécula de *β*-D-tioglucósido unida a una oxima sulfonada, y una cadena lateral variable derivada de un aminoácido (Fig. 11).

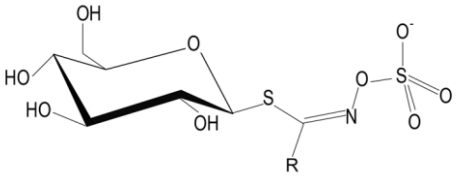

Fig. 11. Estructura general de los glucosinolatos.

De acuerdo a la naturaleza de la cadena lateral, se pueden clasificar en: 1) alifáticos, derivados de metionina, valina, leucina o isoleucina; 2) indólicos, derivados de triptófano; o 3) aromáticos, derivados de fenilalanina o tirosina (Agneta *et al*., 2014). En la actualidad se han identificado más de 100 glucosinolatos diferentes, de los cuales en torno a 50 se encuentran en la familia *Brassicaceae* (Barba *et al*., 2014). En la Tabla 6 se recogen los principales glucosinolatos identificados en diversos cultivos de esta familia.

Los glucosinolatos son moléculas hidrofílicas, no volátiles y estables (Buxdorf *et al*., 2013), y forman parte de los mecanismos de defensa de la planta frente a patógenos y herbívoros. Su modo de acción se basa en la co-existencia con enzimas hidrolíticas específicas en el sistema 'glucosinolato-mirosinasa' (Ahuja *et al*., 2010). Las mirosinasas (*β*-tioglucósido glucohidrolasas) están físicamente separadas en los idioblastos. Cuando la planta está sometida a un daño mecánico se produce una rotura de los tejidos, y enzima y sustrato entran en contacto. Se produce entonces la hidrólisis del glucosinolato, dando lugar a un compuesto inestable. Éste se degrada a su vez en compuestos más estables que incluyen isotiocianatos, nitrilos, tiocianatos, epitionitrilos y oxazolidinas (Fig. 12) (Ahuja *et al*., 2010). La degradación en un tipo de compuesto específico se ve favorecida por condiciones específicas de pH y/o la presencia de proteínas específicas e iones metálicos. Por ejemplo, la actuación de mirosinasas a pH neutro da lugar a isotiocianatos principalmente, y pequeñas cantidades de nitrilos (Hanschen y Schreiner, 2017). Por el contrario, condiciones de pH bajo y la presencia de proteínas específicas e iones metálicos aumentan la liberación de nitrilos y epitionitrilos (Angelino *et al*., 2015; Hanschen y Schreiner, 2017). Más aún, los isotiocianatos derivados de glucosinolatos indólicos son inestables y derivan rápidamente a indoles más estables, como indol-3-carbinol o indol-acetonitrilo (Fahey *et al*., 2001; Ciska *et al*., 2015).

Tabla 6. Principales glucosinolatos (GSLs) e isotiocianatos (ITCs) derivados de su hidrólisis enzimática, flavor asociado y cultivos de la familia *Brassicaceae* en los que se pueden encontrar.

Glucosinolato	Isotiocianato	Flavor	Alimentos destacados
Alifáticos			
Glucobrasicanapina	4-pentenil ITC	Agrio, picante, fragante, a wasabi, a rábano picante	Brócoli, kale, mostaza, rúcula[†]
Glucoerucina	4-metiltiobutilbutil ITC	A rábano, a repollo	Brócoli, coliflor, kale, mostaza, rábano, repollo, rúcula
Glucoiberina	3-metilsulfinilpropil ITC	Picante, a rábano	Brócoli, col de Bruselas, coliflor, kale, rábano, repollo
Gluconapina	3-butenil ITC	Picante, a wasabi, a repollo	Brócoli, col de Bruselas, coliflor, kale, mostaza, repollo
Glucorrafanina	4-metilsulfinilbutil ITC	-	Brócoli, col de Bruselas, coliflor, kale, repollo, rúcula
Progoitrina	2-hidroxi-3-butenil ITC	Muy amargo	Brócoli, col de Bruselas, coliflor, kale, repollo, rúcula
Sinigrina	2-propenil ITC	Amargo, picante, azufrado, a mostaza, a rábano picante, lacrimoso	Brócoli, col de Bruselas, coliflor, kale, mostaza, repollo
Aromáticos			
Gluconasturtina	Feniletil ITC	Picante, a rábano, a berro, sensación de cosquilleo	Brócoli, col de Bruselas, kale, mostaza, repollo, rúcula

Glucosinalbina	4-hidroxibencil ITC	Picante	Brócoli, kale, mostaza, rábano, repollo
Glucotrapeolina	Benzil ITC	Picante	Rúcula
Indólicos			
4-Hidroxi-glucobrasicina	4-hidroxi-3-indolil-metil ITC	-	Col de Bruselas, kale, rúcula
4-Metoxi-glucobrasicina	4-metoxi-3-indolil-metil ITC	-	Col de Bruselas, kale
Glucobrasicina	3-indolilmetil ITC	Amargo, desagradable	Brócoli, col de Bruselas, coliflor, kale, mostaza, rábano, repollo, rúcula

† Bajo este nombre se incluyen dos especies, *Diplotaxis tenuifolia* y *Eruca sativa*. Referencias: Bell *et al*., 2015; Ciska *et al*., 2015; Steindal *et al*., 2015; Taranto *et al*., 2016; Bonasia *et al*., 2017; Fernández-León *et al*., 2017; Wieczorek *et al*., 2017; Bell *et al*., 2018; Cools y Terry, 2018; Hwang *et al*., 2019.

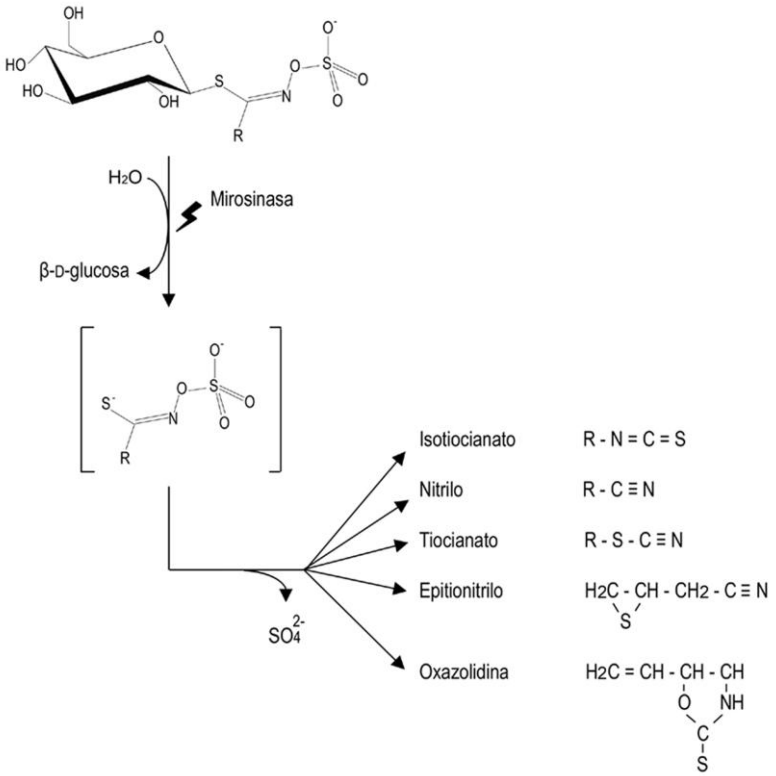

Fig. 12. Hidrólisis enzimática de los glucosinolatos. Adaptado de Bell y Wagstaff, 2014.

Estos derivados de la hidrólisis resultan tóxicos para insectos y patógenos generalistas, si bien algunos organismos han desarrollado mecanismos de detoxificación ante la acción de los glucosinolatos (Buxdorf *et al*., 2013; Badenes-Perez *et al*., 2014; Angelino *et al*., 2015). Se ha observado también un efecto negativo en mamíferos con dietas basadas en alimentos ricos en glucosinolatos. Por ejemplo, ganado alimentado con torta de colza y otros residuos vegetales puede desarrollar problemas tiroideos por deficiencias en la absorción de yodo, especialmente a casusa de los niveles de progoitrina (Fahey *et al*., 2001). Por el contrario, las semillas no se incluyen en la dieta humana –acumula los mayores niveles de glucosinolatos–, y el aporte total dentro de una dieta variada y equilibrada no alcanza niveles tóxicos (Brandt, 2016). Es más, los glucosinolatos y más

específicamente sus derivados han sido extensamente estudiados por su potencial beneficioso sobre la salud humana.

Los isotiocianatos son los productos de mayor potencial como biomoléculas (Romeo *et al*., 2018). En general, se pueden considerar como antioxidantes indirectos que modulan la actividad de diversas enzimas –inhiben enzimas de fase I e inducen enzimas de fase II–, protegiendo así el ADN frente a agentes carcinógenos y especies reactivas (Vig *et al*., 2009) y reduciendo en definitiva el riesgo de enfermedades cardiovasculares, neurodegenerativas y distintos tipos de cáncer (Fimognari *et al*., 2012). Han demostrado, además, potencial como antifúngicos y bactericidas, de interés tanto para la industria farmacéutica como para la alimentaria (Vig *et al*., 2009; Romeo *et al*., 2018). Sin embargo, tal y como ocurría en los compuestos fenólicos, la funcionalidad de los isotiocianatos está condicionada por la estructura química de la molécula (Ishida *et al*., 2014). Entre los distintos isotiocianatos cabe destacar el sulforrafano, predominante en brócoli, por su elevado potencial protector frente a distintos tipos de cáncer (Techapiesancharoenkij *et al*., 2015; Huang *et al*., 2018; Royston *et al*., 2018). Se ha estudiado también el potencial del purificado de alil-isotiocianato frente a células tumorosas (Srivastava *et al*., 2003; Xiao *et al*., 2003; Savio *et al*., 2014; Rajakumar *et al*., 2015; Sávio *et al*., 2015); este compuesto es, además, usado ampliamente como conservante en alimentos (Vig *et al*., 2009). Por otra parte, se ha discutido el efecto detrimental de los isotiocianatos. Fimognari *et al*. (2012) hacen una revisión extensa en este sentido, comparando el potencial genotóxico con el potencial bioactivo de estas moléculas. De esta revisión se puede concluir que, si bien diferentes isotiocianatos tienen capacidad para dañar el ADN, dicho daño sería dependiente del compuesto y, en cualquier caso, difícilmente producido en las dosis habituales de consumo en una dieta equilibrada.

Más allá del potencial funcional, los productos de hidrólisis de los glucosinolatos son también los responsables del aroma y sabor característicos de esta familia, aportando fuertes notas picantes y azufradas (Bell y Wagstaff, 2017); en la Tabla 6 se resume el flavor característico de algunos. Hay una controversia entre los consumidores en cuanto a la aceptación de estos sabores, hecho que determina en gran medida el

consumo de hortalizas de esta familia en la dieta. Factores como hábitos alimenticios y culturales pueden tener una influencia en la aceptación de estos sabores. Pero existe además una componente genética relacionada con receptores de la lengua que determina el grado de sensibilidad a isotiocianatos y otros productos de degradación (Wieczorek *et al*., 2017), pudiendo influir dicha sensibilidad en la aceptación del alimento. El rechazo en parte extendido de estos sabores supone un punto crítico para el desarrollo de variedades de alto valor funcional añadido, considerando que un aumento del contenido en glucosinolatos puede asimismo reducir la apreciación organoléptica del producto final (Ishida *et al*., 2014).

4.4. Nitratos

El nitrógeno es un elemento esencial que forma parte de biomoléculas tales como aminoácidos, ácidos nucleicos y proteínas. De forma general, las plantas incorporan el nitrógeno por absorción a través de las raíces. En el suelo, este elemento puede estar presente en diversas formas, incluyendo iones inorgánicos y compuestos orgánicos. Sin embargo, la mayoría de cultivos hortícolas incorporan este elemento principalmente en forma de ión nitrato (NO_3^-) (Colla *et al*., 2018). Para obtener rendimientos adecuados, los cultivos requieren cantidades de nitrógeno que pueden exceder las cantidades presentes en el suelo de forma natural (Powlson y Addiscott, 2005). Como consecuencia, una práctica común es la fertilización de los suelos agrícolas, generalmente con fertilizantes inorgánicos.

La distribución de nitratos en la planta es órgano-dependiente. Así, hojas –principalmente el peciolo– y tallos tienden a acumular mayores contenidos, puesto que son los órganos de distribución dentro de la planta (Dechorgnat *et al*., 2011; Colla *et al*., 2018); esto explicaría por qué el contenido en nitratos es generalmente mayor en hortalizas consumidas por su hoja y/o penca que en otros alimentos vegetales. Así, las frutas y hortalizas se pueden clasificar de acuerdo a su capacidad de acumular nitratos, estableciéndose por lo general cuatro grupos: alimentos de contenido en nitratos bajo a muy bajo (< 500 mg kg^{-1}), medio (500 – 1.000 mg kg^{-1}), alto (1.000 – 2.000 mg kg^{-1}) y muy alto contenido (> 2.000 mg kg^{-1}) (Blekkenhorst *et al*., 2017). Hierbas aromáticas de la familia *Apiaceae* tienen en general un contenido alto a muy

alto en nitratos; por su parte, la familia *Brassicaceae* incluye desde hortalizas con muy bajo contenido a otras de contenido alto a muy alto (Fig 13). En particular, la rúcula está descrita como uno de los cultivos con mayor acumulación de nitratos dentro de los alimentos vegetales (Santamaria, 2006; Cavaiuolo y Ferrante, 2014; Colla *et al*., 2018).

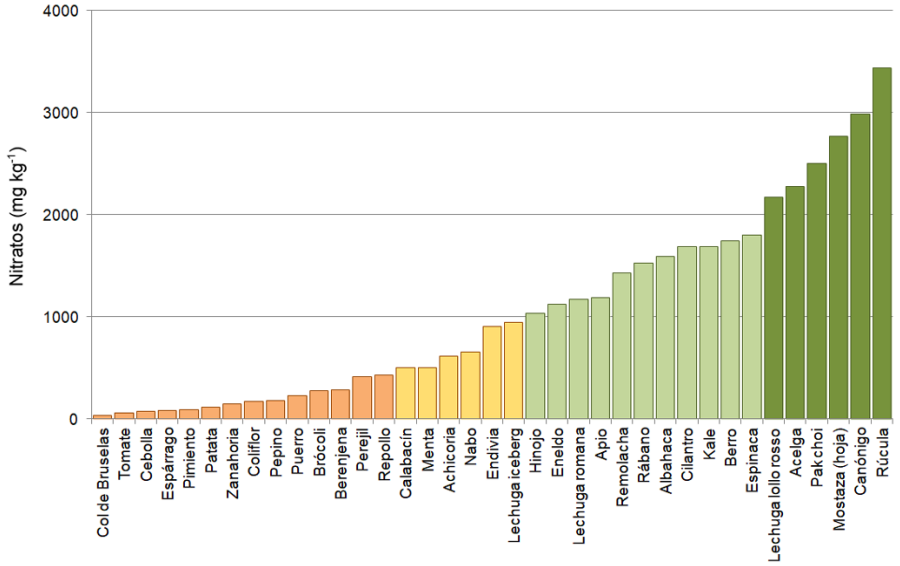

Fig. 13. Contenido en nitratos (mg kg^{-1} MF) de hortalizas y hierbas aromáticas seleccionadas. Adaptado de Blekkenhorst *et al.*, 2017.

Los nitratos contenidos en hortalizas y frutos suponen aproximadamente el 80% del total que el ser humano incorpora con la dieta (Blekkenhorst *et al*., 2017). Los nitratos no tienen en sí un efecto tóxico directo sobre el organismo para individuos adultos y sanos, aunque pueden causar metahemoglobinemia en infantes menores de cuatro meses y grupos altamente sensibles (Bondonno *et al*., 2018). Por el contrario, el riesgo asociado a su consumo para la población en general derivaría de su potencial para formar compuestos *N*-nitroso carcinógenos (como *N*-nitrosaminas y *N*-nitrosamidas) (Lundberg *et al*., 2018), si bien los estudios que relacionan consumo de nitratos y cáncer resultan no concluyentes (Quijano *et al*., 2017). Así pues, el Comité Científico para la Alimentación de la Comunidad

Europea determinó en 1990 el consumo diario de 3,7 mg kg^{-1} de peso como nivel aceptable de seguridad (EFSA, 2008). Posteriormente, se establecieron límites máximos a nivel europeo para la producción comercial de ciertos cultivos, en concreto para la espinaca, lechuga y rúcula, por su alto contenido y/o gran influencia en la dieta (Tabla 7) (European Comission, 2011). Dichos límites pueden ser incluso más restrictivos de acuerdo a legislaciones nacionales concretas, e.g. los límites establecidos en Rusia para la lechuga (Bian *et al.*, 2015). Diversos países, además, han incorporado límites para otros cultivos (Santamaria, 2006).

Tabla 7. Contenido máximo en nitratos (mg kg^{-1}) permitido para la producción comercial de espinaca, lechuga y rúcula en la Unión Europea (European Comission, 2011).

	Contenido	**Especificaciones**
Espinaca	3.500	-
Espinaca procesada (conserva, congelada)	2.000	-
Lechuga fresca excepto lechuga tipo 'iceberg'		*Cosecha: 1 de octubre a 31 de marzo*
	5.000	- Producción protegida[†]
	4.000	- Producción en campo abierto
		Cosecha: 1 de abril a 30 septiembre
	4.000	- Producción protegida
	3.000	- Producción en campo abierto
Lechuga tipo 'iceberg'	2.500	- Producción protegida
	2.000	- Producción en campo abierto
Rúcula[‡]	7.000	*Cosecha: 1 octubre a 31 marzo*
	6.000	*Cosecha: 1 abril a 30 septiembre*

[†]Producción bajo túnel o invernadero. [‡]Incluye las especies *Eruca sativa*, *Diplotaxis* sp., *Brassica tenuifolia* y *Sisymbrium tenuifolium*.

Por otro lado, en las últimas décadas ha aumentado el interés en el beneficio potencial de su consumo. Tal y como recogen las revisiones de Lovegrove *et al*. (2017) y Bondonno *et al*. (2018), los estudios sugieren que los nitratos incorporados con la dieta tienen un efecto positivo en mantener la homeostasis cardiovascular (Larsen *et al*., 2006; Velmurugan *et al*., 2013, 2016). Es más, ya desde la década de 1970 se ha subrayado una correlación negativa entre antioxidantes y la formación de compuestos *N*-nitroso derivados de nitratos (Mirvish, 1975; Helser y Hotchkiss, 1994; Mirvish, 1994), y se ha visto que potentes antioxidantes como ácido ascórbico y compuestos fenólicos tienen un efecto sinérgico de protección (Abraham y Khandelwal, 2013). Es por ello que las dietas ricas en frutas y hortalizas, pese a aportar altos niveles de nitratos, se consideran saludables ya que incorporan igualmente cantidades relevantes de antioxidantes.

5. Factores a considerar para su desarrollo como nuevos cultivos

La explotación de especies silvestres como cultivo conlleva un proceso de selección y adaptación que, idealmente, permite obtener una producción de rendimiento adecuado, a gran escala, uniforme y de calidad óptima dentro de un rango definido –especialmente si se habla de productos de valor funcional añadido, éste debería asegurarse por encima de un mínimo– (Ceccanti *et al*., 2018). Durante este proceso existen diversos puntos críticos a considerar para asegurar el éxito de la adaptación y la calidad del producto obtenido. Dichos puntos incluyen desde la obtención de materiales adecuados y su multiplicación hasta la elección de las prácticas agrícolas que produzcan una calidad óptima.

5.1. Obtención de material reproductivo

Un paso esencial para abordar un programa de domesticación a partir de especies silvestres es la disponibilidad de material reproductivo viable que permita su puesta en cultivo en las instalaciones o campos experimentales destinados para tal fin. Los bancos de germoplasma cuentan con colecciones más o menos extensas de recursos fitogenéticos para

especies cultivadas, incluyendo desde variedades tradicionales hasta especies silvestres emparentadas que pueden utilizarse en programas de mejora de dichos cultivos. Por el contrario, la disponibilidad de colecciones de especies silvestres comestibles es, en la mayoría de casos, nula o muy limitada, al igual que ocurre con los cultivos emergentes (Herraiz *et al*., 2015).

Hasta donde sabemos, no hay entradas de berraza conservadas en bancos de germoplasma. Por el contrario, el grupo de crucíferas de la Universidad Politécnica de Madrid realizó en el pasado importantes tareas de recolección de especies silvestres de la familia (Gómez-Campo, 2007; González-Benito *et al*., 2009), incluyendo una colección considerable de entradas de rabaniza. La colección se fue reduciendo posteriormente y en la actualidad cuenta únicamente con dos entradas de rabaniza y una entrada de la subsp. *longisiliqua* (BGV-UPM, 2019). Así pues, el establecimiento de programas de domesticación de estas dos especies pasa necesariamente por una tarea de prospección y recolección de materiales reproductivos. En el caso de la rabaniza, el material reproductivo incluye la semilla madura todavía retenida en la silicua; para la berraza, por el contrario, se puede recolectar la semilla si se realiza la prospección en el momento de la floración, o bien recolectar material reproductivo vegetativo.

5.1.1. Latencia secundaria y germinación de semillas en rabaniza

En el caso específico de la rabaniza, previamente se ha mencionado la existencia de mecanismos de latencia secundaria en las semillas (Sección 3.2.2). Se define como latencia secundaria la reducción en la capacidad germinativa de la semilla madura, adquirida tras su diseminación y mantenida durante un tiempo más o menos prolongado aun cuando las condiciones ambientales son favorables para su germinación (Naeem *et al*., 2009). Puede desarrollarse en semillas que mantienen todavía cierto grado de latencia primaria o bien en semillas no latentes expuestas a condiciones no favorables para su germinación (Corbineau *et al*., 2014).

La latencia secundaria es un carácter común en especies arvenses; permite que su presencia en el banco de semillas del suelo sea duradera en el tiempo, aumentando así la probabilidad de mantener la población en el

agrosistema (Darmency *et al*., 2017). Se han identificado genes, mecanismos y balances fitohormonales que regulan este carácter, principalmente mediante la relación ácido abscísico/giberelinas; así como factores ambientales tales como la temperatura del suelo, potencial hídrico, presencia/ausencia de luz o contenido en nitratos (Finch-Savage y Footitt, 2017).

El carácter latente está ampliamente descrito entre la familia *Brassicaceae*, principalmente en arvenses pero también en algunas especies cultivadas como la colza (Long *et al*., 2011; Schwabe *et al*., 2019). En el caso de la rabaniza, los resultados de Martínez-Laborde *et al*. (2007) sugieren que las semillas maduras frescas con no latentes; sin embargo, si no están sometidas a condiciones adecuadas para su germinación una vez liberadas de la silicua entran en latencia secundaria y quedan como parte del banco de semillas del suelo. Esto da como resultado una germinación escalonada que aumenta la probabilidad de que alguna población encuentre las condiciones adecuadas para completar su ciclo y producir una nueva generación (Sans y Masalles, 1994). Supone, además, un método de control demográfico de las poblaciones teniendo en cuenta la alta producción de semilla que se puede obtener por individuo.

La entrada en latencia secundaria es, sin embargo, un carácter negativo para su explotación como cultivo. A fin de obtener altos rendimientos y una mayor homogeneidad en el desarrollo del producto final, es necesario que los cultivos tengan una germinación sincronizada; es más, en hortícolas que se comercializan como brotes, la germinación es idealmente rápida, permitiendo varios ciclos de cultivo al año. Se pueden aplicar, no obstante, tratamientos destinados a reducir la latencia de las semillas (Ranil *et al*., 2015; Schwabe *et al*., 2019), los cuales facilitarían una germinación rápida y homogénea.

5.2. Condiciones de cultivo

Otro punto determinante en el desarrollo de nuevos cultivos a partir de especies silvestres es el establecimiento de un método de cultivo adecuado. Existen diversos factores a tener en cuenta para determinar las condiciones

de explotación agrícola de un nuevo cultivo; entre ellos, se podrían destacar como más relevantes: 1) la adaptación de la especie silvestre a las nuevas condiciones de crecimiento; y 2) la obtención de un producto de adecuada calidad visual, organoléptica y funcional (si se busca este valor añadido).

Un método sugerido en diversos trabajos con especies silvestres de hoja es la utilización de sistemas hidropónicos de cultivo, los cuales permiten obtener un producto más limpio al no desarrollarse en contacto con el suelo. El sistema hidropónico se ha utilizado, por ejemplo, en ensayos de colleja, verdolaga, acedera (*Rumex acetosa* L.) o pentinela (*Sanguisorba minor* Scop.) (Egea-Gilabert *et al*., 2013; 2014; Ceccanti *et al*., 2018). Sin embargo, no siempre resulta fácil la adaptación de las infraestructuras a este nuevo sistema. Por el contrario, la producción de hortícolas en campo o invernadero es muy frecuente en regiones mediterráneas. El uso de ambientes similares puede favorecer la aceptación de nuevos cultivos por los productores, siendo mínimas tanto la inversión necesaria como la aplicación de modificaciones en el manejo. Entre estos sistemas existen, sin embargo, diferencias ambientales que pueden afectar la adaptación, desarrollo y calidad del producto final. En condiciones de invernadero se pueden desarrollar cultivos protegidos, con un mayor control de los factores ambientales (principalmente calidad e intensidad de luz, temperatura, humedad y corriente de aire) que minimiza los valores desfavorables extremos para el crecimiento de las plantas. Este sistema, además, limita la actuación de herbívoros y polinizadores.

Las condiciones ambientales tienen efecto sobre la expresión fenotípica de los caracteres. Las plantas responden a condiciones específicas adaptando su desarrollo, morfología y acumulación de metabolitos secundarios, tales como antioxidantes de defensa frente a un aumento de estrés oxidativo. Por ejemplo, Sans y Masalles (1994) determinan en la rabaniza una plasticidad fenotípica o capacidad de adaptación al ambiente según el periodo del año durante el cual se desarrolla el individuo. Así, en comparación con la primavera, las plantas de rabaniza que germinan en otoño desarrollan un ciclo vegetativo más prolongado y una mayor producción de semilla. Otros autores han estudiado el efecto de factores tales como la intensidad de luz, temperatura, disponibilidad de agua e incluso la

fertilización en la morfología, rendimiento y calidad funcional de cultivos hortícolas, pudiéndose encontrar diversos trabajos y revisiones al respecto (e.g., Jin *et al*., 2009; Durazzo *et al*., 2013; Hatfield y Prueger, 2015; Colonna *et al*., 2016; Bell y Wagstaff, 2017; Stagnari *et al*., 2018). Así pues, el análisis de materiales en diversos ambientes (incluyendo diferentes sistemas de cultivo y prácticas, pero también periodo de crecimiento) puede resultar determinante en la obtención de nuevas variedades y cultivos, tal y como apuntan Stommel *et al*. (2015).

En resumen, el proceso de revalorización de especies silvestres y explotación como nuevos cultivos de alto valor funcional añadido pasaría idealmente por la recolección o cesión de materiales, estudio de su perfil funcional e identificación de los metabolitos de mayor interés a considerar en el programa de domesticación, comparación entre distintos materiales para la selección de los más adecuados desde un punto de vista de adaptación y calidad morfológica, funcional y organoléptica, y la selección de las prácticas agrícolas más adecuadas para potenciar dicha calidad.

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Objetivos

El objetivo principal de esta Tesis Doctoral es evaluar el potencial de dos especies silvestres destacadas por su calidad funcional para su domesticación y adaptación a cultivo en condiciones mediterráneas. Esta Tesis pretende además profundizar en el conocimiento del perfil volátil de las especies seleccionadas, dada su importancia en determinar su aroma y sabor y, en consecuencia, la aceptación por el consumidor. Como especies se ha seleccionado la berraza (*A. nodiflorum*) y la rabaniza (*D. erucoides* subsp. *erucoides*), dada la buena adaptación de ambas especies a nuestra región. Con este trabajo se pretende ampliar el conocimiento actual de ambas especies en términos de calidad funcional, volátil y aspectos agronómicos, así como establecer una base para su desarrollo como nuevos cultivos adaptados a nuestras condiciones.

Para cumplir este objetivo principal, el trabajo se ha estructurado en cuatro bloques diferenciados, de los cuales el primero hace referencia a la especie *A. nodiflorum* y el segundo, a *D. erucoides*:

1. Estudiar el valor añadido de la berraza como hortaliza silvestre. Éste incluye el valor funcional centrado en el contenido en fenoles totales y capacidad reductora de radicales libres, y su perfil en agliconas fenólicas; así como el valor de su perfil volátil.

2. Estudiar el potencial e interés de materiales autóctonos de rabaniza para su selección y domesticación. El bloque en su conjunto incluye la caracterización morfológica, nutracéutica y aromática de materiales prospectados, así como el estudio de la variabilidad registrada. Incluye además un primer análisis de bioaccesibilidad como consecuencia de una digestión *in vitro*.

3. Evaluar la capacidad de adaptación de la rabaniza a condiciones de cultivo y la superación de problemas asociados a la puesta en cultivo.

3.1. Superar la germinación deficiente de semilla madura de rabaniza consecuencia de la entrada en latencia secundaria con el fin de aumentar la capacidad germinativa de las poblaciones y obtener una germinación uniforme.

3.2. Evaluar la adaptación a cultivo en dos sistemas modelo, invernadero y campo, y el efecto del ciclo de cultivo. La capacidad de adaptación se evalúa a partir de la calidad visual y funcional obtenida en distintas condiciones.

4. Evaluar la aceptación de la rabaniza en distintos estados fenológicos por consumidores potenciales, y su relación con el perfil volátil.

Resultados

Capítulo 1. Valor añadido de la berraza como hortaliza silvestre: capacidad antioxidante y perfil aromático

1. 1. Wild edible fool's watercress, a potential crop with high nutraceutical properties

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Keywords: Antioxidants, *Apium nodiflorum*, DPPH, flavonoids, new crops, quercetin, total phenolics, wild edible plants

Guijarro-Real, C., Prohens, J., Rodriguez-Burruezo, A., Adalid-Martínez, A., López-Gresa, M.P., Fita, A. 2019. Wild edible fool's watercress, a potential crop with high nutraceutical properties. PeerJ, 7, e6296

DOI: 10.7717/peerj6296

Abstract

Background. Fool's watercress (*Apium nodiflorum*) is an edible vegetable with potential as a new crop. However, little information is available regarding the antioxidant properties of the plant and the individual phenolics accounting for this capacity are unknown.

Methods. The antioxidant properties of twenty-five wild populations were analysed and individual phenolics present in the species reported and compared with celery and parsley. The antioxidant activity was measured as the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging capacity, and the total phenolics content (TPC) via the Folin-Ciocalteu procedure. The individual phenolics constituents were determined via high performance liquid chromatography (HPLC) as aglycones.

Results. The average DPPH and TPC of fool's watercress were 28.1 mg Trolox g^{-1} DW and 22.3 mg of chlorogenic acid equivalents g^{-1} DW, respectively, much higher than those of celery and parsley. Significant differences for both DPPH and TPC, which may be explained by either genotype or environmental factors, were detected among groups established according to geographical origin. Quercetin was identified as the major phenolic present in the leaves of the species, unlike parsley and celery, in which high amounts of apigenin and luteolin were determined. Quercetin represented 61.6% of the phenolics targeted in fool's watercress, followed by caffeic acid derivatives as main hydroxycinnamic acids.

Discussion. The study reports the high antioxidant properties of fool's watercress based on a large number of populations. Results suggest that quercetin accounts for an important share of the antioxidant capacity of this potential new crop. The study also provides a basis for future breeding programs, suggesting that selection by geographical locations may result in differences in the antioxidant properties.

Introduction

Wild fruits and vegetables are part of the traditional cuisine of many countries of the Mediterranean region. Besides enriching the cuisine with particular tastes, many of them have also been used in the past as dietary supplements or sources of bioactive compounds, as well as in traditional medicine (Shikov *et al.*, 2017). In the last decades, there has been an increasing interest in wild vegetables by consumers. Consequently, several works have evaluated the nutritional value of wild edible species and also assessed their bioactive health promoting properties (Motamed & Naghibi, 2010; Egea-Gilabert *et al.*, 2013; García-Herrera *et al.*, 2014). Moreover, domestication of wild species to be grown as new crops is an opportunity for increasing the offer in food markets. As examples, salad rocket (*Eruca sativa* Mill. and *Diplotaxis tenuifolia* (L.) DC.) and watercress (*Rorippa nasturtium-acquaticum* Hayek) have been adapted and developed as common crops (Molina, Pardo-de-Santayana & Tardío, 2016).

Apium nodiflorum (L.) Lag., commonly known as fool's watercress or water celery, is a perennial herb from *Apiaceae* family. Well adapted to damp soils, it can be easily found, forming clamps, in fresh, shallow water courses such as streams or ditches. The species is broadly distributed along the temperate areas of central and southern Europe, northern Africa and western and central Asia (Tardío *et al.*, 2016). It is widely distributed in Spain, including the Mediterranean coast (Knees, 2003), a region with an ancient agricultural tradition. However, the alteration in the irrigation system to drip irrigation in agriculture and the reduction of river flows may negatively affect its natural distribution.

Wild fool's watercress has been traditionally harvested and consumed in several Mediterranean countries, such as Spain, Italy, Portugal or Morocco (Tardío *et al.*, 2016). The edible parts are the young leaves and tender shoots, which are used as a vegetable and mainly consumed raw in salads, or to a lesser extent boiled or included as a condiment in soups and other dishes (Parada, Carrió & Vallès, 2011; Guarrera and Savo, 2016). The species has been reported as appetite enhancer, diuretic, intestinal anti-inflammatory, antimicrobial and antifungal (Menghini *et al.*, 2010; Maxia *et al.*, 2012; Guarrera & Savo, 2013; Tardío *et al.*, 2016). However, the

nutritional and bioactive value of the species has not been extensively studied. García-Herrera (2014) classified fool's watercress as a vegetable with high content in calcium and sodium, although its consumption should be moderate for people with kidney damage due to the content in oxalic acid, as revealed by Morales (2011). The plant may be also considered as a source of vitamin E and B_9 (Tardío *et al.*, 2016). But the greatest interest considering its nutritional capacity is probably due to the high content in phenolic compounds together with the strong antioxidant activity that presents (Morales *et al.*, 2012).

Phenolic compounds can be included into different categories attending to their chemical composition, being flavonoids and phenolic acids the most common classes in plants (Zhou *et al.*, 2016). They commonly appear as glycosides in plants, conjugated to other molecules such as sugars, amines, organic acids or other phenolic compounds (Barba, Esteve & Frígola, 2014). Besides the importance of these metabolites for plants defence and survival (Cartea *et al.*, 2011), flavonoids and phenolic acids are considered of great importance for human health due to their antioxidant capacity (Kaushik *et al.*, 2015; Sahidi & Ambigaipalan, 2015). As antioxidants, they neutralize reactive oxygen species, which in excess can produce molecular and cellular disorders causing several diseases (Prasad, Gupta & Tyagi, 2017). However, this capacity is greatly dependent on the chemical structure of each molecule (Zaluski, CIesla & Janeczko, 2015).

Flavonoids and phenolic acids are commonly found in *Apiaceae* (Sayed-Ahmad *et al.*, 2017) and daily used spices and aromatic herbs of the family have been studied for these compounds. For instance, leaves of parsley (*Petroselinum crispum* (Mill.) Nyman) and celery (*Apium graveolens* L. var. *dulce*) are good sources of apigenin (Pápay *et al.*, 2016; Zhou *et al.*, 2017), with levels that can reach 630 mg 100 g^{-1} FW (Justesen & Knuthsen, 2001) and 970 mg 100 g^{-1} FW (Yao & Ren, 2011), respectively. Polyphenol glycosides including apigenin, quercetin, chlorogenic acid, caffeic acid or ferulic acid derivatives have been detected in fennel (*Foeniculum vulgare* Mill.) (Salami, Rahimmalek, & Ehtemam, 2016). And Barros *et al.* (2012) determined that coriander leaves (*Coriandrum sativum* L.) are rich in quercetin derivatives, with a total value of 494 mg 100 g^{-1} DW, and also

present relevant contents of *p*-coumaric acid derivatives. However, we have not found references to the phenolic constituents of the edible organs of fool's watercress.

We consider that there is a need to evaluate the antioxidant properties and phenolic composition of fool's watercress since this edible species has potential as a source of antioxidants. So far, little information on the diversity for phenolics content and antioxidant activity in the species is available (Morales *et al.*, 2012). The study of several populations may offer more accurate information for the antioxidant properties and phenolic content of the species. Thus, in the present study we evaluated the antioxidant activity of a set of populations of fool's watercress. We also determined the main phenolic compounds in an attempt to correlate them with the antioxidant properties of this species. We included two related crops with similar uses in the analysis (celery and parsley) in order to compare data of wild and related cultivated species. The results obtained may be also useful for considering the domestication of fool's watercress.

Materials and methods

Plant material and sample preparation

The Horta Nord of Valencia (Spain), an area with many irrigation ditches used for centuries by the farmers, was prospected. The prospection took place in the spring season of 2015 and was focused on the locations where ditches are still in use and a regular water flow is provided (Fig. 1). A total of twenty-five wild isolated masses of fool's watercress were sampled. Samples were grouped by their geographical origin and seven groups were established according to the following geographical areas: Puerto de Sagunto (FW1), Puzol-El Puig (FW2), Masamagrell (FW3), Albuixech-Albalat dels Sorells (FW4), Foios-Meliana town (FW5), Meliana beach-Alboraya-Valencia (FW6) and Pueblo Nuevo-Alfara del Patriarca (FW7) (Table 1).

Table 1. Geographical situation of the wild populations of fool's watercress harvested in the region of Valencia (Spain) and identification of the groups established by their origin.

Geographical group[a]	Population[b]	Location	Coordinates
FW1	Nod-001	Puerto de Sagunto	39º 37' 36" N 0º 16' 49" W
	Nod-002	Puerto de Sagunto	39º 37' 55" N 0º 16' 11" W
FW2	Nod-003	Puzol	39º 36' 08" N 0º 18' 08" W
	Nod-004	El Puig	39º 35' 09" N 0º 17' 45" W
	Nod-005	El Puig	39º 36' 00" N 0º 18' 05" W
	Nod-006	El Puig	39º 35' 33" N 0º 19' 16" W
FW3	Nod-007	Masamagrell	39º 34' 04" N 0º 19' 42" W
	Nod-008	Masamagrell	39º 33' 59" N 0º 19' 48" W
	Nod-009	Masamagrell	39º 33' 42" N 0º 18' 25" W
FW4	Nod-010	Albuixech	39º 33' 04" N 0º 19' 39" W
	Nod-011	Albuixech	39º 32' 37" N 0º 19' 55" W
	Nod-012	Albuixech	39º 32' 29" N 0º 19' 27" W
	Nod-013	Albuixech	39º 32' 43" N 0º 19' 07" W
	Nod-014	Albalat dels Sorells	39º 32' 04" N 0º 19' 26" W
FW5	Nod-015	Foios	39º 31' 59" N 0º 20' 31" W
	Nod-016	Foios	39º 32' 16" N 0º 20' 49" W
	Nod-017	Meliana (town)	39º 31' 26" N 0º 20' 52" W
FW6	Nod-018	Meliana (beach)	39º 31' 01" N 0º 19' 36" W
	Nod-019	Alboraya	39º 30' 59" N 0º 19' 37" W
	Nod-020	Valencia	39º 28' 57" N 0º 20' 11" W
FW7	Nod-021	Pueblo Nuevo	39º 30' 39" N 0º 22' 57" W
	Nod-022	Pueblo Nuevo	39º 30' 39" N 0º 23' 10" W
	Nod-023	Pueblo Nuevo	39º 31' 19" N 0º 23' 17" W
	Nod-024	Alfara del Patriarca	39º 31' 37" N 0º 22' 36" W
	Nod-025	Alfara del Patriarca	39º 32' 21" N 0º 23' 06" W

[a] Codes FW1 to FW7 refer to the seven geographical groups in which the populations of fool's watercress have been clustered. [b] Nod-001 to Nod-025 refer to the codes given to the twenty-five populations of fool's watercress analysed in the study.

The aerial part was air-dried in oven at 37 ºC, with low humidity conditions, for three days, in order to prevent water activity (Fig. 1). Dried samples were powdered with a commercial grinder and used for the determinations, which were carried out in triplicates. For comparison of results, celery and parsley species were also analysed. Thus, two commercial samples of celery, and two commercial samples of parsley, each one coming from different local markets, were acquired and processed in the same way than samples of fool's watercress.

Fig. 1. Plant of fool's watercress. A. Wild population growing in an irrigation ditch. B. Sample representing the edible part of this plant.

Evaluation of the antioxidant activity and total phenolics content

The antioxidant activity was measured as the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging capacity as described by Rufino *et al.* (2007). Subsamples of 0,1 g were extracted with 5 mL methanol (50 % v/v) plus 5 mL acetone (70 % v/v), then samples of fool's watercress diluted (1:2). Absorbance was measured at 515 nm after 25 minutes of incubation with DPPH (Sigma-Aldrich, Sant Louis, MO, USA) solution (0.025 g/L in methanol). The antioxidant Trolox (Scharlab S.L., Sentmenat, Barcelona, Spain) was used as standard and results were expressed as milligrams of Trolox equivalents per gram of dry weight (mg Trolox g^{-1} DW).

Total phenolics were extracted from subsamples of 0.125 g with 5 mL acetone (70 % v/v) containing glacial acetic acid (0.5 % v/v) and determined

according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) as indicated in Plazas *et al.* (2014). Absorbance was measured at 750 nm after 95 minutes of incubation with the diluted Folin-Ciocalteu reagent (10 % v/v) (Scharlab S.L.). Chlorogenic acid (Sigma-Aldrich) was used as standard and results were expressed as milligrams of chlorogenic acid equivalents per gram of dry weight (mg CAE g^{-1} DW).

Phenolics profile

Phenolic compounds were extracted from subsamples of 0.1 g with 1.5 mL methanol (80 % v/v) including 0.1 % (w/v) 2,6-di-*tert*-butyl-4-methylphenol (BHT) (Sigma-Aldrich) (Plazas *et al.*, 2014). Then, a hydrolysis was performed by adding 1.2 M HCl for two hours at 95 ºC for fool's watercress and celery, and 2 M HCl for four hours for parsley (Justesen and Knuthsen, 2001), to a final methanol solution of 50% (v/v).

Samples were analysed on a HPLC 1220 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA). A BRISA C18 column (150 mm x 4.6 mm i.d., 3 µm particle size; Teknokroma, Barcelona, Spain) was used and the injection volume was 10 µl. Mobile phase consisted of two solvents, (A) 0.1% formic acid in water and (B) methanol with gradient elution. Hydroxycinnamic acids profile was studied under the following conditions (Yildiz *et al.*, 2008): starting with 7% (B) the first 8 min, raising up to 30% (B) at 13 min, 66% (B) at 48 min, 75% (B) at 50 min, 100% (B) at 54 min and maintained for 2 min, then decreasing to initial conditions of 7% (B) at 60 min and equilibrated for 5 minutes. Flow rate was 1 mL min^{-1} and the absorbance was fixed at 320 nm. The study of flavonoids was performed as described by Bae *et al.* (2012) with a flow rate of 0.8 mL min^{-1} and a fixed absorbance of 360 nm, using the same solvents as above. The gradient elution started with 40% (B) following to 100% (B) at 10 min and maintained for 5 minutes, then decreasing to the initial conditions at 20 min and equilibrated for 5 minutes.

A tentative identification of the compounds was performed by comparison of the retention time from the peaks with commercial standards (Sigma-Aldrich), and with published data. Standards from common phenolics described in *Apiaceae* were selected and used in this study (Table

2), which their general chemical structures are represented in Fig. 2. Due to the possible partial hydrolysis of chlorogenic acid to caffeic acid under the above cited conditions, concentration of both compounds were added and considered together as caffeic acid derivatives.

Table 2. Phenolic aglycones commonly cited in the literature for the family *Apiaceae*. It is indicated the class classification according to the chemical structure as well as the retention time in the current conditions (Rt, min).

Rt[†]	Compound	Class	References
8.0	Quercetin	Flavonol	Justesen, 2000; Justesen & Knuthsen, 2001; Barros *et al.*, 2012; Vallverdú-Queralt *et al.*, 2014; Salami, Rahimmalek & Ehtmam, 2016
8.4	Luteolin	Flavone	Crozier *et al.*, 1997; Justesen, 2000; Justesen & Knuthsen, 2001; Viña & Chaves, 2007; Yildiz *et al.*, 2008; Barros *et al.*, 2012
9.0	Kaempferol	Flavonol	Justesen, Knuthsen & Leth, 1998; Justesen, 2000; Justesen & Knuthsen, 2001; Yao *et al*,. 2010, ; Yao & Ren, 2011; Barros *et al.*, 2012
9.2	Apigenin	Flavone	Crozier *et al.*, 1997; Justesen & Knuthsen, 2001; Yildiz *et al.*, 2008; Hossain *et al.*, 2011; Barros *et al.*, 2012
16.3	Chlorogenic acid	Hydroxy-cinnamic acid	Viña & Chaves, 2007; Barros *et al.*, 2012; Vallverdú-Queralt *et al.*, 2014; Salami, Rahimmalek & Ehtmam, 2016
17.3	Caffeic acid	Hydroxy-cinnamic acid	Yao *et al.*, 2010; Hossain *et al.*, 2011; Yao & Ren, 2011; Vallverdú-Queralt *et al.*, 2014, Salami, Rahimmalek & Ehtmam, 2016
20.7	*p*-Coumaric acid	Hydroxy-cinnamic acid	Yao *et al.*, 2010; Yao & Ren, 2011; Barros *et al.*, 2012; Vallverdú-Queralt *et al.*, 2014, Salami, Rahimmalek & Ehtmam, 2016
21.9	Ferulic acid	Hydroxy-cinnamic acid	Yao *et al.*, 2010; Yao & Ren, 2011; Barros *et al.*, 2012; Vallverdú-Queralt *et al.*, 2014, Salami, Rahimmalek & Ehtmam, 2016

[†]Rt obtained in the conditions described by Yildiz *et al.*, 2008 (hydroxycinnamic acids) or Bhae *et al.*, 2012 (flavones and flavonols).

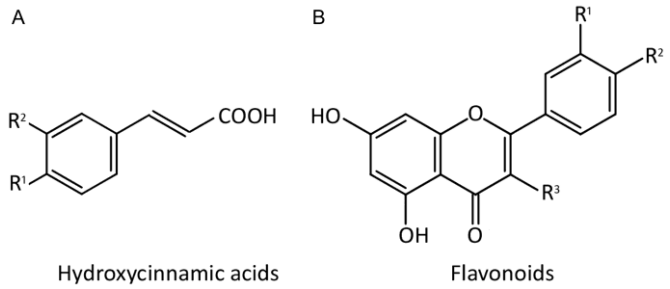

Fig. 2. Chemical structure of the phenolic compounds evaluated in the samples of fool's watercress, celery and parsley. The phenolics targeted included the following hydroxycinnamic acids: caffeic acid (R^1=R^2=OH), chlorogenic acid (R^1=R^2=OH plus the carboxylic group esterified with quinnic acid), *p*-coumaric acid (R^1= OH, R^2=H) and ferulic acid (R^1= OH, R^2=OCH_3); and the flavonoids: apigenin (R^1=OH, R^2=R^3=H), kaempferol (R^1=R^3=OH, R^2= H), luteolin (R^1=R^2=OH, R^3= H) and quercetin (R^1=R^2=R^3=OH).

Data analysis

The average values of DPPH and total phenolics content (TPC) in each sample were used to obtain the mean value and the average standard error of the seven geographical groups of fool's watercress (FW1-FW7), celery (GRAV, as average of two samples, Grav-01 and Grav-02), and parsley (CRI, as average of two samples, Cri-01 and Cri-02). Data were analysed using a one-way factorial analysis of variance (ANOVA) considering the groups as a factor and significant differences between groups were calculated with the Student-Newman-Keuls test. Ten selected populations of fool's watercress with low and high antioxidant activities and overall representing four geographical groups, plus the two samples of celery and the two of parsley, were analysed for phenolic profile by HPLC, in triplicates. Finally, Pearson pairwise comparisons were performed in order to evaluate correlations between DPPH, TPC and the content in phenolics determined as sum of the individual phenolics targeted.

Table 3. Mean values and range for DPPH radical-scavenging activity and TPC for fool's watercress, celery, and parsley groups. N is the number of populations included in each group. Statistics includes the mean squares values (MS) for accession and residuals, and the value of the *F*-test for differences among groups.

		(mg Trolox g^{-1} DW)		*(mg CAE g^{-1} DW)*	
Group[a]	**N**	**DPPH[†]**	**Range**	**TPC[†]**	**Range**
FW1	2	28.19 cd	(22.84-33.55)	24.16 c	(23.12-25.21)
FW2	4	15.17 bc	(10.34-18.20)	15.90 ab	(13.26-18.91)
FW3	3	28.61 cd	(19.95-36.20)	23.73 c	(21.31-26.97)
FW4	5	28.29 cd	(19.86-38.22)	22.73 c	(18.58-27.56)
FW5	3	25.67 cd	(21.14-29.59)	19.82 bc	(18.55-21.82)
FW6	3	32.23 d	(30.74-33.82)	23.89 c	(23.17-24.39)
FW7	5	36.97 d	(27.96-43.51)	26.19 c	(21.19-28.91)
GRAV	2	8.09 ab	(7.47-8.71)	13.32 a	(12.71-13.93)
CRI	2	2.20 a	(1.87-2.52)	14.98 ab	(12.58-17.38)
MS group		*373.28*		*64.28*	
MS residual		*36.98*		*7.02*	
Prob *F*-test		<0.0001		<0.0001	

[a]Groups FW1 to FW7 refer to the seven geographical groups in which the populations of fool's watercress have been clustered (See Table 1). GRAV refers to the celery group, including Grav-01 and Grav-02 samples. CRI refers to the parsley group, including Cri-01 and Cri-02 samples. [†]Different letters indicate significant differences according to the Student-Newman-Keuls test (confidence level 95.0%).

Results

DPPH radical-scavenging activity and TPC

A highly significant variation ($P < 0.001$) between fool's watercress and celery and parsley was found for the DPPH scavenging capacity (Table 3). The average DPPH capacity of fool's watercress was 28.12 mg Trolox g^{-1} DW. This value was 3.5-fold higher than the DPPH value for celery (8.09 mg Trolox g^{-1} DW) and 12.8-fold higher compared to parsley (2.20 mg Trolox g^{-1} DW). Values of the seven geographical groups considered in fool's watercress ranged from 15.17 to 36.97 mg Trolox g^{-1} DW (FW2 and

FW7, respectively; $P < 0.01$), with continuous variation among them (Table 3).

In the case of TPC, differences were less remarkable in absolute values but also highly significant ($P < 0.001$) (Table 3). Celery and parsley presented similar average values of 13.32 mg of chlorogenic acid equivalents (CAE) g^{-1} DW and 14.98 mg CAE g^{-1} DW, respectively, while the average content determined for fool's watercress was 22.35 mg CAE g^{-1} DW. However, the variation in this species ranged from 15.90 (FW2) to 26.19 mg CAE g^{-1} DW (FW7), which meant that geographical groups with the lowest content were comparable to celery and parsley. As in the DPPH radical-scavenging activity, a continuous variation was observed for the total of groups established with significant differences ($P < 0.01$).

Phenolic profile of fool's watercress, celery, and parsley

Ten populations of fool's watercress from diverse geographical groups (FW2, FW4, FW5 and FW7) and overall representing samples with low and high antioxidant capacity and TPC were used for analysing the phenolic profile of the species and comparing it with those of celery and parsley (Fig S1). The content in phenolics of fool's watercress, obtained as sum of individual phenolics, ranged from 1.20 to 7.12 mg g^{-1} DW (for populations Nod-004 and Nod-021, respectively), with a mean value of 4.19 mg g^{-1} DW (Table 4). Samples with the highest values corresponded to the geographical group FW7 (mean value 6.71 mg g^{-1} DW) while those with low content belonged to the geographical groups FW2 and FW5 (mean values 1.92 and 2.76 mg g^{-1} DW, respectively). Populations from geographical group FW4 showed intermediate content (mean value 4.15 mg g^{-1} DW). On the other hand, the mean values for celery and parsley were 7.63 and 4.76 mg g^{-1} DW, respectively.

In addition, the relative content of each compound against the sum of the phenolics targeted was determined (%, indicating mg of each compound mg^{-1} of total compounds) (Fig. 3). In general, flavonoid compounds comprised the most representative group considering the total identified, with an average relative abundance of 76.4%, 92.7% and 97.0% for fool's

watercress, celery and parsley, respectively. Nevertheless, the profile of individual phenolics varied considerably between species, both qualitatively and quantitatively. Quercetin was the major flavonoid in fool's watercress (61.6%), while this flavonoid represented less than 0.3% in celery and parsley. On the contrary, apigenin was found as the major phenolic in parsley (90.7%) but it only represented 0.8% in fool's watercress. This flavonoid ranked second in concentration in celery (35.8%), after luteolin (56.3%). Compared to celery, luteolin abundance was 6.6-fold lower in fool's watercress and 11-fold lower in parsley. Finally, kaempferol was present in fool's watercress in a relative abundance of 4.3% in contrast with parsley, in which represented only 0.9%. This compound was not detected in celery.

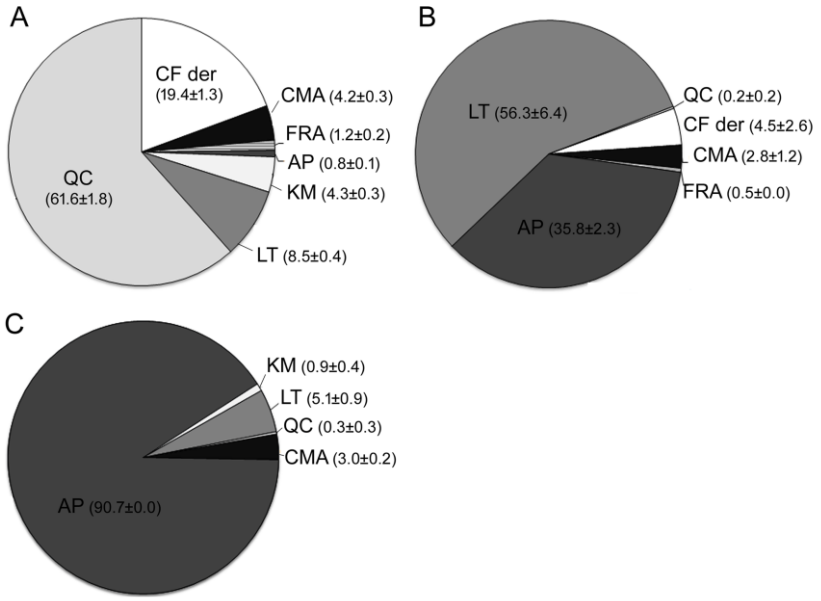

Fig. 3. Relative abundance (µg compound μg^{-1} total) of individual phenolics identified. A. Phenolics identified in the samples of fool's watercress. B. Phenolics identified in the samples of celery. C. Phenolics identified in the samples of parsley. The abbreviations correspond to: apigenin (AP), caffeic acid derivatives (CF der), *p*-coumaric acid (CMA), ferulic acid (FRA), kaempferol (KM), luteolin (LT) and quercetin (QR).

Table 4. Mean values for the content of individual phenolics targeted, DPPH radical-scavenging activity and TPC in individual samples of fool's watercress, celery, and parsley. The content of each phenolic targeted is expressed as μg g^{-1} DW, the sum of the individual phenolics targeted (Σ i.p.) as mg g^{-1} DW, the DPPH as mg Trolox g^{-1} DW and the TPC as mg CAE g^{-1} DW. Statistics includes the mean squares values (MS) for accession and residuals, and the value of the *F*-test for differences among groups.

	CF der	**CMA**	**FRA**	**AP**	**KM**	**LT**	**QR**	**Σ i.p.**	**DPPH**	**TPC**
Nod-003[a]	640.9	139.9	55.8	12.2	91.2	214.8	1478.0	2.6	17.6	15.1
Nod-004	203.4	43.9	12.3	15.1	67.7	105.8	751.1	1.2	10.3	13.3
Nod-011	445.6	89.7	28.7	19.9	172.5	215.2	2232.7	3.2	21.2	18.6
Nod-013	935.5	238.8	43.3	33.7	207.8	400.3	2567.7	4.4	38.2	27.6
Nod-014	1100.7	213.1	96.3	43.2	146.4	490.3	2729.0	4.8	37.9	24.8
Nod-016	449.7	107.7	32.0	21.7	132.1	184.9	1920.0	2.8	21.1	18.5
Nod-017	703.7	146.9	42.6	22.2	112.4	226.6	1407.6	2.7	26.3	19.1
Nod-021	1178.7	260.8	56.7	54.9	235.7	652.0	4684.2	7.1	39.2	28.2
Nod-022	1041.6	228.9	48.6	36.1	295.7	448.7	4004.1	6.1	39.6	26.5
Nod-024	1299.5	250.5	65.3	59.0	231.4	747.1	4252.3	6.9	43.5	28.9
Mean	799.9	172.0	48.1	31.8	169.3	368.6	2602.6	4.2	29.5	22.0
Cri-01	-	138.7	-	3828.1	54.1	179.8	23.0	4.2	1.9	12.6
Cri-02	-	149.0	-	4815.7	27.3	319.6	-	5.3	2.5	17.4

Mean	-	143.8	-	4321.9	40.7	249.7	11.5	4.8	2.2	15.0
Grav-01	511.0	287.8	33.9	2742.5	-	3593.3	32.6	7.2	7.5	12.7
Grav-02	148.9	128.0	36.2	2695.3	-	5058.0	-	8.1	8.7	13.9
Mean	329.9	207.9	35.0	2718.9	-	4325.6	16.3	7.6	8.1	13.3
MS										
species	$1.3 \cdot 10^{6}$	$2.1 \cdot 10^{3}$	$2.9 \cdot 10^{2}$	$1.9 \cdot 10^{7}$	$2.8 \cdot 10^{4}$	$1.3 \cdot 10^{7}$	$9.6 \cdot 10^{6}$	9.9	$8.6 \cdot 10^{2}$	90.6
residual	$1.3 \cdot 10^{3}$	$5.9 \cdot 10^{3}$	$4.7 \cdot 10^{2}$	$4.5 \cdot 10^{4}$	$4.8 \cdot 10^{3}$	$1.4 \cdot 10^{5}$	$1.4 \cdot 10^{6}$	3.4	$1.1 \cdot 10^{2}$	28.5
P. *F*-test	0.022	0.712	0.454	<0.001	0.037	<0.001	0.013	0.098	0.007	0.082

[a]Samples Nod-003 to Nod-024 refer to the ten samples of species fool's watercress evaluated. Samples Cri-01 and Cri-02 refer to the two samples of parsley evaluated. Samples Grav-01 and Grav-02 refer to the two samples of celery evaluated. CF der: caffeic acid derivatives. CMA: *p*-coumaric acid. FRA: ferulic acid. AP: apigenin. KM: kaempferol. LT: luteolin. QR: quercetin.

Differences in the composition of phenolic acids were also determined (Fig. 3). The only hydroxycinnamic acid detected in leaves of parsley was *p*-coumaric and represented 3.0% of the total phenolics. On the contrary, the four hydroxycinnamic acids targeted were detected in fool's watercress and celery. The relative concentration of these compounds in leaves of celery ranged from 0.5% for ferulic acid to 4.5% for caffeic acid derivatives. Ferulic acid was also the minor phenolic acid detected in fool's watercress (1.2%), followed by *p*-coumaric acid (4.2%). Caffeic acid derivatives were determined as the main hydroxycinnamic acids of fool's watercress (19.4%).

Correlation between antioxidant parameters

The Pearson linear correlation coefficient (r) between the DPPH radical-scavenging activity and TPC in fool's watercress populations was r = 0.903 ($P < 0.001$). For those samples of fool's watercress that were analysed by HPLC, correlation coefficient values between DPPH, TPC and the content in targeted phenolics were also studied. The content in targeted phenolics as sum of the individual compounds presented high correlation coefficients with both the DPPH scavenging activity and TPC (r = 0.924 and r = 0.933, respectively; $P < 0.001$).

Discussion

The present study highlights the antioxidant capacity of fool's watercress in terms of DPPH scavenging activity and TPC. These results are in agreement with published data. For example, Morales *et al.* (2012) analysed four wild leafy vegetables (*A. nodiflorum, F. vulgare*, *Montia fontana* L. and *Silene vulgaris* (Moench) Garcke.) and obtained the highest values for DPPH and TPC in fool's watercress. In addition, fool's watercress also has high antioxidant activity compared to other common aromatic herbs and spices from the same family. Hossain *et al* (2011) evaluated those parameters for fennel, celery, cumin and parsley. Values of DPPH-radical scavenging activity in these spices were 1.7 to 9.7-fold lower than the antioxidant capacity of fool's watercress. On the contrary, the TPC

calculated there for the four spices were quite similar to the range determined in fool's watercress.

Comparison among different geographical groups of fool's watercress revealed moderate differences for the TPC and the DPPH radical scavenging activity. The production and accumulation of these secondary metabolites can be affected by the environmental conditions and stress situations as well as by genetic diversity (Kaulmann *et al.*, 2014; Galieni *et al.*, 2015). Thus, differences between geographical groups may result from genotypic differences or by divergence of particular environmental conditions. The evaluation of genetic and environmental effects needs to be studied in future selection programmes aimed at the development of fool's watercress as a new crop with high antioxidant properties as added value.

Our results also reveal differences in the phenolic profile of the three species. Fool's watercress displayed low amounts of apigenin and luteolin in contrast to parsley and celery, vegetables described as sources of these compounds (Zhou *et al.*, 2016). On the contrary, quercetin was detected as the main flavonoid of fool's watercress. Quercetin has been also detected within the *Apiaceae* in fresh herbs such as coriander, dill or fennel (Barros *et al.*, 2012; Salami, Rahimmalek & Ehtmam, 2016; El-Zaeddi *et al.*, 2017). Regarding to the hydroxycinnamic acids studied (caffeic acid, chlorogenic acid, *p*-coumaric acid and ferulic acid), the four of them have been previously detected in fennel (Salami, Rahimmalek & Ehtmam, 2016); these authors found that chlorogenic acid was the main phenolic acid. Chlorogenic acid is rapidly hydrolysed to caffeic acid in alkaline conditions (Mattila & Kumpulainen, 2002), while it is considered more stable at low pH. However, we noted a partial hydrolysis of chlorogenic acid to caffeic acid under the current conditions. Due to this reason, we considered both hydroxycinnamic acids, it is, chlorogenic and caffeic acids, together as caffeic acid derivatives, and the individual values were not given.

In agreement with our results, positive, strong correlation between DPPH and TPC has been previously established in other vegetables, including common spices and wild species (Morales *et al.*, 2014; Skotti *et al.*, 2014; Tang *et al.*, 2015). However, in other cases this correlation was not so clear (Albano & Miguel, 2011). Discrepancies may be explained in part

by the composition of the evaluated matrix, as well as the possible interferences of different compounds others than phenolics with the reagents, as they could be tocopherols, aminoacids or, commonly to many fruits, ascorbic acid (Craft *et al.*, 2012). However, the ascorbic acid is an unstable metabolite highly sensitive that can be easily degraded with the consequence of losing the antioxidant capacity. Conditions such as exposure to oxygen (e.g., during the extraction step and storage of extracts), humidity, or temperature of drying and store can affect the stability of ascorbic acid (e.g. Kaya, Aydin & Kolayli, 2010; Van Bree *et al.*, 2012). Moreover, this molecule is commonly stabilized with *meta*-phosphoric, in order to preserve it from degradation during storage of the extract (Chebrolu *et al.*, 2012). On the contrary, phenolic compounds are stable molecules, not affected by the drying process (Bianchi & Lo Scalzo, 2018). Our results suggest that the antioxidant capacity of the dried leaves in this species is mainly due to the phenolic compounds, according to the high correlation established between the two parameters. In the same way, the high correlation between the sum of the individual phenolics of fool's watercress detected by HPLC and both the DPPH radical-scavenging activity and TPC indicated that the compounds identified account for the antioxidant activity of the species.

Although celery presented the highest values of phenolics measured by HPLC and contents determined in parsley were also remarkable, the antioxidant capacity measured in these species was lower than the obtained in fool's watercress, especially in the case of DPPH radical-scavenging activity. A possible reason may be found in the chemical structure of the major compounds detected in the different species, as well as the synergistic effects of different phenolic compounds present in specific species. The number and position of hydroxyl groups affect the antioxidant capacity of polyphenols (Cartea *et al.*, 2011; Zaluski, Ciesla & Janeczko, 2015). The antioxidant capacity of these compounds would decrease in the following order: quercetin > luteolin > apigenin (Yildiz *et al.*, 2008), which could explain the relatively poor DPPH activity of parsley in comparison with the two other species. In celery, both apigenin and luteolin would account for an important share of the antioxidant capacity. Finally, the highly remarkable DPPH radical-scavenging activity of fool's watercress would be correlated to the content in quercetin, which is in addition a molecule that has been related

with a protective and inhibition action against several cancers, in cellular models but also *in vivo* in mammals (Sharmila *et al.*, 2014).

Conclusions

Our results reveal that fool's watercress is a leafy vegetable with high antioxidant activity, especially in comparison to the related cultivated parsley and celery. The high correlation between DPPH radical scavenging activity and TPC suggested that the antioxidant activity of this species is mainly caused by the phenolic compounds accumulated in the leaves. When the phenolic profile was analysed, we observed that, unlike celery and parsley, quercetin was the main compound present in the species. This finding may explain the greatest antioxidant activity of fool's watercress, resulting from the higher antioxidant capacity of this flavonoid compared to apigenin and luteolin, the main compounds detected in parsley and celery, respectively. In addition, results revealed differences among the geographical groups established for the total of populations of fool's watercress, indicating that selection among geographical origins may result in differences in bioactive properties. Although these differences may be caused by either genetic variation or environmental conditions, our results offer a starting point for future domestication and breeding programs.

Acknowledgements

Authors thank Mr. Manuel Figueroa for his help in the botanical identification of the plant.

Funding

Carla Guijarro-Real is supported by the Ministerio de Educación, Cultura y Deporte of Spain (MECD) with a predoctoral FPU grant (FPU14-06798). There was no additional external funding received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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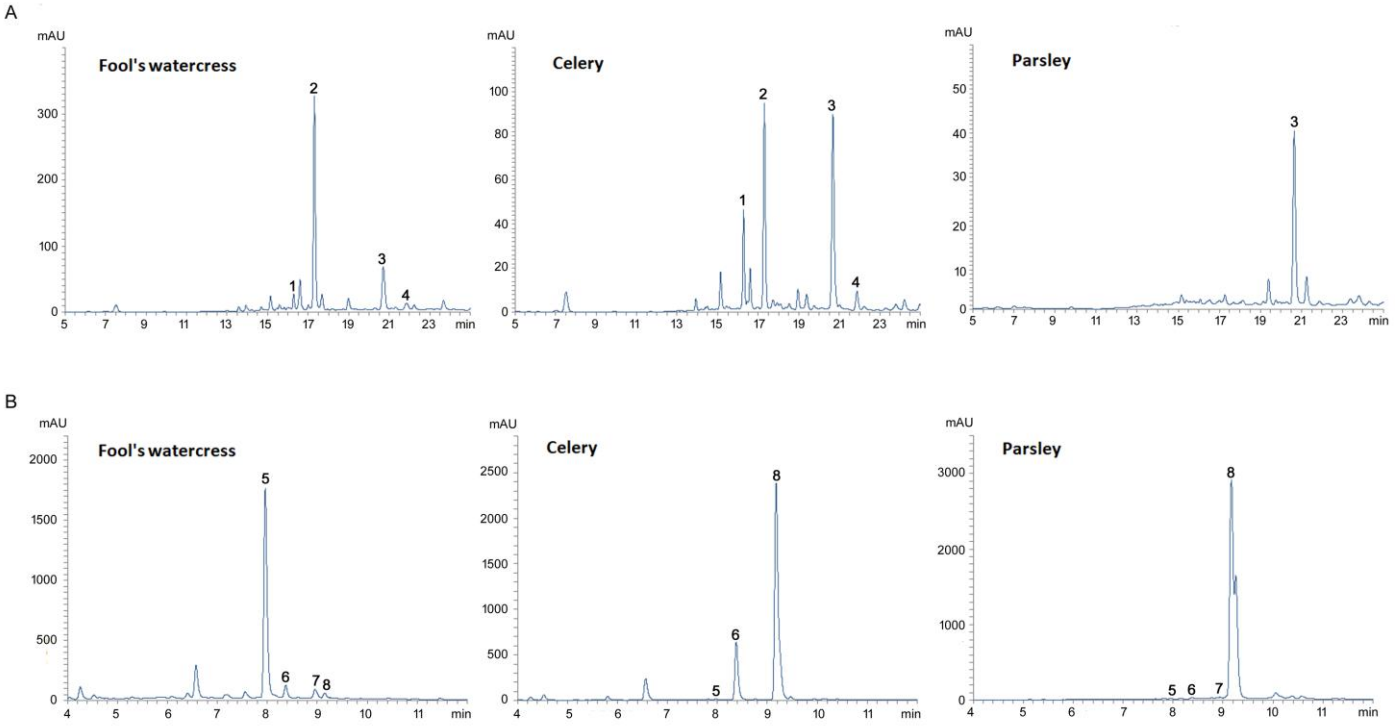

Fig. S1. Representative chromatograms of fool's watercress, celery and parsley. (A) Chromatogram obtained with the conditions described by Yildiz *et al.* (2008), with identification of the hydroxycinnamic acids targeted. Peaks correspond to: 1 chlorogenic acid, 2 caffeic acid, 3 *p*-coumaric acid, and 4 ferulic acid. (B) Chromatogram obtained with the conditions described by Bhae *et al.* (2012), with identification of the flavonoids (aglycones) targeted. Peaks correspond to: 5 quercetin, 6: luteolin, 7: kaempferol, and 8: apigenin.

1. 2. HS-SPME analysis of the volatiles profile of water celery (*Apium nodiflorum*), a wild vegetable with increasing culinary interest

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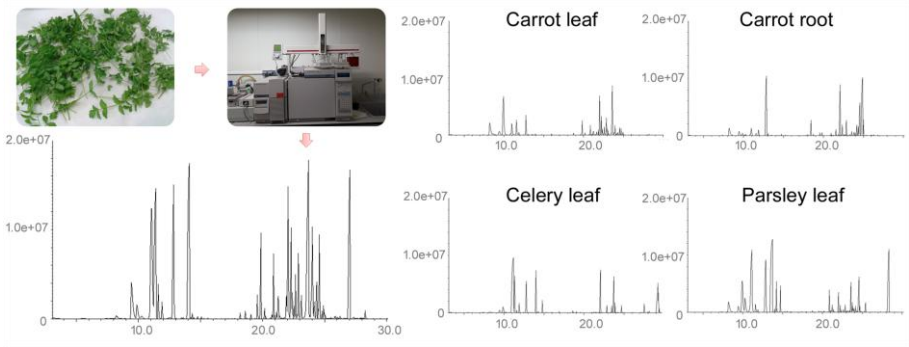
Keywords: Aroma; carrot; celery; diversity; fool's watercress; parsley; phenylpropanoids; sesquiterpenes

Guijarro-Real, C., Rodríguez-Burruezo, A., Prohens, J., Raigón, M.D., Fita, A. 2019. HS-SPME analysis of the volatiles profile of water celery (Apium nodiflorum), a wild vegetable with increasing culinary interest. Food Research International, 121, 765–775

DOI: 10.1016/j.foodres.2018.12.054

Abstract

Water celery (*Apium nodiflorum*) is a wild plant traditionally harvested in some Mediterranean areas for being consumed raw. Despite its appreciated organoleptic properties, the aromatic profile of the fresh vegetable remains to be studied. In the present study, volatile compounds from five wild populations were extracted by the headspace-solid phase microextraction technique, analysed by gas cromatography-mass spectrometry, and compared to related crops. The wild species had a high number of aromatic compounds. It was rich in monoterpenes (49.2%), sesquiterpenes (39.4%) and phenylpropanoids (9.6%), with quantitative differences among populations, in absolute terms and relative abundance. On average, germacrene D was the main compound (16.6%), followed by *allo*-ocimene (11.9%) and limonene (11.1%). Only in one population, the levels of limonene were greater than those of germacrene D. Among phenylpropanoids, dillapiol displayed the highest levels, and co-occurred with myristicin in all populations except one. These differences may have a genetic component, which would indicate the possibility of establishing selection programmes for the development of water celery as a crop adapted to different market preferences. On the other hand, comparison with related crops revealed some similarities among individual volatiles present in the different crops, which would be responsible of the common aroma notes. However, water celery displayed a unique profile, which was in addition quantitatively richer than others. Thus, this differentiation may promote the use of water celery as a new crop.



Introduction

Aromatic plants, rich in volatile organic compounds (VOCs), have been highly appreciated by humans since the early beginning of civilization (Evergetis & Haroutounian, 2014). These plants are used in the cuisine to enhance the taste of many dishes, either as fresh herbs or dried spices. There are different botanical families including species rich in VOCs such as *Alliaceae*, *Apiaceae*, *Labiateae*, *Lauraceae*, *Myrtaceae* or *Rutaceae* (Raut & Karuppayil, 2014). Among these, *Apiaceae* and *Labiateae* families are probably the most relevant for culinary uses as flavour enhancers. Within the *Apiaceae* family it is possible to find species used in cuisine by the aerial parts such as celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.), coriander (*Coriandrum sativum* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* Mill.) or parsley (*Petroselinum crispum* (Mill.) Nyman), or for the seeds such as anise (*Pimpinella anisum* L.) or cumin (*Cuminum cyminum* L.). Due to the importance of the family, it is possible to find several works studying the volatile profiles, specially focused on essential oils (e.g., Cioanca, Hancianu, Mircea, Trifan & Hritcu, 2016; El-Zaeddi *et al.*, 2016; Filly *et al.*, 2014). In addition, the interest on the volatile fraction and/or essential oils from other wild species and neglected vegetables has increased recently, attending mainly to pharmacological uses and, to a lesser extent, culinary purposes (e.g., Dev *et al.*, 2010; Landoulsi *et al.*, 2016; Maggi, Bartolucci & Conti, 2017; Quassinti *et al.*, 2013; Tabanca *et al.*, 2007).

Water celery (*Apium nodiflorum* (L.) Lag., also known as fool's watercress) is a perennial wild herb from the *Apiaceae* family. It is distributed in Central and Southern Europe (especially in the South-West), Northern Africa and Western and Central Asia (Knees, 2003; Molina, Pardo-de-Santayana, & Tardío, 2016), and can be easily found in fresh, shallow water courses such as natural streams or irrigation ditches. The young leaves and tender shoots have been consumed in the Mediterranean cultures in salads and different traditional dishes (Guarrera & Savo, 2016; Licata *et al.*, 2016; Tardío *et al.*, 2016). In the last years wild water celery is marketed in the United Kingdom in mixtures of wild vegetables due to its flavour and crunchy texture (Evans & Irving, 2018), and its use may increase if the species becomes cultivated.

The taste of water celery has been described as spicy (Guarrera & Savo, 2016) with a flavour that resembles celery, carrot (*Daucus carota* L.) or a mixture of both of them (Heshmati Afshar, Maggi, Iannarelli, Cianfaglione, & Isman, 2017; Nebel, Pieroni, & Heinrich, 2006; "Wild Food UK," 2018). In addition, the volatile fraction has been previously studied in essential oils extracted from dried materials (Benelli, Pavela, Ricciutelli, Lupidi, & Maggi, 2017; Heshmati Afshar *et al.*, 2017; Maxia *et al.*, 2012; Menghini, Leporini, Tirillini, Epifano, & Genovese, 2010). Nevertheless, dried samples do not represent the common way to consume this vegetable. In addition, the extraction of VOCs by hydrodistillation, the common methodology applied for essential oils, has as a disadvantage the occurrence of thermal artifacts, which may modify the real volatile profile, as well as tedious and time-consuming extraction protocols (Rodríguez-Burruezo, Kollmannsberger, González-Mas, Nitz, & Nuez, 2010). As an alternative, the headspace-solid phase microextraction (HS-SPME) technique has been successfully used for the isolation of VOCs in a number of fruits and vegetables (e.g., González-Mas, Rambla, Alamar, Gutiérrez & Granell, 2011; López-Gresa *et al.*, 2017; Mzoughi *et al.*, 2018; Taveira *et al.*, 2009), providing a more accurate profile of volatiles present in fresh vegetables. In the present work, we evaluated the volatile profile of water celery based on the analysis of the fresh edible leaves and tender shoots. Considering that the most common use of water celery is as vegetable eaten raw in salads, HS-SPME technique will provide a more realistic and accurate description of its aroma and flavour factors, as they are perceived by the consumer. We also compared the volatile profile of water celery with related cultivated species (i.e., carrot, celery and parsley) in order to identify qualitative and quantitative differences and similarities, which will be helpful to explain the distinctive features of the aroma and flavour of this species.

Materials and methods

Plant material

Irrigation ditches with regular water flows of the Horta Nord shire of Valencia (Spain) were prospected for water celery populations during the spring of 2015 (Fig. 1). Five isolated populations were sampled: Nod-001 (Puerto de Sagunto, 39º37'30" N, 0º16'49" W), Nod-015 (Foios, 39º31'59" N,

0º20'31" W), Nod-018 (Meliana, 39º31'01" N, 0º19'36" W), Nod-023 (Pueblo Nuevo, 39º31'19" N, 0º23'17" W) and Nod-025 (Alfara del Patriarca, 39º32'21" N, 0º23'06" W). The edible shoots were harvested, cleaned and stored at 4 ºC until analysed within the next two days (Fig. 1).

Fig. 1. A. Population of water celery growing in a ditch. B. Material representing the edible part of this wild vegetable.

In addition, two commercial samples of aerial parts of celery and parsley, and one commercial sample of carrot (including both root and aerial parts) were acquired from local markets and used as reference crops. The samples were also analysed within the next 24 hours after acquisition. For all the materials, three independent replicates were prepared.

Preparation of samples and extraction of VOCs

Extraction and analysis of volatiles was performed by the HS-SPME technique according to Moreno, Fita, González-Mas and Rodríguez-Burruezo (2012). For that, 1.5 g of fresh leaves were weighted, finely chopped with a knife and immediately placed into 20 mL sealed headspace vials. In the case of carrot roots, 1.5 g were weighted and cut in small, regular pieces, then placed into the sealed vials. Pre-incubation of samples was performed at 40ºC during 30 min, then in the extraction step, VOCs were adsorbed on a fibre (50/30 µm DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) for 40 min at the same temperature. The thermal desorption was carried out at 250ºC for 30 s in the splitless mode. Prior to the first analysis,

the fibre was conditioned at 270ºC for 1 h and reconditioned after each sample for 30 min at 250ºC to ensure no cross-contamination between samples.

Analysis of volatiles

VOCs were analysed by gas chromatography-mass spectrometry (GC-MS) using a 6890N Network GC System with autosampler coupled to a 5973 Inert Mass Selective Detector (Agilent Techonologies, Santa Clara, CA, USA) and equipped with a HP-5MS J&W silica capillary column (5% phenyl-95% methylpolysiloxane as stationary phase, 30 m length x 0.25 mm i.d., 0.25 µm thickness film; Agilent Technologies). Helium was used as carrier gas at a constant flow of 1 mL min^{-1}. The temperature of the column was programmed to raise from 100ºC to 250ºC at a rate of 5ºC min^{-1} and then maintained at 250ºC for 10 minutes. The transfer line was maintained at 220ºC. The electron impact (EI) mode (70 eV ionization energy, source temperature 225ºC) was used for the detection by the mass spectrometer, and acquisition was performed in scanning mode (mass range *m/z* 35-350 amu).

Chromatograms and spectra were processed using the MSD ChemStation D.02.00.275 (Agilent Technologies). Identification of compounds was performed by comparing the GC retention time and mass spectra with reference substances (Sigma-Aldrich, Saint Louis, MO, USA), when available, or tentatively by comparing the mass spectra with the NIST 2005 Mass Spectral Library, previous literature and also with a customized library from our laboratory and, when available. Due to the number of volatiles detected, it was not possible to find suitable standards and estimate response factors. Thus, quantification of VOCs was based on the integration of peak areas by the total ion current chromatogram (TIC), as previously reported (e.g., González-Mas *et al.*, 2011; Moreno *et al.*, 2012; Rodríguez-Burruezo *et al.*, 2010). The percentage of each compound was also estimated as the ratio of its peak area relative to the total of compounds identified.

Statistical analysis

The five populations of water celery data were used for determining the mean value of the species, and coefficients of variation (CV) were

calculated. Data were log_2-transformed for normalization. Analysis of variance (ANOVA) was performed and significant differences were calculated with the multiple range Student-Newman-Keuls test. In addition, the relative abundance of each compound in the different samples was calculated as the ratio between the peak area of the compound against the total area of the compounds identified in the sample.

Finally, an illustrative comparison of the profiles in the different species was performed by means of both Principal Component Analysis (PCA) and Hierarchical Cluster Analysis, using the ClustVis Tool (Metsalu & Vilo, 2015). Unit variance scaling for the normalized and centred data was applied for PCA. The Hierarchical Cluster Analysis was performed with the distance measures based on Pearson correlations.

Results

VOCs detected among the different species analysed

A total of 64 VOCs were identified among the different species (Table 1). Terpenes represented the largest group in all the materials studied (41 compounds, 16 monoterpenes and 25 sesquiterpenes). In addition, terpenoid-derived compounds were also found, including several alcohols (two monoterpene-derived, four sesquiterpene-derived and one diterpene-derived), three monoterpene-derived esters, one monoterpene-derived ketone and one sesquiterpene-derived ether. Phenylpropanoids, including myristicin and the derivatives apiol and dillapiol were also detected. Finally, other compounds detected included two aromatic hydrocarbons, two ketones, one ester, one cyclic alkene, two furanes and one thiazole.

The occurrence of the identified compounds differed among species. At a qualitative level, water celery and parsley presented the most diverse volatile fraction, with forty-four compounds identified in each species. On the contrary, celery showed the least diverse volatile composition with only thirty-one VOCs, while carrot (both roots and leaves) showed an intermediate level with thirty-seven VOCs (Table 1).

Table 1. List of volatile organic compounds (VOC) identified in the present study with its retention index (RI), identification method (Id) and their presence in the materials studied: water celery (WaCel), carrot leaf (CtL), carrot root (CtR), celery (Cel) and parsley (Par).

VOC	Code	RI	Id[d]	Wa Cel	CtL	CtR	Cel	Par
Alcohols								
p-cymen-8-ol[a]	A1	1197	MS	X		X	X	
(Z)-carveol[a]	A2	1206	MS	X			X	
germacrene D-4-ol[b]	A3	1660	MS	X				X
spathulenol[b]	A4	1536	MS	X				
α-cadinol[b]	A5	1580	MS	X				X
α-bisabolol[b]	A6	1625	R	X	X	X		
phytol[c]	A7	2045	MS	X	X		X	X
Esters								
(*Z*)-3-hexenyl acetate	E1	992	MS		X			X
bornyl acetate[a]	E2	1277	MS			X		X
carvyl acetate[a]	E3	1346	MS				X	
α-terpinyl acetate[a]	E4	1432	MS			X		
Furanes								
4,7-dimethyl benzofuran	F1	1244	MS					X
3-butylidenephtalide	F2	1655	MS				X	
Aromatic hydrocarbons								
1-Methyl-4-sec-butylbenzene	H1	1141	MS					X
β-methylnaphthalene	H2	1345	R	X	X	X	X	X
Ketones								
p-methylacetophenone	K1	1142	MS					X
carvone[a]	K2	1190	R	X			X	
4-hydroxy-3-methyl acetophenone	K3	1363	MS	X				
Monoterpenes								
α-thujene	M1	902	MS					X
α-pinene	M2	948	R	X	X	X	X	X
camphene	M3	943	R		X	X	X	X
β-pinene	M4	943	R	X	X	X	X	X

β-myrcene	M5	958	R	X	X	X	X	X
α-phellandrene	M6	969	R	X	X	X	X	X
α-terpinene	M7	998	R	X	X	X	X	X
p-cymene	M8	1042	R	X	X	X	X	X
limonene	M9	1018	R	X	X	X	X	X
β-phellandrene	M10	964	MS					X
β-(*Z*)-ocimene	M11	976	R	X	X	X	X	X
β-(*E*)-ocimene	M12	976	R	X	X	X	X	X
γ-terpinene	M13	998	R	X	X	X	X	X
terpinolene	M14	1052	R	X	X	X	X	X
1,3,8-*p*-menthatriene	M15	1029	MS	X		X	X	X
allo-ocimene	M16	993	R	X	X	X	X	X
Phenylpropanoids								
myristicin	P1	1516	R	X	X	X		X
dillapiol	P2	1705	MS	X				
apiol	P3	1705	MS					X
Sesquiterpenes								
δ-elemene	S1	1367	MS	X	X	X		X
α-cubebene	S2	1344	MS	X	X	X		X
ylangene	S3	1221	MS	X	X	X		X
α-copaene	S4	1221	R	X	X	X	X	X
β-bourbonene	S5	1339	MS	X	X			X
β-cubebene	S6	1339	MS	X	X	X	X	X
β-elemene	S7	1398	R	X	X	X	X	X
α-gurjunene	S8	1419	R	X				
α-cedrene	S9	1403	R				X	X
β-caryophyllene	S10	1494	R	X	X	X	X	X
α-bergamotene	S11	1430	R	X	X	X	X	X
β-sesquiphellandrene	S12	1446	MS		X	X		
γ-muurolene	S13	1435	MS	X	X			X
α-caryophyllene	S14	1579	R	X	X	X	X	X
(*Z*)-β-farnesene	S15	1440	R		X	X		
α-curcumene	S16	1524	MS				X	
germacrene D	S17	1515	R	X	X	X		X
β-selinene	S18	1469	MS				X	
α-zingiberene	S19	1451	MS			X		

α-selinene	S20	1474	MS				X	
β-bisabolene	S21	1500	MS		X	X		X
γ-cadinene	S22	1435	MS	X	X	X		X
δ-cadinene	S23	1469	MS	X	X		X	
cadala-(1,10)-3,8-triene	S24	1423	MS	X	X	X		X
cadalene	S25	1706	MS	X	X			
Ethers								
caryophyllene oxide[b]	SE1	1507	R	X	X	X	X	X
Thiazols								
1,2-benzothiazole	T1	1208	MS	X				

[a]Monoterpenoid derived compound. [b]Sesquiterpenoid derived compound. [c]Diterpenoid derived compound. [d]The identification of the VOCs is indicated by: R, if the VOC matched to the GC retention time and MS with substances of reference; or MS, if the MS matched with the NIST 2005 Mass Spectral library and considering the literature available and our customized library

Volatile profile of water celery

The volatile profile of water celery was mainly characterized by terpene hydrocarbons. The average relative abundance of monoterpenes ranged between 39.1 (Nod-025) and 61.3% (Nod-001); however, the highest absolute levels were determined for Nod-018 (Table 2). On the contrary, Nod-025 presented the highest level in sesquiterpenes, more than 2-fold the levels of Nod-001. In relative terms, these contents corresponded to 53.9 and 25.8%, respectively.

The main terpenes targeted in the species were germacrene D (S17, 16.6% on average), *allo*-ocimene (M16, 11.9%), limonene (M9, 11.1%), β-(*Z*)-ocimene (M11, 9.7%), and terpinolene (M14, 7.3%) (Table 3). However, significant differences in quantitative terms and relative abundance were found among populations (Table 2). Nod-025 displayed the highest levels of germacrene D, 2.3-fold higher than Nod-001, and represented 22.5% of the total VOCs within this population. On the contrary, Nod-001 displayed the greatest area of limonene (M9), corresponding to a relative abundance of 18.0%. Terpinolene (M14) was also relevant in Nod-018, with a content up to 5-fold higher than the other populations; while β-caryophyllene (S10) was

Table 2. Mean values (n=3) and coefficient of variation (CV) of the individual VOCs targeted in the five populations of water celery (Nod-001, Nod-015, Nod-018, Nod-023, and Nod-025), expressed as GC peak area ($x10^6$). The relative abundance is also indicated, in parentheses, as percentage from each VOC against the total identified.

VOC	Nod-001	Nod-015	Nod-018	Nod-023	Nod-025	CV
p-cymen-8-ol	1.42[a]	0.43[a]	4.71[b]	1.64[a]	1.23[a]	0.82
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	
(Z)-carveol	1.39[a]	tr[a]	tr	tr	tr	2.09
	(0.0)					
germacrene D-4-ol	3.73[a]	6.80[b]	6.31[b]	4.97[ab]	6.69[b]	0.28
	(0.0)	(0.1)	(0.1)	(0.1)	(0.1)	
spathulenol	8.08[a]	tr	8.62[a]	7.07[a]	3.51[a]	0.71
	(0.1)		(0.1)	(0.1)	(0.0)	
α-cadinol	6.31[a]	5.14[a]	-	3.24[a]	4.90[a]	0.78
	(0.1)	(0.1)		(0.0)	(0.1)	
α-bisabolol	31.72[a]	27.36[a]	28.57[a]	17.99[a]	20.63[a]	0.35
	(0.4)	(0.3)	(0.3)	(0.2)	(0.2)	
phytol	93.35[a]	85.89[a]	74.39[a]	83.67[a]	97.99[a]	0.23
	(1.2)	(1.0)	(0.7)	(1.0)	(1.1)	
β-methylnaphthalene	1.56[a]	1.43[a]	0.96[a]	28.90[c]	3.49[b]	1.56
	(0.0)	(0.0)	(0.0)	(0.3)	(0.0)	
carvone	1.48[a]	2.05[a]	0.73[a]	1.68[a]	1.00[a]	0.65
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	
4-hydroxy-3-methyl acetophenone	13.72[a]	17.06[ab]	11.32[a]	22.01[b]	14.16[a]	0.29
	(0.2)	(0.2)	(0.1)	(0.3)	(0.2)	
α-pinene	43.93[b]	34.21[ab]	33.35[ab]	31.45[ab]	22.22[a]	0.28
	(0.5)	(0.4)	(0.3)	(0.3)	(0.2)	
β-pinene	612.22[c]	506.07[bc]	306.07[ab]	349.34[abc]	247.75[a]	0.41
	(7.4)	(5.6)	(2.8)	(3.9)	(2.7)	
β-myrcene	130.54[a]	108.38[a]	137.54[a]	120.44[a]	111.20[a]	0.26
	(1.6)	(1.2)	(1.3)	(1.3)	(1.2)	
α-phellandrene	28.10[ab]	21.31[a]	40.20[b]	30.78[ab]	25.71[ab]	0.25
	(0.3)	(0.2)	(0.4)	(0.3)	(0.3)	
α-terpinene	tr	2.85[a]	2.16[a]	1.83[a]	tr	1.14
		(0.0)	(0.0)	(0.0)		

p-cymene	33.30[a]	65.37[b]	45.64[a]	36.05[a]	31.67[a]	0.36
	(0.4)	(0.7)	(0.4)	(0.4)	(0.3)	
limonene	1476.22[d]	1002.70[bc]	804.87[ab]	1132.49[c]	767.76[a]	0.27
	(18.0)	(11.0)	(7.5)	(12.7)	(8.4)	
β-(*Z*)-ocimene	874.69[a]	858.05[a]	1190.80[a]	910.65[a]	722.43[a]	0.24
	(10.6)	(9.4)	(11.0)	(9.8)	(7.9)	
β-(*E*)-ocimene	97.56[a]	85.12[a]	137.94[a]	95.42[a]	65.71[a]	0.35
	(1.2)	(0.9)	(1.3)	(1.0)	(0.7)	
γ-terpinene	94.41[a]	214.61[a]	145.11[a]	114.40[a]	102.55[a]	0.46
	(1.2)	(2.3)	(1.3)	(1.3)	(1.1)	
terpinolene	529.08[b]	251.54[a]	1263.25[c]	769.46[bc]	620.50[bc]	0.56
	(6.5)	(2.7)	(11.7)	(8.3)	(6.8)	
1,3,8-*p*-menthatriene	2.83[ab]	1.29[a]	14.23[c]	5.06[b]	4.79[b]	0.87
	(0.0)	(0.0)	(0.1)	(0.1)	(0.1)	
allo-ocimene	1127.08[a]	1043.03[a]	1445.55[a]	1092.00[a]	850.75[a]	0.25
	(13.6)	(11.4)	(13.4)	(11.7)	(9.3)	
myristicin	695.22[b]	29.70[a]	304.86[b]	35.49[a]	-	1.35
	(8.4)	(0.3)	(2.9)	(0.4)		
dillapiol	209.61[a]	647.04[ab]	1437.32[b]	681.37[ab]	466.60 [ab]	0.80
	(2.5)	(7.0)	(13.0)	(7.0)	(5.2)	
δ-elemene	212.13[b]	106.95[a]	308.11[b]	262.85[b]	182.78[b]	0.39
	(2.6)	(1.2)	(2.9)	(2.9)	(2.0)	
α-cubebene	17.82[a]	37.89[bc]	26.88[b]	27.05[b]	42.46[c]	0.34
	(0.2)	(0.4)	(0.3)	(0.3)	(0.5)	
ylangene	9.42[a]	18.27[b]	15.13[b]	14.87[b]	23.24[b]	0.34
	(0.1)	(0.2)	(0.1)	(0.2)	(0.3)	
α-copaene	126.84[a]	270.68[b]	191.92[b]	191.59[b]	267.91[b]	0.30
	(1.5)	(3.0)	(1.8)	(2.2)	(2.9)	
β-bourbonene	31.50[a]	38.81[a]	33.72[a]	24.94[a]	37.14[a]	0.26
	(0.4)	(0.4)	(0.3)	(0.3)	(0.4)	
β-cubebene	65.69[a]	125.79[a]	97.80[a]	82.21[a]	128.60[a]	0.36
	(0.8)	(1.4)	(0.9)	(0.9)	(1.4)	
β-elemene	44.61[a]	40.34[a]	36.59[a]	37.62[a]	55.69[a]	0.38
	(0.5)	(0.4)	(0.3)	(0.4)	(0.6)	
α-gurjunene	5.19[b]	3.17[a]	7.16[c]	6.81 [c]	5.53[bc]	0.29
	(0.1)	(0.0)	(0.1)	(0.1)	(0.1)	

β-caryophyllene	397.24[a]	847.67[b]	513.18[ab]	589.96[ab]	907.06[b]	0.37
	(4.8)	(9.3)	(4.8)	(6.7)	(9.9)	
α-bergamotene	26.55[a]	98.95[bc]	61.60[ab]	272.64[cd]	413.56[d]	1.01
	(0.3)	(1.1)	(0.6)	(3.2)	(4.2)	
γ-muurolene	92.31[a]	185.30[b]	152.68[b]	144.44[b]	226.46[b]	0.34
	(1.1)	(2.0)	(1.4)	(1.6)	(2.5)	
α-caryophyllene	126.70[a]	282.00[b]	221.88[b]	203.10[b]	311.64[b]	0.33
	(1.5)	(3.1)	(2.1)	(2.3)	(3.4)	
germacrene D	906.92[a]	1800.27[b]	1502.69[b]	1516.03[b]	2063.93[b]	0.28
	(11.0)	(19.7)	(14.0)	(17.0)	(22.5)	
γ-cadinene	60.87[a]	267.10[b]	276.45[b]	129.64[b]	143.26[b]	0.60
	(0.7)	(2.9)	(2.6)	(1.4)	(1.6)	
δ-cadinene	-	-	-	-	395.55[a]	2.09
					(4.3)	
cadala-(1,10)-3,8-triene	8.89[a]	17.41[b]	13.87[b]	14.18[b]	20.96[b]	0.33
	(0.1)	(0.2)	(0.1)	(0.2)	(0.2)	
cadalene	2.20[a]	3.25[a]	2.70[a]	2.94[a]	4.28[a]	0.31
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	
caryophyllene oxide	6.20[a]	6.89[a]	4.34[a]	5.68[a]	7.12[a]	0.34
	(0.1)	(0.1)	(0.0)	(0.1)	(0.1)	
1,2-benzothiazole	0.74[a]	-	0.78[a]	1.04[a]	0.91[a]	0.58
	(0.0)		(0.0)	(0.0)	(0.0)	
Total	5050[ab]	4187[ab]	5566[b]	4689[ab]	3573[a]	0.2
monoterpenes	(61.3)	(45.8)	(51.4)	(51.0)	(39.1)	
Total	2134[a]	4144[bc]	3462[b]	3521[b]	4954[c]	0.29
sesquiterpenes	(25.8)	(45.3)	(32.3)	(39.7)	(53.9)	
Total	904[a]	667[a]	1641[b]	705[a]	467[a]	0.6
phenylpropanoids	(10.9)	(7.2)	(15.0)	(7.3)	(5.2)	
Total terpenoid	153[a]	134[a]	128[a]	125[a]	143[a]	0.19
derived compounds	(1.9)	(1.5)	(1.2)	(1.4)	(1.6)	
Total	16 [a]	19[a]	13[a]	52[b]	18[a]	0.64
others	(0.2)	(0.2)	(0.1)	(0.6)	(0.2)	
Total	8259[a]	9151[a]	10810[a]	9091[a]	9156[a]	0.13

Different letters within rows indicate significant differences at $P < 0.05$, according to the Student-Newman-Keuls test. [a] tr indicates compound detected as traces

accumulated in high, similar content in Nod-015 and Nod-025. By contrast, *allo*-ocimene (M16) and β-(*Z*)-ocimene (M11) had similar levels in the five populations.

Phenylpropanoids were also relevant in the species, with a relative abundance between 5.2% (Nod-025) and 15.0% (Nod-018) (Table 2). Dillapiol was the main phenylpropanoid in all populations except Nod-001, with levels of expression similar to relevant terpenes. Only in Nod-001, myristicin (P1) was found in higher relative abundance than dillapiol (P2), representing the former 8.4% of total. Interestingly, Nod-025 did not accumulated myristicin (P1).

Comparison of the volatile profile of water celery with related crops

Water celery was determined as the species with the highest values of total VOCs, followed by parsley, while celery and the carrot samples presented low contents (Table 3). As in water celery, terpenoids were also the main group in all related species, with monoterpenes relative abundances between 49.8% and 75.3% (carrot leaves and parsley, respectively). Sesquiterpenes represented also the second major class in celery and carrot materials, although the total absolute levels were significantly lower compared to water celery. By contrast, the relative abundance of phenylpropanoids in parsley was greater than the sesquiterpenes fraction (16.2% and 7.3% on average, respectively) (Table 3).

In parsley, the main monoterpenes were 1,3,8-*p*-menthatriene (M15, 24.0% on average), β-phellandrene (M10, 15.1%) and terpinolene (M14, 13.6%) (Table 3). As phenylpropanoids, the species presented myristicin (P1) and apiol (P3) at relative abundances of 7.6 and 9.0%, respectively. Celery also had great amounts of terpenes, specially limonene (M9, 31.0%), and β-caryophyllene (S10, 23.6%) (Table 3). β-Selinene (S18) was the third in abundance (12.0%). Phenylpropanoids were not found in celery, and other compounds such as terpenoid derived compounds were present in low amounts, representing only 1.8 % of the VOCs profile of this species. On the other hand, the leaves and roots of carrot, even belonging to the same species, had important differences (Table 3).

Table 3. Mean values of the individual VOCs targeted in water celery (WaCel, n=5), carrot leaves (CtL, n=3), carrot roots (CtR, n=3), celery (two samples, Cel1, n=3, and Cel2, n=3) and parsley (two samples, Par1, n=3, and Par2, n=3), expressed as GC peak area ($x10^6$). The relative abundance is also given, in parentheses, as percentage of each compound against the total identified.

	WaCel	CtL	CtR	Cel1	Cel2	Par1	Par2
p-cymen-8-ol	1.89^{a} (0.0)	-	0.89^{a} (0.0)	-	0.20^{a} (0.0)	-	-
(Z)-carveol	0.28^{a} (0.0)	-	-	1.54^{b} (0.0)	0.43^{b} (0.0)	-	-
germacrene D-4-ol	5.70^{b} (0.1)	-	-	-	-	0.95^{a} (0.0)	1.06^{a} (0.0)
spathulenol	5.46^{a} (0.1)	-	-	-	-	-	-
α-cadinol	3.92^{a} (0.0)	-	-	-	-	2.98^{a} (0.0)	1.74^{a} (0.0)
α-bisabolol	25.25^{c} (0.3)	3.23^{b} (0.1)	0.66^{a} (0.0)	-	-	-	-
phytol	87.06^{e} (0.9)	29.58^{bc} (1.0)	-	71.50^{de} (1.5)	41.03^{cd} (1.6)	16.38^{a} (0.2)	22.83^{ab} (0.4)
(*Z*)-3-hexenyl acetate	-	181.37^{a} (6.4)	-	-	-	-	261.92^{a} (5.1)
bornyl acetate	-	-	60.11^{b} (2.7)	-	-	-	0.77^{a} (0.0)
carvyl acetate	-	-	-	5.34^{b} (0.1)	1.10^{a} (0.0)	-	-
α-terpinyl acetate	-	-	0.77^{a} (0.0)	-	-	-	-
4,7-dimethyl benzofuran	-	-	-	-	-	1.83^{a} (0.0)	1.47^{a} (0.0)
3-butylidenephtalide	-	-	-	4.75^{a} (0.1)	-	-	-
1-Methyl-4-sec-butylbenzene	-	-	-	-	-	1.50^{a} (0.0)	1.34^{a} (0.0)
β-methylnaphthalene	7.27^{ab} (0.1)	0.44^{a} (0.0)	tr[†]	20.30^{b} (0.4)	8.26^{ab} (0.3)	1.04^{ab} (0.0)	tr
p-methylacetophenone	-	-	-	-	-	9.23^{a} (0.1)	6.86^{a} (0.1)
carvone	1.39^{b} (0.0)	-	-	0.55^{a} (0.0)	0.29^{a} (0.0)	-	-
4-hydroxy-3-methyl	15.65^{a} (0.2)	-	-	-	-	-	-

acetophenone							
α-thujene	-	-	-	-	-	tr	tr
α-pinene	33.03^{c} (0.3)	258.01^{e} (9.0)	152.61^{d} (6.9)	8.74^{a} (0.2)	16.89^{b} (0.6)	173.80^{d} (2.3)	167.93^{d} (3.3)
camphene	tr	tr	tr	tr	2.82^{a} (0.1)	tr	tr
β-pinene	404.29^{d} (0.0)	110.47^{c} (3.9)	75.36^{bc} (3.4)	19.55^{a} (0.4)	42.93^{b} (1.6)	61.69^{bc} (0.8)	109.21^{c} (2.1)
β-myrcene	121.62^{c} (1.3)	750.64f (26.3)	30.67^{a} (1.4)	195.28^{d} (4.2)	86.72^{b} (3.3)	569.54^{e} (7.4)	466.73^{e} (9.1)
α-phellandrene	29.22^{b} (0.3)	tr	13.19^{a} (0.6)	tr	tr	223.73^{c} (2.9)	36.54^{b} (0.7)
α-terpinene	2.28^{a} (0.0)	tr	1.40^{a} (0.1)	tr	2.42^{a} (0.1)	14.64^{b} (0.2)	tr
p-cymene	42.41^{b} (0.5)	21.77^{a} (0.8)	22.08^{a} (1.0)	23.74^{a} (0.5)	33.06^{ab} (1.3)	171.21^{d} (2.2)	89.12^{c} (1.7)
limonene	1036.81^{d} (11.1)	91.19^{b} (3.2)	52.08^{a} (2.3)	1478.66^{e} (31.9)	786.04^{d} (30.2)	141.67^{c} (1.8)	81.70^{b} (1.6)
β-phellandrene	-	-	-	-	-	1084.18^{a} (14.1)	826.50^{a} (16.2)
β-(*Z*)-ocimene	911.32^{e} (9.7)	6.83^{a} (0.2)	tr	178.84^{c} (3.9)	257.28^{d} (9.9)	10.39^{ab} (0.1)	18.60^{b} (0.4)
β-(*E*)-ocimene	96.35^{c} (1.0)	133.20^{c} (4.7)	14.98^{a} (0.7)	26.11^{b} (0.6)	12.65^{a} (0.5)	68.28^{c} (0.9)	113.72^{c} (2.2)
γ-terpinene	134.22^{d} (1.4)	27.02^{ab} (0.9)	51.66^{c} (2.3)	40.27^{bc} (0.9)	66.46^{c} (2.6)	22.82^{ab} (0.3)	20.60^{a} (0.4)
terpinolene	686.77^{c} (7.3)	167.98^{ab} (5.9)	687.45^{c} (31.0)	122.60^{a} (2.6)	247.06^{b} (9.5)	1063.08^{c} (13.8)	684.95^{c} (13.4)
1,3,8-*p*-menthatriene	5.64^{a} (0.1)	-	2.15^{a} (0.1)	4.39^{a} (0.1)	2.33^{a} (0.1)	2164.54^{b} (28.2)	1010.39^{b} (19.1)
allo-ocimene	1111.68^{d} (11.9)	14.82^{b} (0.5)	0.55^{a} (0.0)	291.12^{c} (6.3)	321.99^{c} (12.4)	12.59^{b} (0.2)	20.74^{b} (0.4)
myristicin	213.05^{a} (2.3)	tr	253.71^{a} (11.4)	-	-	526.86^{a} (6.9)	385.92^{a} (7.6)
dillapiol	688.39^{a} (7.3)	-	-	-	-	-	-
apiol	-	-	-	-	-	682.78^{a} (8.9)	465.83^{a} (9.1)

δ-elemene	214.56^{e} (2.3)	44.58^{d} (1.6)	15.75^{c} (0.7)	-	-	4.55^{b} (0.1)	2.06^{a} (0.0)
α-cubebene	30.42^{d} (0.3)	16.73^{c} (0.6)	tr	-	-	8.71^{b} (0.1)	5.33^{a} (0.1)
ylangene	16.19^{d} (0.2)	4.77^{c} (0.2)	tr	-	-	2.22^{b} (0.0)	1.04^{a} (0.0)
α-copaene	209.79^{g} (2.2)	38.21^{d} (1.3)	1.60^{b} (0.1)	2.73^{c} (0.1)	0.97^{a} (0.0)	112.94^{f} (1.5)	57.26^{e} (1.1)
β-bourbonene	33.22^{c} (0.4)	8.62^{b} (0.3)	-	-	-	11.04^{b} (0.1)	3.06^{a} (0.1)
β-cubebene	100.02^{e} (1.1)	17.21^{c} (0.6)	1.56^{a} (0.1)	5.94^{b} (0.1)	1.73^{a} (0.1)	46.93^{d} (0.6)	24.16^{c} (0.5)
β-elemene	42.97^{d} (0.5)	8.80^{c} (0.3)	2.65^{b} (0.1)	4.96^{c} (0.1)	1.35^{a} (0.0)	8.57^{c} (0.1)	2.96^{b} (0.1)
α-gurjunene	5.57^{a} (0.1)	-	-	-	-	-	-
α-cedrene	-	-	-	13.34^{d} (0.3)	5.97^{c} (0.2)	1.92^{b} (0.0)	0.95^{a} (0.0)
β-caryophyllene	651.02^{d} (6.9)	278.24^{c} (9.7)	525.64^{d} (23.7)	1088.77^{e} (23.5)	338.32^{c} (13.0)	145.41^{b} (1.9)	89.20^{a} (1.7)
α-bergamotene	174.66^{c} (1.9)	34.86^{b} (1.2)	49.74^{b} (2.2)	2.07^{a} (0.0)	2.36^{a} (0.1)	7.54^{a} (0.1)	6.97^{a} (0.1)
β-sesquiphellandrene	-	9.25^{b} (0.3)	2.24^{a} (0.1)	-	-	-	-
γ-muurolene	160.24^{d} (1.7)	37.48^{c} (1.3)	-	-	-	16.86^{b} (0.2)	5.69^{a} (0.1)
α-caryophyllene	229.06^{c} (2.4)	90.82^{b} (3.2)	84.33^{b} (3.8)	138.79^{b} (3.0)	33.21^{a} (1.3)	39.00^{a} (0.5)	24.77^{a} (0.5)
(*Z*)-β-farnesene	-	29.63^{b} (1.0)	7.07^{a} (0.3)	-	-	-	-
α-curcumene	-	-	-	14.35^{b} (0.3)	6.75^{a} (0.3)	-	-
germacrene D	1557.97^{e} (16.6)	376.43^{d} (13.2)	21.50^{a} (1.0)	-	-	211.18^{c} (2.7)	68.46^{b} (1.3)
β-selinene	-	-	-	693.87^{b} (15.0)	233.83^{a} (9.0)	-	-
α-zingiberene	-	-	18.13^{a} (0.8)	-	-	-	-
α-selinene	-	-	-	167.18^{b} (3.6)	43.72^{a} (1.7)	-	-
β-bisabolene	-	9.94^{a} (0.3)	61.18^{d} (2.8)	-	-	28.14^{c} (0.4)	17.80^{b} (0.3)

γ-cadinene	175.46^{c} (1.9)	11.80^{b} (0.4)	-	-	-	8.67^{b} (0.1)	2.59 ^{a} (0.0)
δ-cadinene	79.11^{a} (0.8)	32.12^{a} (1.1)	-	tr	1.50^{a} (0.1)	-	-
cadala-(1,10)-3,8-triene	15.07^{e} (0.2)	2.29^{d} (0.1)	0.34^{a} (0.0)	-	-	1.05^{c} (0.0)	0.54^{b} (0.0)
cadalene	3.07^{b} (0.0)	0.71^{a} (0.0)	-	-	-	-	-
caryophyllene oxide	6.05^{d} (0.1)	3.28^{c} (0.1)	5.77^{d} (0.3)	5.78^{d} (0.1)	1.49^{b} (0.1)	0.61^{a} (0.0)	1.30^{b} (0.0)
1,2-benzothiazole	0.73^{a} (0.0)	-	-	-	-	-	-
Total monoterpenes	4615^{e} (49.2)	1582^{b} (55.5)	1104^{a} (49.8)	2389^{c} (51.6)	1879^{b} (72.2)	5782^{f} (75.3)	3647^{d} (71.4)
Total sesquiterpenes	3698^{e} (39.4)	1052^{c} (36.9)	792^{bc} (35.7)	2132^{d} (46.0)	670^{b} (25.7)	655^{b} (8.5)	313^{a} (6.1)
Total phenylpropanoids	901^{a} (9.6)	tr	254^{a} (11.4)	-	-	1210^{a} (15.8)	852^{a} (16.7)
Total terpenoid derived compounds	137^{e} (1.6)	36^{bc} (1.3)	68^{d} (3.1)	85^{d} (1.8)	44^{c} (1.7)	21^{a} (0.3)	28^{ab} (0.5)
Total others	24^{a} (0.2)	181^{ab} (6.4)	-	25^{ab} (0.5)	8^{a} (0.3)	14^{a} (0.2)	272^{b} (5.3)
Total	9376^{e}	2852^{b}	2218^{a}	4631^{c}	2601^{ab}	7681^{d}	5111^{c}

Different letters within rows indicate significant differences at $P < 0.05$, according to the Student-Newman-Keuls test. [†]tr indicates compound detected as traces

The most abundant VOC in leaves was β-myrcene (M5), representing 26.3% of its aroma. Other terpenes of relevance were germacrene D (S17, 13.2%), β-caryphyllene (S10, 9.7%) and α-pinene (M2, 9.0%). By contrast, terpinolene was the main terpene of carrot roots (M14, 31.0%), followed by β-caryophyllene and α-pinene (23.7 and 6.9%, respectively). Myristicin was the unique phenylpropanoid found in the species, and accounted for 11.4% of total in carrot root but was only present as traces in carrot leaf (Table 3).

The hierarchical cluster analysis showed the differences and similarities among species in an illustrative way. The analysis separated two main clusters: A and B, including different subclusters (Fig. 2). Subcluster A1 grouped the sesquiterpenes found in water celery, which were at the highest contents compared to the related crops. Germacrene D (S17), main compound of water celery, was found in this subcluster. Levels of this VOC were on average 4-fold higher than in carrot leaves, although representing similar relative abundances, and even higher compared to the other materials (Table 3). In this subcluster was also included the phenylpropanoid myristicin (P1), determined at similar levels in water celery, parsley and carrot roots but accounting for different relative abundances (Table 3). Within the subcluster A2, A2.1 included the most relevant monoterpenes in water celery: limonene (M9), found at similar content in celery but representing in the latter a higher relative abundance, and *allo*-ocimene (M16), present in all materials but displaying the highest content in water celery (Fig. 2, Table 3). Subcluster A2.2 grouped the isomers α-caryophyllene (S14) and β-caryophyllene (S10) and the derived caryophyllene oxide (SE1) (Fig. 2), whose content varied among samples. In particular, β-caryophyllene (S10) had similar levels to carrot leaves and celery although the relative abundance in water celery was lower (Table 3). Finally, subcluster A2.3, which clearly separated the volatile profile of water celery from the rest of species, included among others, the compounds reported as unique for water celery (1,2-benzothiazole, T1; spathulenol, A4; dillapiol, P2; 4-hydroxy-3-methyl acetophenone, K3; and α-gurjunene, S8) (Fig. 2).

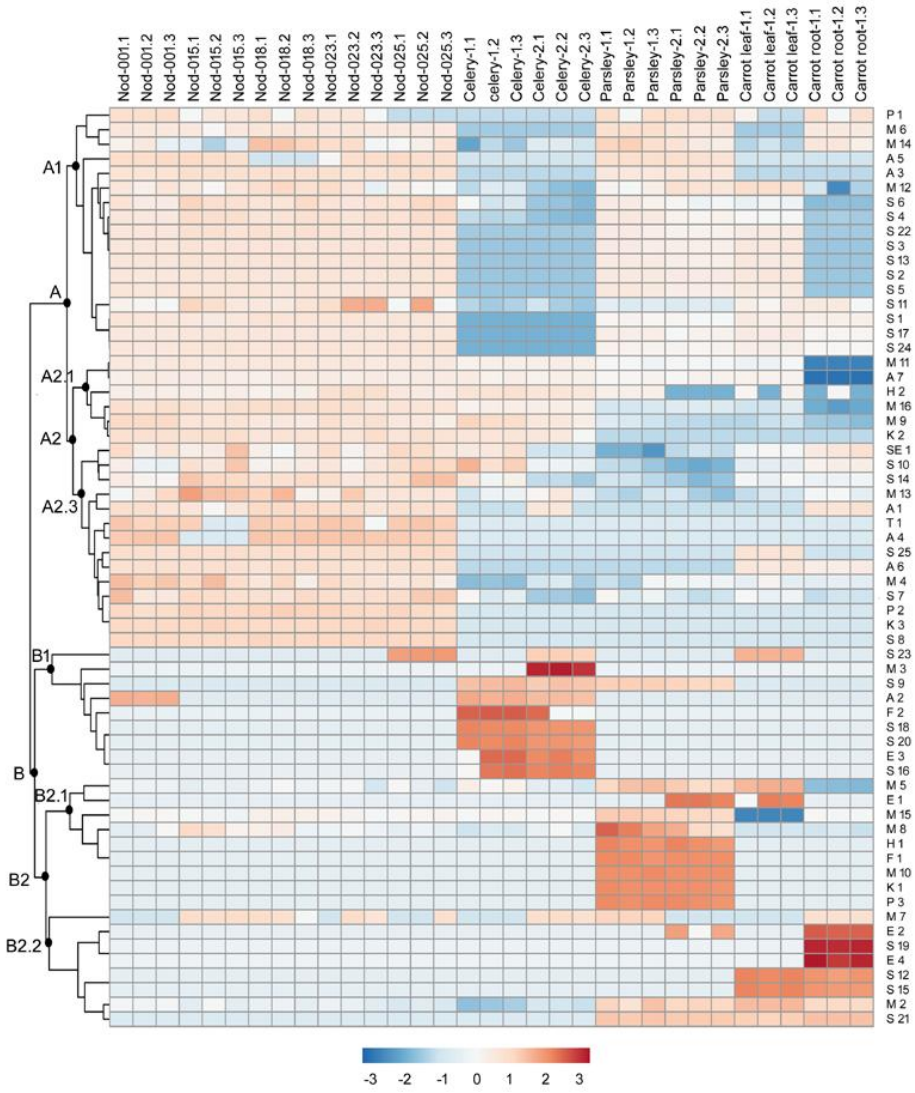


Fig. 2. Hierarchical cluster analysis of the VOCs targeted in the materials studied: water celery (Nod), celery, parsley, and carrot leaf and root, including the three biological replicates of each material. VOCs are clustered in two clusters, A and B, and the correspondent subclusters. Codes of the VOCs correspond to codes indicated in Table 1.

Subclusters in cluster B grouped compounds of relevance for the other species (Fig. 2). Most compounds grouped in subcluster B1 were exclusive of celery, such as β-selinene (S18), one of the main VOCs of celery, and α-selinene (S20); or found in the species at the highest content, like α-cedrene (S9), also present in parsley but at significant lower levels (Table 3). On the contrary, subcluster B2.1 grouped some of the main compounds of parsley (Fig. 2). In this subcluster was included the compound 1,3,8-*p*-menthatriene (M15), on average 280-fold higher than in water celery; but also those exclusive for the species including β-phellandrene (M10), with similar levels to limonene (M9) in water celery, and apiol (P3), close to the levels of dillapiol (P2) in water celery (Table 3). Finally, subcluster B2.2 grouped compounds unique from carrot (e.g. β-sesquiphellandrene, S12; and (*Z*)-β-farnesene) together with others found also in parsley at considerable levels like α-pinene (M2), which level in these species was higher than in water celery.

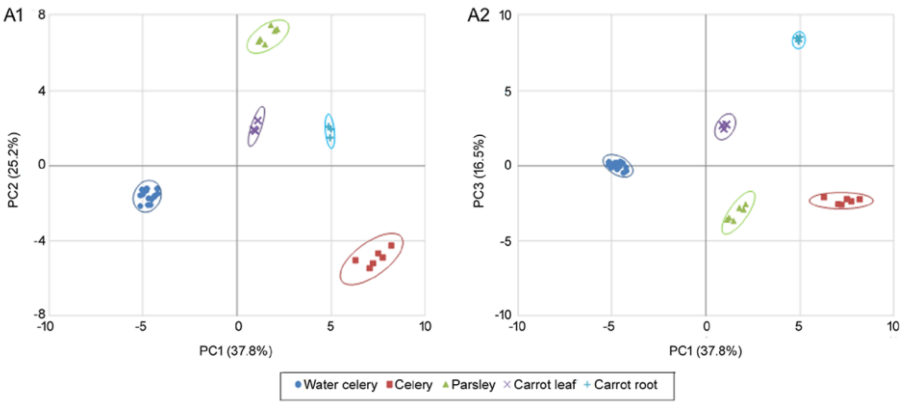


Fig. 3. Scores plot from the Principal Component Analysis of the materials studied: water celery, celery, parsley, and carrot leaf and root, including the three biological replicates. A1. Analysis of the first and second principal components, A2. Analysis of the first and third principal components.

The PCA score plot clearly separated the materials evaluated according to the qualitative and quantitative differences described among them (Fig. 3). The first two principal components accounted for 63.0% of the

variance (PC1 37.8%, PC2 25.2%), which increased to 79.5% when the third principal component was considered. Water celery and celery were clearly separated in the PC1, while the samples of parsley and leaves of carrot overlapped. By contrast, the second principal component grouped the materials by species, overlapping the roots and aerial parts of carrot and with water celery and celery being very close to each other. Finally, the third principal component separated the roots of carrot from the other leafy materials (Fig. 3).

Discussion

Water celery has been traditionally gathered from the wild and included in the culinary tradition of several countries, especially among the Mediterranean cultures, that lived in close connection with the nature. Despite the popularity of this wild vegetable, described as an aromatic herb with spicy, intense flavour (Guarrera & Savo, 2013, 2016), there are few works focused on the aromatic profile of the species, based in the analysis of the extracted essential oil from dried leaves and with pharmacological and environmental purposes (Benelli *et al.*, 2017; Heshmati Afshar *et al.*, 2017; Maxia *et al.*, 2012; Menghini *et al.*, 2010). Here we provide for the first time the volatile profile of the unprocessed water celery shoots using the HS-SPME technique instead of other extracting methods.

Previous works reported this species as rich in monoterpene hydrocarbons (20.9 to 58.7%) and phenylpropanoids (33.9 to 70.8%) (Benelli *et al.*, 2017; Heshmati Afshar *et al.*, 2017; Maxia *et al.*, 2012; Menghini *et al.*, 2010), while the sesquiterpene fraction represented less than 7.0% of total volatiles. By contrast, in our study, sesquiterpenes ranged from 25.7 to 53.9%. These differences probably derived from the extraction method and analysis employed. In this way, Stashenko, Jaramillo and Martínez (2004) found that the HS-SPME technique significantly increased the percentage of total sesquiterpenes, in comparison to other techniques. On the other hand, drying methods may also affect the extraction of VOCs, although differences in these terms are not clear and apparently depend on the spice, drying method and compound considered (Díaz-Maroto, Pérez-Coello & Cabezudo, 2002; Pirbalouti, Mahdad & Craker, 2013).

Consequently, monoterpenes and phenylpropanoids, specially limonene, dillapiol and myristicin were previously described as the main VOCs (Benelli *et al.*, 2017; Heshmati Afshar *et al.*, 2017; Maxia *et al.*, 2012; Menghini *et al.*, 2010). By contrast, the HS-SPME technique allowed in our work to identify the sesquiterpene germacrene D as the main VOC in fresh leaves of water celery, while the relative abundance of phenylpropanoids were not higher than 15% in any of the populations.

Our results showed that the volatile profile of water celery is quantitative and qualitatively very rich, at the same or even higher level than parsley. The main aroma constituents of water celery were germacrene D (S17), which provides weak spicy and fruity flavour, and limonene (M9), with citrus and fresh notes (Acree and Arn, 2004; Jirovetz, Buchbauer, Ngassoum & Geissler, 2002; "The Good Scents Company", 2018); as well as *allo*-ocimene (M16), terpinolene (M14), and β-caryophyllene (S10), which provide different woody, spicy and sweet notes (Acree and Arn, 2004; Jirovetz *et al.*, 2002; "The Good Scents Company", 2018). Also, the great content in dillapiol (P2) contributed to the spicy and woody notes that can be detected in this vegetable.

Moreover, the specific aroma of a species is due not only to the main components, but also the relative abundance of them (Auda, Pineau, Mestdagh, Poisson, & Rytz, 2016). The aroma of water celery has been described as a mixture of carrot and celery (Heshmati Afshar *et al.*, 2017), with notes that related it to parsley. The aromatic profile described here reinforces this idea. On the one hand, all of them were rich in terpenes. This family of VOCs has been described as the main group in the essential oil in *Apiaceae* species, together with the phenylpropanoids (e.g., El-Zaeddi *et al.*, 2016; Jawdat, Al-Faoury, Odeh & Al-Safadi, 2015; Valente *et al.*, 2013). Many sesquiterpenes determined here provide herbal and woody notes (Acree and Arn, 2004; Jirovetz *et al.*, 2002; "The Good Scents Company", 2018), whose presence would characterise the common, basal aroma in all the materials. Moreover, some of the most prominent VOCs of water celery were present in a similar relative abundance in celery, carrot and, to a lesser extent, in parsley. For instance, limonene (M9), β-caryophyllene (S10), *allo*-ocimene (M16), β-(*Z*)-ocimene (M11) and terpinolene (M14) were the

predominant compounds of celery and were in high relative abundances also in water celery. All these VOCs are probably the reason for similarities in the aroma of both vegetables. The same happened with the presence of β-caryophyllene (S10) and terpinolene (M14) in carrot samples. And probably the notes of parsley come from the phenylpropanoid myriscitin (P1) present in parsley and water celery. On the contrary, the presence of other unique compounds, such as dillapiol (P2) or spathulenol (A4), and the specific combinations of VOCs present, would contribute further to differentiate the aroma of water celery.

Also, our findings showed that the volatile fraction of water celery was quantitatively richer compared to the related species evaluated, particularly in the levels of sesquiterpenes. An increasing content in these compounds would be reflected in a more intense aroma. In fact, the aroma and taste of wild edible vegetables is frequently cited as one of the sociocultural reasons behind the consumption of these plants (Serrasolses *et al.*, 2016); and, in the case of water celery, this is a critical point for its consumption since the vegetable is described as an aromatic ingredient and, therefore, is used for adding flavour to several dishes (Guarrera and Savo, 2013, 2016).

Finally, differences among the five water celery populations were described, in terms of absolute GC areas and for relative abundance of individual compounds. The genotype can be responsible in the production and accumulation of volatile compounds (Darriet, Andreani, De Cian, Costa, & Muselli, 2014). Thus, our results may be used in future works of selection and adaptation for developing a new crop adapted to the consumers' preferences. For instance, Nod-001 presented the lowest levels in sesquiterpenes, so the intensity of the woody and herbal odour that many of these compounds provide, would be the lowest. On the contrary, this population may be selected for developing a variety with more intense citrus and minty notes, due to the higher content and relative abundance in limonene (M9) of this accession. By contrast, Nod-025 could be selected for the high content combined with relative abundance in germacrene D (S17) and β-caryophyllene (S10), two main sesquiterpenes that would provide woody and spicy notes with high intensity due to their content. Moreover,

based on the variation found in our materials and considering that only populations of the Horta Nord shire of Valencia were studied, our results suggest that more diversity on the volatile composition for this species could be found in other areas. This suggestion is supported by the differences that Maxia *et al.* (2012) found between specimens coming from Italy or Portugal. Therefore, further surveys are advisable in order to widen the genetic pool available for breeding based on the organoleptic quality of this species. In addition, the use of controlled conditions for growing materials in the subsequent steps of the breeding program would be useful for minimising the environmental effect.

Conclusion

As a whole, the volatile profile of water celery was determined as rich in terpenes, but also the phenylpropanoids dillapiol and myristicin presented relevant contents in the fresh leaves of this vegetable. In contrast to previous studies, the sesquiterpene fraction was accumulated in higher percentage in our water celery materials, differences that may be related to the extraction protocol and analysis of the VOCs. In fact, the HS-SPME technique employed in the current study allowed identifying germacrene D as the main compound of water celery, together with limonene and *allo*-ocimene. Differences found among the populations for these contents and relative abundance may present a genetic component, thus allowing the selection of materials according to consumers' preferences.

The particular aroma of water celery, although unique, presented similarities in the relative abundance of different VOCs with their relatives. These similarities would account for the similarities in the aroma of water celery and the other species. In addition, the sesquiterpene family of VOCs was accumulated in higher concentration in water celery, which would be reflected in a more intense aroma with herbal, spicy and citrus notes. Therefore, this distinct aroma quality may be useful for the differentiation and enhancement of water celery as new salad ingredient, enhancing its value as a potential new crop for vegetable diversification.

Declarations of interest

None.

Acknowledgments

C. Guijarro-Real thanks the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for the financial support with a predoctoral FPU grant (FPU14-06798). Authors also thank Manuel Figueroa for his unvaluable ethnobotanical knowledge and advice, as well as his support in the survey of water celery in the Horta Nord shire.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Capítulo 2. Potencial de la rabaniza como cultivo: caracteres morfológicos, nutracéuticos y aromáticos

2.1. Morphological diversity and nutraceutical properties in wall rocket (*Diplotaxis erucoides* (L.) DC.): The basis for the development of a new crop

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Keywords: Antioxidants, morphology, nitrates, phenotypic variability, wall rocket

Abstract

Wall rocket is a species traditionally consumed along the Mediterranean regions, with interest as a potential crop of high functional quality. However, the information regarding morphological variability is scarce. Thus, there is a need of characterizing and determining such variation, as a tool for the development of breeding programmes. In addition, the composition evaluation of these materials is also of interest if they are aimed at developing a crop with enhanced quality. In this study, 45 populations of wall rocket were evaluated for selected morphoagronomic traits and for the content in ascorbic acid (AA), total phenolics (TP) and nitrates (NO_3^-). Plants had on average moderate growth habit and good response to transplant. Moderate variability was determined among populations, mainly for size-related traits, with low to moderate heritability estimates (<35% for plant and leaf traits). Significant correlations among traits were established. These results have implications for breeding programmes as they limit the quantity of morphologically different varieties that can be developed. Nevertheless, some different materials may be selected according to the PCA. On the other hand, materials were on average rich in AA (53 mg 100 g^{-1}) and TP (116 mg CAE 100 g^{-1}), and also accumulated high levels of NO_3^- (892 mg 100 g^{-1}). Significant correlations were determined for nutritional traits, which could be exploited for enhancing the quality of the final product. In summary, this information increases the knowledge regarding the variation that wall rocket displays as species, and can be useful for the establishment of current and future breeding programmes.

Introduction

It is estimated that more than 7,000 plant species have been used during History as food (Shikov *et al.*, 2017). These edible species include established crops, neglected and underutilized crops, and wild edible plants (WEPs) directly collected in the wild or modified systems where are found as weeds (Shin *et al.*, 2018). Changes in lifestyle, detachment from the nature or large-scale cultivation, among other reasons, gradually decreased

the use of WEPs (Łuczaj *et al.*, 2012; Pinela *et al.*, 2017). However, Pinela *et al.* (2017) highlighted a recent phenomenon of revalorization of WEPS emerging in modern societies. Such renewed interest offers an opportunity for the development of new crops by means of domestication and adaptation programmes.

Wall rocket (*Diplotaxis erucoides* (L.) DC.) represents an example of WEP traditionally consumed in the Mediterranean countries (e.g., Parada *et al.*, 2011; Couplan, 2015; Licata *et al.*, 2016; Pinela *et al.*, 2017). This wild vegetable is consumed by their leaves and tender shoots, raw or cooked, in salads, soups, omelettes or pasta (Guarrera and Savo, 2016; Licata *et al.*, 2016). The species has a characteristic, little pungent flavour resembling other *Brassicaceae* like mustard seeds, horseradish or wasabi. Regarding the functional quality, wall rocket may accumulate high concentrations of vitamin C and phenolic compounds as the cultivated rocket crops (Spadafora *et al.*, 2016). However, these species can accumulate high amounts of nitrates as well (Egea-Gilabert *et al.*, 2009; Disciglio *et al.*, 2017; Schiattone *et al.*, 2018). Nitrates have been considered for decades as antinutrients with potential negative effects for health (Bondonno *et al.*, 2018; Lundberg *et al.*, 2018); thus, the accumulation in foodstuff must be controlled, especially for leafy vegetables.

The appreciation of wall rocket flavour by consumers, together with its promising functional quality makes it a good candidate for being established as a crop. As far as we know there is only one commercial cultivar of wall rocket, but it is not cultivated extensively. Domestication and adaptation programmes for establishing WEPs as crops require, in a first step, the collection and evaluation of materials searching for characters of interest. However, it is common to find a lack of information regarding these two key points in emerging crops, which represents a weakness for breeding programmes (Herraiz *et al.*, 2015a). In the case of wall rocket, the number of accessions currently available in germplasm banks is very low (BGV-UPM, 2019). Thus, collecting materials is an imperative step for starting the domestication process. In addition, we have not found previous studies that analyse the morphoagronomic diversity present in the species. By contrast, previous studies have been developed in these terms for the taxonomically

related salad rocket (*Eruca sativa*) and wild rocket (*Diplotaxis tenuifolia*), demonstrating a high degree of variation especially in the former (Taranto *et al.*, 2016; Bell *et al.*, 2017). Characterization can be used to identify whether there is high degree of variation among the materials or not; to identify, if present, accessions with specific characters of interest; and to establish adequate selection or breeding strategies. The use of standardized descriptors allows an effective characterization, needed for breeding programmes and related tasks and for comparison of experimental data (Herraiz *et al.*, 2015b). However, standardized descriptors are not usually found for underutilized crops and wild vegetables. In fact, no standardized descriptors have been described for wall rocket, although they can be found for *Eruca* spp. (IPGRI, 1999). Due to the taxonomic relationship between both species, IPGRI descriptors may be used as a basis for characterizing wall rocket germplasm.

We have started a domestication and crop-adaptation programme at the Universitat Politècnica de València (UPV, Valencia, Spain), in which this work is encompassed. The programme is addressed to the release of commercial cultivars of wall rocket well adapted to our climatic conditions. In this context, the present work was focused on the phenotypic characterization of the local germplasm collected, mainly leaf characterization as this is the product of commercial interest, as well as in evaluating key composition traits. *Eruca* spp. descriptors (IPGRI, 1999) were selected for this task, and adapted when needed. This work offers a starting point for selection in breeding programmes, and is also a key for developing descriptors for wall rocket.

Material and methods

Plant material and cultivation conditions

Forty-five populations of wall rocket were evaluated in the current study. Forty-three represented seedlings from wild populations collected in the Valencian Community (CV, Spain) during the spring of 2015. Two other populations (BGV-UPM 1235, Alicante, CV, Spain; and BGV-UPM 1549, Teruel, Aragon, Spain) were provided by the "Banco de Germoplasma Vegetal-UPM César Gómez Campo" (Madrid, Spain). All materials are conserved at the UPV.

The experiment was performed during the months of May to July 2016 at the UPV (39º 29' 00" N; 0º 20' 27" W). Seeds were treated with 2.5% commercial sodium hypochlorite plus 100 ppm gibberellic acid (Duchefa Biochemie, Haarlem, The Netherlands) solution (Guijarro-Real *et al.*, 2018). Treated seeds were sown in seedling trays using commercial Neuhaus Humin-substrat N3 substrate (Klasmann-Deilmann Gmbh, Geeste, Germany) and placed for one week in a growing chamber with long day conditions (16 h light/ 8 h dark, T=25 ºC), then moved to a greenhouse. Three weeks after sowing, seedlings were transplanted to larger pots (15L) filled with a mixture of commercial N3 substrate and coconut fibre (Horticoco, Valimex, Valencia, Spain) (1:1). For each population, three pots with 25 plants each were filled. Populations were placed following a randomised design. A drip irrigation system was used for watering and fertilizing the pots. The final concentration of the main anions and cations added with the irrigation was: 11.47 mM NO_3^-, 1.00 mM NH_4^+, 1.50 mM $H_2PO_4^-$, 6.75 mM K^+, 3.25 mM Ca^{2+}, 2.50 mM Mg^{2+}, 2.82 mM SO_4^{2-}. For microminerals supply, the following salts were added to the system: 50 µM H_3BO_3, 10 µM Fe-EDTA, 4.5 µM $MnCl_2$, 3.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$, 0.1 µM $(NH_4)_6Mo_7O_{24}$. Leaves were harvested with the appearance of the first flower bud.

Morphoagronomic characterization

Morphoagronomic traits for characterization were selected from normalized descriptors for *Eruca* spp. (IPGRI, 1999), and adapted when needed. Selected traits related to the whole plant as well as traits used for describing the leaves were used as summarized in Table 1.

Plant habit traits were described considering the whole set of plants in each population, and included growth rate (Growth), adaptation level to transplant and greenhouse conditions (Adaptation), and leaf growth attitude (Attitude). In addition, five plants per population were used to measure plant height ($Height_{Plant}$) and width ($Width_{Plant}$), stem height ($Height_{Stem}$), and length of the longest internode ($Length_{Internode}$). These plants were also used for describing the stem thickening (Thickening) and colour ($Colour_{Stem}$).

Table 1. Morphological and agronomic descriptors used for the characterization of wall rocket populations.

Descriptor[†]	Code	Type[‡]	Scale/ units
Plant habit			
Plant growth rate	Growth	Q. ord	3 = slow; 7 = fast
Adaptation level[a]	Adaptation	Q. ord	3 = low; 7 = high
Leaf growth attitude	Attitude	Q. cat	1 = semi-prostrate; 2 = horizontal; 3 = semi-erect
Whole plant traits			
Size-related traits			
Plant height	$Height_{Plant}$	Quant	cm
Plant width	$Width_{Plant}$	Quant	cm
Stem height	$Height_{Stem}$	Quant	cm
Internode length	$Length_{Internode}$	Quant	cm
Descriptive traits			
Stem thickening	Thickening	Q. ord	3 = thin; 7 = thick
Stem colour	$Colour_{Stem}$	Q. cat	1 = light green; 2 = green, 3 = dark green; 4 = red/purple green; 5 = red/purple
Stem hairiness	Hairiness	Q. ord	3 = sparse; 7 = dense
Foliage[b]	Foliage	Quant	(number of leaves)
Leaf traits			
Size-related traits			
Leaf length[c]	Length	Quant	cm
Leaf width	Width	Quant	cm
Petiole length	$Length_{Petiole}$	Quant	cm
Leaf perimeter[c]	Perimeter	Quant	cm
Leaf area[c]	Area	Quant	cm^2
Descriptive traits			
Leaf margin shape	Margin	Q. cat	1 = entire; 2 = crenate; 3 = dentate
Leaf blade shape	Shape	Q. cat	1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate
Leaf apex shape	Apex	Q. cat	1 = acute; 2 = rounded; 3 = broadly rounded
Leaf lobation intensity	Lobation	Q. ord	0 = absent; 5 = deep lobation
Petiole/midvein	$Colour_{Petiole}$	Q. cat	1 = white; 2 = light green; 3 = green; 4

color	= purple; 5 = red

[†]Traits were measured in the pre-flowering stage, it is, when the first flower bud became visible but not fully developed. [‡]Quant: quantitative; Q. cat: qualitative categorical; Q. ord: qualitative ordinal. [a]Refers to the adaptation of plants to greenhouse conditions, after transplant. [b]Refers to the number of leaves developed. [c]Traits measured including the petiole.

Finally, ten descriptors were evaluated in the leaves (Table 1). Five descriptors were size-related traits, including leaf length (Length), and width (Width), petiole length ($Length_{Petiole}$), and total perimeter (Perimeter) and area (Area). Measurements were performed using the Tomato Analyzer v 3.0 software (Rodríguez *et al.*, 2010). On the other hand, the descriptive traits included the shape of blade (Shape), leaf apex (Apex) and margin (Margin), intensity of lobation (Lobation), and petiole/midvein colour ($Colour_{Petiole}$). All traits were measured in ten leaves per population corresponding to second (lower) and fourth (upper) true leaves.

Chemical analysis

When the floral bud appeared, leaves were harvested, cleaned and divided for the analyses. Fresh material was used for the determination of ascorbic acid (AA), while frozen (-80 ºC) and freeze-dried material was destined to the analysis of total phenolics (TP) and nitrates (NO_3^-) contents. Three replicates were used for each analysis. The AA content was measured according to Cano and Bermejo (2011) with slight modifications. Briefly, 1.0 g was homogenated with 5 mL 3.0% (w/v) cold *meta*-phosphoric acid solution and filtered through a 0.22 μm PVDF filter (Teknokroma, San Cugat del Vallès, Spain). Determinations were performed with a 1220 Infinity HPLC system (Agilent Technologies; Santa Clara, CA, USA) using a Brisa C_{18} column (150mm × 4.6 mm id, 3μm particle zixe; Teknokroma, San Cugat del Vallès, Spain). Conditions were as follows: an isocratic phase of methanol: 1.0% acetic acid (5:95) during 15 min; injection volume of 5 μL; flow rate of 1 mL min^{-1}. Quantification was performed at 254 nm using *L*-ascorbic acid for external standard calibration. Results were expressed as mg AA 100 g^{-1} of fresh weight (FW).

Content in TP was evaluated using the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) according to Plazas *et al.* (2014). Briefly, 0.125 g were extracted with 70% acetone containing 0.5% acetic acid for 24 h. Aliquots of 65 µl reacted with 500 µl of diluted Folin-Ciocalteu (1:10) for 5 min, plus 500 µl of 60 g L^{-1} sodium carbonate for 90 min. Absorbance was read at 765 nm and an external standard of chlorogenic acid was used for quantification. Results were expressed as mg of chlorogenic acid equivalents in 100 g of fresh weight using the percentage of humidity for conversion (mg CAE 100 g^{-1} FW).

The content in NO_3^- was determined with a nitrate-selective ion (Crison Instruments S.A., Alella, Barcelona, Spain). The extraction protocol was adapted from Egea-Gilabert *et al.* (2014). Thus, 0.1 g was homogenated with 50 mL of distilled water for 15 min, in continuous stirring. Measurement was obtained after adding 1 mL of 2 M diammonium sulfate ($(NH_4)_2 SO_4$) buffer, and results expressed as mg NO_3^- 100 g^{-1} FW.

Folin-Ciocalteu reagent, sodium carbonate, glacial acetic acid and acetone were purchased from Scharlab S.L. (Mas d'En Cisa, Spain). *L*-ascorbic acid, chlorogenic acid, *meta*-phosphoric acid and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade reagents were used for HPLC analyses, and ACS grade for the rest of analyses.

Statistical analysis

Mean and ranges of each population were obtained for quantitative data. Data were subjected to a one-way analysis of variance (ANOVA). Negligible differences were obtained when comparing original and transformed data; thus, original data were used for analysis and calculation of heritabilities, as they provide information on actual data (Rodríguez-Burruezo *et al.*, 2002). The total sum of squares was partitioned in the sum of squares of accession and residual effects (Clewer and Scarisbrick, 2001), and expressed as percentages. Broad sense heritability (H^2) was calculated as $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2)$, where σ_G^2 and σ_E^2 were the estimates of genotypic and residual variance, respectively (Wrickle and Weber, 1986). In addition, Pearson linear correlations among traits were studied. For qualitative data,

percentages for each category were obtained and compared using the Marascuilo procedure ($P = 0.05$).

Finally, a Principal Component Analysis (PCA) was performed using the Clustvis tool (Metsalu and Vilo, 2015), including quantitative and ordinal qualitative traits. Prior to the analysis, data were $\log_2$-transformed and centered, and vector scaling was applied to rows.

Results

Plant habit and whole plant characterization

The plant growth was mainly intermediate, with 64.4% of populations displaying a 4.5 to 5.5 score in a 3-7 scale (Fig. 1). Populations DER040, DER051, DER055 and DER073 had the fastest growth, while DER069 had the lowest growth rate (Table S1). In addition, all populations had a good response to transplant and subsequent greenhouse growing conditions, determined as intermediate only for DER017 (Fig. 1, Table S1). Moreover, the leaves growth attitude was mainly semi-erect, as described in 84.5% of populations (Fig. 1). Only DER041 displayed a semi-prostrate growth attitude of leaves (Table S1).

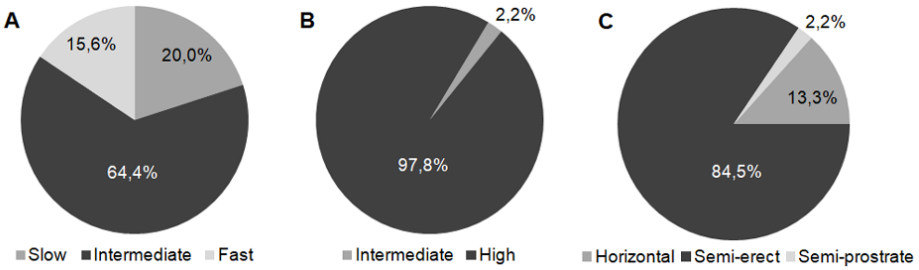

Fig. 1. Percentage of populations included in the different categories described for the qualitative categorical descriptors. A) Plant growth rate with three categories ranging from slow rate (< 4 in the scale) to fast rate (> 6 in the scale). B) Plant adaptation with two categories: intermediate (5 in the scale) and high adaptation (> 6 in the scale). C) Leaf growth attitude with three defined categories: semi-prostrate, horizontal and semi-erect. For more details refer to Table 1.

The mean values and ranges for the plant size-related traits are shown in Table 2 (for individual values of each population refer to Table S1). Significant differences ($P < 0.05$) were observed among populations for all traits. The contribution of population effect to the total sum of squares ranged between 26.6% ($Width_{plant}$) and 49.7% ($Height_{stem}$) (Table 2). Therefore, the broad-sense heritabilities were moderate, in the case of $Height_{Plant}$, $Height_{stem}$ and $Lengh_{internode}$ (28.9%, 37.6% and 33.1%, respectively), to low for Foliage and $Width_{Plant}$ (15.4% and 8.5%, respectively) (Table 2). On the other hand, positive correlations were established among these traits (Table 3). All Pearson correlations were significant ($P < 0.05$) except for $Width_{Plant}$/$Length_{Internode}$. The greatest correlation coefficient was found for $Height_{Stem}$/$Length_{Internode}$ ($r = 0.9335$), and relative high correlations ($r > 0.5$) were also established between these traits and $Height_{Plant}$.

Table 2. Mean value, range, total sum of squares for the effects of population and residuals, and broad sense heritability (H^2) for plant quantitative traits.

Descriptor[a]	Mean	Range	Sum of squares (%)		H^2 (%)
			Population	Residual	
$Height_{Plant}$ (cm)	8.92	(6.48–10.74)	42.8^{***}	57.2	28.9
$Width_{Plant}$ (cm)	13.33	(10.56–16.60)	26.6^{*}	73.4	8.5
$Height_{Stem}$ (cm)	4.03	(2.38–6.57)	49.7^{***}	50.3	37.6
$Length_{Internode}$ (cm)	1.06	(0.56–2.10)	46.3^{***}	53.7	33.1
Foliage	8.32	(7.00–9.40)	32.1^{**}	67.9	15.4

*, ** and *** indicate significant differences at $P < 0.05$, 0.01 and 0.001, respectively.
[a]For details refer to Table 1.

Table 3. Pearson linear correlations between plant size-related traits ($n = 45$).

	$Height_{Plant}$	$Height_{Stem}$	$Length_{Internode}$
$Width_{Plant}$	0.4335^{**}	0.3431^{*}	0.2328^{ns}
$Height_{Plant}$		0.6280^{***}	0.5559^{***}
$Height_{Stem}$			0.9335^{***}

^{ns}, *, ** and *** indicate no significant or significant at $P < 0.05$, 0.01 or 0.001, respectively.

Finally, traits such as stem thickening, colour and hairiness, and foliage, were evaluated. Overall, populations developed a thin to intermediate stem, with a mean value of 3.9 on a 3-7 scale; and a sparse to intermediate degree of hairiness (4.05 on average on a 3-7 scale) (Table S1). The stem colour was mainly described as green considering the total set of plants in each population. The foliage developed at the moment of the flower bud emergence was similar in all populations, between seven and nine leaves on average (Table S1).

Leaf related traits

A first analysis was performed in order to compare the lower and upper leaves developed in wall rocket materials. Significant differences ($P < 0.05$) were found for quantitative traits, except for leaf length, which displayed a mean value of 8.96 cm (Fig. 2). Compared to lower leaves, upper ones were on average wider (3.57 cm vs. 3.27 cm), with longer perimeter (25.55 cm vs. 22.35 cm) and larger area (16.37 cm^2 vs. 14.25 cm^2). By contrast, lower leaves displayed longer petioles, on average 1.12 cm more than upper leaves (Fig. 2).

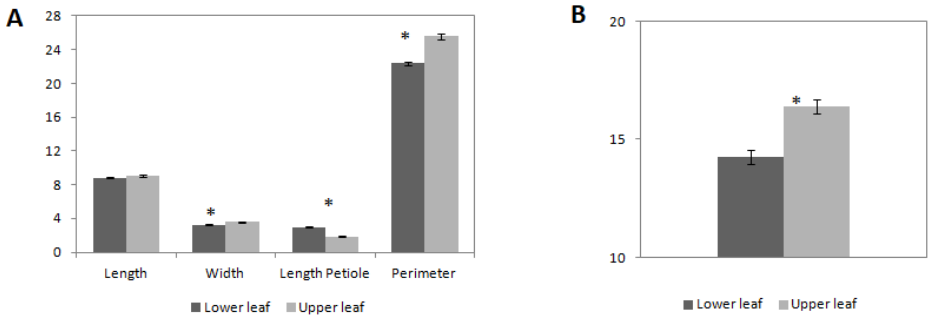

Fig. 2. Mean values ± SE ($n = 225$) for size-related traits measured in the lower and upper leaves. A) Comparison between lower and upper leaves for length, width, length of the petiole and perimeter (cm). B) Comparison between lower and upper leaves for area (cm^2). * indicate significant differences between lower and upper leaves according to the LSD test ($P = 0.05$).

Table 4. Percentage of categorical traits analyzed in the populations of wall rocket, differentiating whether traits were analyzed in the lower or upper leaves (n = 225).

Descriptor[a]	Lower leaf	Upper leaf
Shape	1: 15.6%[b]	1: 2.2%[a]
	-	2: 9.8%
	3: 82.7%[ns]	3: 76.4%[ns]
	4: 1.8%[a]	4: 11.6%[b]
Apex	1: 2.2%[a]	1: 30.2%[b]
	2: 88.0%[b]	2: 62.2%[a]
	3: 9.8%[ns]	3: 7.6%[ns]
Margin	1: 5.3%	-
	1.5: 48.0%[b]	1.5: 0.9%[a]
	2: 45.3%[b]	2: 30.2%[a]
	2.5: 1.3%[a]	2.5: 52.0%[b]
	-	3: 16.9%
Lobation	0: 99.6%[b]	0: 6.7%[a]
	1: 0.4%[a]	1: 25.3%[b]
	-	2: 46.7%
	-	3: 21.3%

Different letters among rows correspond to significant differences according to the Marascuilo procedure. [ns] indicates non significant differences. [a]Shape (leaf blade shape): 1 = orbicular, 2 = elliptic, 3 = obovate, 4 = spatulate. Apex (leaf apex shape): 1 = acute; 2 = rounded; 3 = broadly rounded. Margin (leaf margin shape): 1 = entire; 1,5 = entire-crenate; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate. Lobation (leaf lobation intensity): 0-5 scale where 0 = absent; 5 = deep lobation

Upper and lower leaves also differed for qualitative traits (Table 4). As exception, the petiole/midvein colour was light-green to green in all leaves. Lower leaves were mainly obovate (82.7 %) to orbicular (15.6 %), with a rounded (88.0%) to broadly rounded (9.8%) apex. On the contrary, upper leaves combined obovate shape (76.4%) with remarkable percentages of elliptic (9.8%) and spatulate leaves (11.6%). The percentage of upper leaves displaying rounded apex (62.2%) decreased compared to lower leaves, while the development of acute shape increased significantly, from 2.2% in lower leaves to 30.2% in the upper ones (Table 4). The margin

shape also displayed differences. While lower leaves had a soft margin, mainly entire-crenate (48.0%) or crenate (45.3%), the upper leaves had sharper margins, with 52.0% developing a crenate-dentate margin and 16.9% displaying dentate margins. The intensity of lobation also increased in the upper leaves. Lower leaves were entire (0-score), while 46.7% of upper leaves displayed a 2-score in a 0-5 scale (Table 4).

Table 5. Mean value, range, percentage of the total sum of squares for the effects of population and residuals, and broad sense heritability (H^2) for quantitative leaf-related traits in upper leaves of wall rocket.

Descriptor[a]	Mean	Range	Sum of squares (%)		H^2 (%)
			Population	Residual	
Length (cm)	9.08	(7.27–10.47)	32.8***	67.2	16.6
Width (cm)	3.57	(2.71–4.37)	43.6***	56.4	30.1
Perimeter (cm)	25.55	(20.50–32.00)	45.4***	54.6	32.4
Area (cm^2)	16.37	(10.68–24.17)	43.2***	56.8	29.7
$Length_{Petiole}$ (cm)	1.85	(0.75–2.95)	28.4*	71.6	11.1

*, ** and *** indicate significant differences at $P < 0.05$, 0.01 and 0.001, respectively.
[a]For details refer to Table 1.

Table 6. Pearson linear correlations between leaf size-related traits for upper leaves ($n = 45$).

	$Length_{Petiole}$	Width	Perimeter	Area
Length	0.6781***	0.7032***	0.9413***	0.8006***
$Length_{Petiole}$		0.3149***	0.5471***	0.3802***
Width			0.7663***	0.9229***
Perimeter				0.7852***

*** indicates significant differences at $P < 0.001$.

Considering that the upper leaves were more representative as commercial product, size-related differences among populations were analysed only for the upper leaves (Table 5). As for plant traits, the contribution of population effects to the total sum of squares was below

50.0% for all traits. $Length_{Petiole}$ displayed the lowest contribution (28.4%), while Width, Perimeter and Area had a population effect close to 50.0% (43.6%, 45.4% and 43.2%, respectively). Thus, broad-sense heritability estimates for these traits were also low (for Length and $Length_{Petiole}$) to moderate (for Width, Perimeter and Area) (Table 5). All Pearson correlations among these traits were significant ($P < 0.05$) and positive (Table 6). $Length_{Petiole}$ was great correlated to Length ($r = 0.68$). On the other hand, Length and Width were highly correlated to both Perimeter and Area ($r > 0.75$).

Chemical characterization

As nutritional traits, the content in AA and TP were evaluated in the 45 populations of wall rocket. The average content in AA was 53.3 mg 100 g^{-1} FW, with values ranging between 31.0 (BGV-UPM 1235) and 86.7 (DER064) mg AA 100 g^{-1} FW (Table S2). The content in TP, measured by means of the Folin-Ciocalteu procedure, had a mean value of 116.3 mg CAE 100 g^{-1} FW. A 1.24-fold difference was established between the lowest (DER051) and the highest (DER045) value. On the other hand, the content in NO_3^- was evaluated as antinutrient. The average value was 892.4 mg 100 g^{-1} FW, with a difference of 1.8-fold times between the lowest (DER069) and highest (BGV-UPM 1549) values (Table S2).

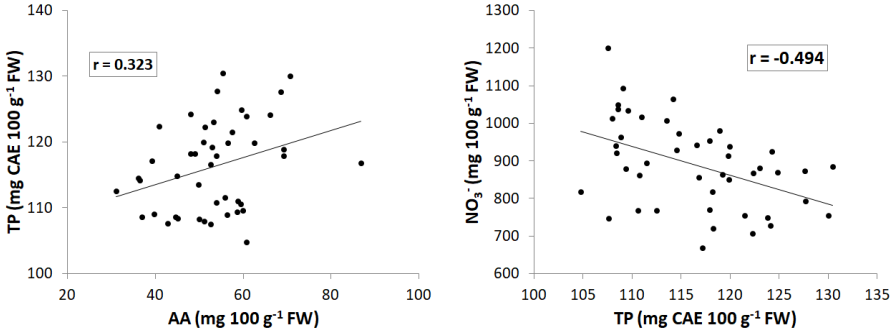

Fig. 3. Correlation between the content in ascorbic acid (AA) and total phenolics (TP), and between TP and the content in nitrates (NO_3^-).

Pearson linear correlations were established between AA - TP and between TP - NO_3^-, while no significant correlation was determined between AA - NO_3^- (Fig. 3). Correlation coefficients were moderate in both cases, and positive in the case of AA - TP (r = 0.323) but negative for TP - NO_3^- (r = -0.449).

Principal component analysis

A PCA was performed with the quantitative and qualitative ordinal data (Fig. 4). Only data from upper leaves were included, due to the high similarity of the lower leaves among the different populations. The first two principal components accounted for 40.7% of the total variability registered. The first component explained 28.8% of the variability registered. It was positively correlated to most of the characters, with leaf-size descriptors displaying the strongest correlations (Fig. 4A). The content in AA and TP and the plant growth attitude were negatively correlated to the first component, with only the content in TP having a correlation coefficient greater than 0.15 in absolute terms. On the other hand, the second principal component accounted for 11.9% of total variability (Fig. 4A). It was negatively correlated to plant-size descriptors, especially height-related traits; from them, plant width had a correlation coefficient lower than 0.10 in absolute terms. The content in NO_3^- had also a negative, weak correlation (< -0.150) with this component. On the contrary, the strongest positive correlations were obtained for leaf size traits, leaf lobation, and TP content. The loading plot analysis grouped in the graphic those descriptors that displayed great correlations (Fig. 4A, Table 3, Table 6). Moreover, the analysis situated the leaf lobation trait close to leaf size-related descriptors in the graph, indicating a positive relationship among these characters.

The PCA score plot showed the distribution of populations in the first and second components (Fig. 4B). The analysis clearly separated the population DER017 from the rest. This population displayed on average the lowest values for leaf-size traits, and had also low values for plant-size traits and low transplant adaptation (Table S1). On the contrary, populations with similarities grouped together. Thus, populations on the right side of the PCA displayed greater values for leaf-size traits compared to other populations, especially in terms of Length and Perimeter (Fig. 4B, Table S1). Considering

plant size traits, the PCA grouped populations DER051, DER008, DER023, DER075 and BGV-UPM 1549 on the right-bottom of the graphic, with high and similar values for $Height_{Plant}$, $Height_{Stem}$ and $Length_{Internode}$. In contraposition, populations DER001, DER057, DER081 and DER103 had low values for those traits and were grouped on the left-up of the graphic (Fig. 4B, Table S1). Finally, no correlations between geographic origin and distribution of populations in the analysis were determined.

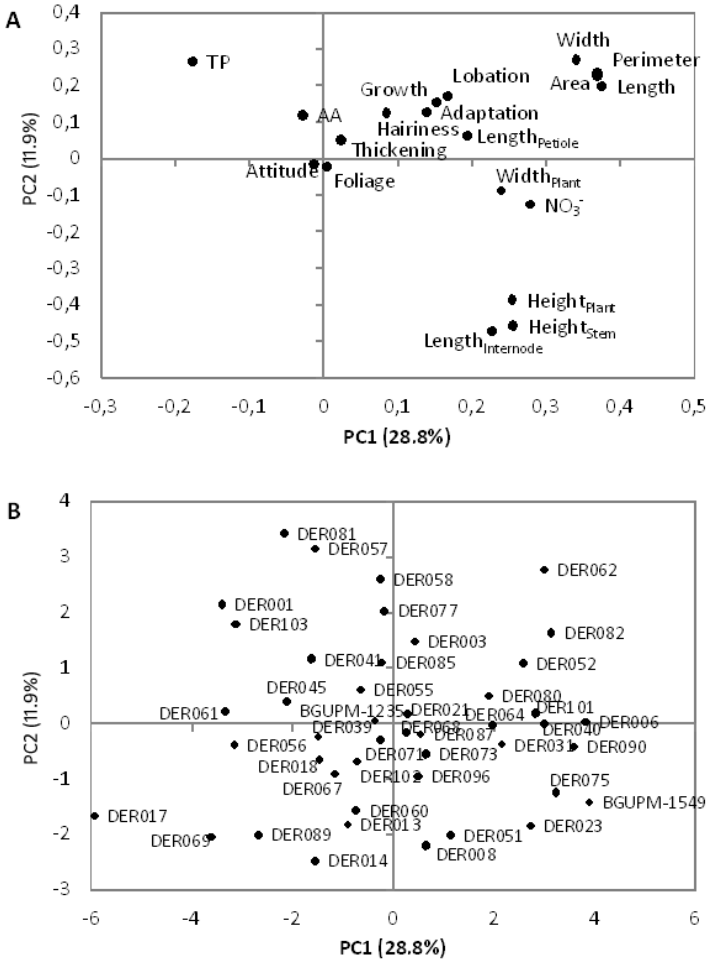

Fig. 4. Principal Component Analysis for the first and second principal components, performed with the quantitative plus qualitative ordinal data. A) PCA loading plot. B) PCA score plot.

Discussion

Characterization of the germplasm available for target traits is an essential step for selection and breeding programmes (Egea-Gilabert *et al.*, 2009). As far as we know, this is the first report that characterizes and compares wall rocket germplasm. Some adaptations of the *Eruca* spp. IPGRI descriptors were required. For instance, the intensity of lobation for *Eruca* do not easily match with the lobation patterns found in wall rocket. Moreover, other descriptors, although not used in the current study, should be also revised and adapted, such as the petal colour –wall rocket is characterized by white flowers (Martínez-Laborde, 1990), with no colour variation–.Thus, specific descriptors lists may be established for the species, useful for future breeding programmes and varieties characterization.

Low to moderate differences were found among populations. The low diversity registered may be explained by genetic factors 1) related to the species, or 2) related to the material collected. On the one hand, it may be that wall rocket as species does not display great morphological variability for the traits evaluated. A similar situation has been observed for wild rocket, as contraposition to salad rocket, which displays greater diversity as species (Taranto *et al.*, 2016; Bell *et al.*, 2017). On the other hand, this low variability may be also related to the area prospected. The strategy followed for our breeding program is the Focused Identification of Germplasm Strategy (FIGS). It is based on the assumption that wild germplasm from a specific origin carry adaptative traits as result of the natural selection pressures in that environment (Prohens *et al.*, 2017). Thus, collecting local germplasm may increase success in breeding programmes aimed at obtaining cultivars for our region and other similar Mediterranean regions. However, the FIGS strategy may have resulted in the collection of low variability, and using populations from other, farer regions may result in a diversity increase. Interestingly, the accessions transferred from the BGV-UPM, which were collected five decades ago also in Spain, did not greatly differ from the current populations either.

Principal Component Analysis is considered a useful tool for screening germplasm in breeding programmes (Mousavizadeh *et al.*, 2015), widely used when morphoagronomic traits are analysed. It should be noticed

that only the upper leaves were included. The lower leaves were greatly different, and consequently should be avoided during the harvest in order to increase the homogeneity of the final product. The PCA results suggest that DER017 could be a good candidate for obtaining small plants developing small leaves. By contrast, populations on the right of the graphic may be selected for increasing the leaf size of the future cultivar. However, broad-sense heritabilities for these descriptors were estimated as low to moderate. The moderate heritability estimates are a key point for current and future selection programmes in wall rocket, and should be considered for adequate selections. Nevertheless, the high Pearson correlations determined between leaf length, width, perimeter and area indicated that leaf proportions are maintained despite the leaf size. These correlations may be also considered as indicators of low variance among populations in terms of leaf shape and intensity of lobation, reinforcing the results obtained for these traits.

Regarding composition traits, results were promising for the classification of wall rocket as a crop with enhanced antioxidant properties. Thus, wall rocket can be considered as a leafy vegetable with relevant amounts of AA, as rocket crops and other *Brassicaceae* (Colonna *et al.*, 2016; Spadafora *et al.*, 2016). The contents determined in our work were significantly greater than the levels determined by Salvatore *et al.* (2005), although the different treatment of materials (fresh vs. boiled) difficult comparisons. In addition, our results highlighted the content in TP in concordance with Disciglio *et al.* (2017). In contraposition to AA, phenolics are less studied in *Brassicaceae* than in other botanical families, usually included in works that are focused on the study of glucosinolates as important secondary metabolites (e.g., Bennett *et al.*, 2006; D'Antuono *et al.*, 2008; Francisco *et al.*, 2010). However, our results suggest that phenolic compounds should be considered as part of the functional quality of wall rocket together with AA. On the other hand, a high content in NO_3^- was also determined. Values were significantly greater than the ones previously established for the species (Bianco *et al.*, 1998; Disciglio *et al.*, 2017). The accumulation of NO_3^- can vary among genotypes (Tang *et al.*, 2016), and it is also affected by growing conditions and crop practices. Factors such as light intensity, air temperature and moisture, growth density, duration of growing period and fertilization, among others, can determine these values

(Bahadoran *et al.*, 2016). Our results suggest that the growing conditions used in this assay may be not adequate for wall rocket cultivation, while modifying the cultivation practices may result in a quality improvement.

Finally, the negative correlation established between the content in TP and NO_3^- was highly promising. According to these results, it would be possible to select materials of wall rocket that combine high antioxidant capacity with low nitrate accumulation, being both desirable traits for markets. Moreover, it is known that reducing the nitrogen fertilization can increase the accumulation of antioxidants including AA and TP (Stagnari *et al.*, 2015), and also decrease the content in NO_3^- (Schiattone *et al.*, 2018). It is, cultivation under conditions of low nitrogen fertilization may enhance the quality of the product. To confirm these results, selected materials could be tested under different environments, thus allowing a better understanding of the environment effect as well as the possibility of detecting genotype x environment interaction effects. In fact, Stommel *et al.* (2015) pointed out the need of testing materials across different environments as it can become determinant for developing new varieties.

Conclusions

This is the first study that analyse morphological aspects of wall rocket by comparing a large quantity of materials. First of all, differences between lower and upper leaves were established. Thus, avoiding the harvest of lower leaves may increase the homogeneity considering a commercial purpose. Results showed a moderate variability among the populations tested, mainly for size related traits, with heritability estimates determined from low to moderate. The limited variation found must be considered in breeding programmes addressed to obtain new cultivars, as it will determine the amount of different materials that can be developed. Despite this moderate variation, the PCA analysis revealed that some materials may be selected, such as DER017 or those ones with the bigger leaves.

Regarding composition traits, wall rocket has been confirmed as a vegetable with high content in AA and TP. It is also a NO_3^- bioaccumulator. However, the establishment of negative correlations among these traits is a promising result to be exploited in terms of genetic selection and/or selection

of adequate cultivation practices. In summary, the information revealed in this study can be considered as a tool for understanding the expected variation for wall rocket as species. Nevertheless, including new materials from other regions may increase such variability so this possibility should be considered.

Acknowledgements

C. Guijarro-Real thanks the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for the financial support by means of a predoctoral FPU grant (FPU14-06798). Authors also thank the "Banco de Germoplasma Vegetal-UPM César Gómez Campo" (Madrid, Spain) for transfer of seeds.

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Table S1. Scores (for qualitative traits) or mean values (for quantitative traits) determined in the populations of wall rocket evaluated for traits related to plant habit, whole plant traits and leaf traits.

Plant traits[a]										
	Plant habit			Whole plant traits						
Population	**Growth**	**Adaptation**	**Attitude**	**$Height_{Plant}$**	**$Width_{Plant}$**	**$Height_{Stem}$**	**$Length_{Internode}$**	**Thickening**	**Hairiness**	**Foliage**
BGV-UPM 1235	5	7	3	8.60	14.80	3.54	0.90	4.0	4.8	8.6
BGV-UPM 1549	4.5	7	3	10.22	13.80	6.30	1.52	3.4	4.0	8.8
DER001	5	7	3	7.70	13.10	2.56	0.60	4.2	4.0	8.4
DER003	4.5	7	3	7.60	13.72	3.38	0.84	3.2	5.0	8.4
DER006	4.5	7	3	10.02	12.82	4.88	1.18	3.6	3.8	8.2
DER008	5	7	3	9.80	13.94	5.72	1.56	3.8	3.6	8.2
DER013	4.5	7	3	8.70	13.96	4.46	1.34	4.4	3.8	7.8
DER014	4	7	3	9.30	13.64	4.68	1.38	4.4	2.8	8.0
DER017	4	5	3	7.84	12.16	3.10	0.66	3.4	3.0	8.6
DER018	5	7	3	8.22	13.32	3.92	1.12	3.8	4.8	8.0
DER021	5	6.5	3	8.46	13.14	3.82	1.06	4.0	5.0	8.4
DER023	5	7	3	10.74	14.30	5.88	1.50	4.8	4.8	8.6
DER031	4	7	2	9.48	15.22	3.82	1.02	4.0	5.6	8.6
DER039	4.5	7	3	8.54	11.60	4.16	1.00	4.0	4.2	8.6
DER040	7	7	3	10.38	14.36	4.30	1.12	3.8	4.0	7.4
DER041	5.5	7	1	8.10	11.50	2.38	0.64	4.4	4.4	7.8
DER045	5	7	3	8.08	12.74	3.60	0.96	4.4	4.4	9.4

DER051	7	7	2	10.56	13.96	5.16	1.42	3.4	2.0	8.2
DER052	5.5	7	3	9.56	12.62	4.42	1.28	3.2	5.0	8.8
DER055	7	7	3	9.76	13.94	3.42	0.90	4.0	4.8	8.0
DER056	4	7	3	8.62	10.56	3.26	0.98	3.0	5.4	7.6
DER057	4.5	7	3	7.26	11.54	2.56	0.64	4.0	2.8	8.0
DER058	6	7	3	7.42	14.14	3.18	0.70	5.0	5.0	8.8
DER060	4	6	2	9.16	12.84	4.48	1.12	4.2	5.4	7.8
DER061	4.5	7	3	8.20	11.80	3.06	0.68	3.6	3.4	9.0
DER062	5.5	7	3	8.98	15.34	3.72	0.74	4.4	5.0	9.0
DER064	5	6.5	3	8.88	16.60	5.10	1.35	4.3	5.0	8.3
DER067	4	7	3	9.04	13.36	4.60	1.24	4.2	2.0	8.2
DER068	5	7	3	8.42	14.48	3.66	1.04	4.2	5.6	9.0
DER069	3	7	3	8.66	13.10	4.42	1.06	4.0	4.6	8.8
DER071	4	7	3	10.16	14.30	3.30	0.68	4.4	3.6	9.0
DER073	7	6.5	2	9.98	12.68	4.24	1.02	3.8	4.0	8.2
DER075	5	7	3	10.68	14.50	5.42	1.46	4.0	3.8	8.8
DER077	6	6.5	2	7.90	13.16	2.68	0.68	4.2	3.4	7.8
DER080	4	7	3	8.78	14.16	4.06	1.00	3.8	4.6	8.6
DER081	5	7	3	6.48	10.56	2.52	0.56	3.2	3.4	7.4
DER082	5	7	3	10.20	13.92	3.52	0.92	4.2	3.8	9.0
DER085	5	7	3	9.24	14.20	3.60	0.82	4.6	3.6	7.6
DER087	5	7	3	8.72	12.30	4.58	1.24	2.8	4.4	8.0
DER089	5	7	3	9.10	11.28	4.46	1.42	3.4	2.2	8.8

DER090	6	7	3	8.47	12.77	6.57	2.10	3.3	3.0	7.0
DER096	5	7	3	9.46	13.94	5.06	1.34	3.8	4.4	8.4
DER101	5	7	2	9.26	14.20	5.02	1.44	4.2	3.6	8.2
DER102	5	6.5	3	9.86	12.96	3.32	1.20	3.8	3.0	7.4
DER103	4.5	7	3	7.36	12.80	2.54	0.64	3.6	3.2	8.2

Leaf traits[a]									
Population	**Length**	**Width**	**Length$_{Petiole}$**	**Perimeter**	**Area**	**Margin**	**Shape**	**Apex**	**Lobation**
BGV-UPM 1235	8.62	3.69	1.31	23.52	17.84	2: 20%; 2.5: 80%	3: 100%	2: 20%; 3: 80%	1.4
BGV-UPM 1549	9.82	4.12	1.88	29.00	19.92	2.5: 20%; 3: 80%	1: 20%; 2: 40%; 3: 40%	2: 60%; 3: 40%	2.4
DER001	7.89	3.36	1.14	22.03	14.16	2: 20%; 2.5: 60%; 3: 20%	2: 60%; 3: 40%	2: 80%; 3: 20%	2.2
DER003	9.42	4.03	1.82	26.18	19.01	2: 40%; 2.5: 40%; 3: 20%	3: 100%	2: 20%; 3: 40%; 4: 40%	1.8
DER006	10.08	4.28	2.69	30.38	21.71	2: 40%; 2.5: 60%	1: 20%; 3: 80%	3: 40%; 4: 60%	2.6
DER008	8.47	3.64	1.78	23.67	16.19	2: 80%; 2.5: 20%	2: 20%; 3: 80%	2: 20%; 3: 60%; 4: 20%	1.2
DER013	8.25	3.38	0.75	22.29	15.63	2: 20%; 2.5: 60%; 3: 20%	3: 100%	2: 20%; 3: 40%; 4: 40%	1.4
DER014	7.57	3.33	1.25	21.60	13.63	2: 20%; 2.5: 60%; 3: 20%	2: 20%; 3: 80%	2: 60%; 3: 40%	1.8
DER017	7.27	2.71	1.23	20.52	10.68	3: 100%	2: 20%; 3: 40%; 4: 40%	2: 60%; 3: 40%	1.4
DER018	8.38	3.34	2.05	22.35	13.73	2: 20%; 2.5: 40%; 3: 40%	3: 100%	2: 60%; 3: 40%	1.2
DER021	9.01	3.76	0.76	25.30	18.42	2: 60%; 2.5: 20%; 3: 20%	2: 20%; 3: 80%	2: 20%; 3: 80%	2.2
DER023	9.68	3.54	2.33	26.76	16.80	2: 40%; 2.5: 20%;	3: 80%; 4: 20%	2: 40%; 3: 60%	2.0

						3: 40%			
DER031	10.15	3.62	2.95	27.46	16.94	2: 20%; 2.5: 80%	3: 100%	3: 100%	1.4
DER039	8.25	3.40	1.67	23.86	14.23	2: 80%; 3: 20%	3: 100%	2: 60%; 3: 40%	1.8
DER040	9.83	3.93	1.88	27.46	19.27	2: 40%; 2.5: 40%; 3: 20%	3: 100%	2: 40%; 3: 60%	2.2
DER041	8.89	3.14	1.52	24.14	13.21	2: 20%; 2.5: 80%	3: 60%; 4: 40%	2: 20%; 3: 80%	2.0
DER045	8.19	3.15	1.46	25.25	12.28	2: 20%; 2.5: 80%	3: 80%; 4: 20%	2: 40%; 3: 60%	1.8
DER051	9.06	3.36	2.15	24.76	15.16	2: 60%; 2.5: 40%	3: 60%; 4: 40%	3: 80%; 4: 20%	2.2
DER052	10.40	4.06	1.90	29.48	21.81	2: 40%; 2.5: 20%; 3: 40%	2: 40%; 3: 40%; 4: 20%	2: 60%; 3: 40%	2.2
DER055	8.61	3.43	1.73	23.19	15.17	2.5: 80%; 3: 20%	3: 100%	2: 20%; 3: 80%	1.4
DER056	8.10	3.14	0.98	21.26	13.99	1.5: 20%; 2: 40%; 2.5: 40%	3: 100%	3: 100%	1.6
DER057	8.97	3.77	2.15	27.51	16.19	2: 80%; 2.5: 20%	2: 20%; 3: 80%	2: 20%; 3: 80%	2.4
DER058	9.26	3.69	1.74	25.65	17.01	2: 40%; 2.5: 60%	2: 20%; 3: 80%	2: 80%; 3: 20%	1.8
DER060	8.16	3.34	1.62	24.88	13.64	2.5: 40%; 3: 60%	2: 20%; 3: 40%; 4: 40%	2: 20%; 3: 80%	2.4
DER061	8.58	3.13	1.99	22.43	13.18	2: 20%; 2.5: 80%	3: 100%	2: 40%; 3: 60%	0.6
DER062	10.38	4.37	1.30	31.19	24.17	2: 40%; 2.5: 60%	3: 100%	2: 40%; 3: 60%	2.4
DER064	10.27	3.46	2.29	28.27	16.65	2: 20%; 2.5: 80%	3: 60%; 4: 40%	2: 60%; 3: 40%	2.4
DER067	8.72	3.37	1.61	24.11	15.00	2: 40%; 3: 60%	3: 60%; 4: 40%	2: 40%; 3: 60%	2.4
DER068	8.92	3.39	1.74	24.50	15.52	2: 40%; 2.5: 60%	3: 60%; 4: 40%	3: 80%; 4: 20%	2.2
DER069	7.55	2.97	1.40	20.63	11.66	2: 40%; 2.5: 60%	3: 100%	2: 20%; 3: 80%	1.8
DER071	9.36	3.34	2.26	25.40	14.64	2.5: 80%; 3: 20%	3: 80%; 4: 20%	2: 40%; 3: 60%	1.2
DER073	9.43	3.33	1.63	26.76	14.97	2: 40%; 2.5: 40%; 3: 20%	2: 20%; 3: 60%; 4: 20%	2: 40%; 3: 60%	2.2

DER075	10.03	3.97	2.02	27.00	20.62	2: 20%; 2.5: 80%	2: 20%; 3: 80%	3: 60%; 4: 40%	1.4
DER077	9.69	3.67	2.27	26.05	17.06	2: 20%; 2.5: 80%	3: 80%; 4: 20%	2: 20%; 3: 80%	1.6
DER080	10.23	3.73	2.24	27.89	18.80	2: 20%; 2.5: 80%	3: 80%; 4: 20%	2: 20%; 3: 80%	2.0
DER081	9.16	3.78	2.15	25.63	17.42	2: 20%; 2.5: 20%; 3: 60%	3: 80%; 4: 20%	3: 100%	1.8
DER082	10.40	4.24	2.33	32.02	20.95	2: 20%; 2.5: 80%	2: 20%; 3: 80%	2: 40%; 3: 60%	2.2
DER085	9.80	3.52	2.39	25.21	16.76	2: 40%; 2.5: 60%	3: 80%; 4: 20%	2: 40%; 3: 60%	1.2
DER087	9.25	3.71	2.00	26.35	17.60	2: 40%; 2.5: 40%; 3: 20%	3: 80%; 4: 20%	3: 100%	1.4
DER089	8.45	3.18	2.39	22.68	12.48	2.5: 80%; 3: 20%	1: 40%; 3: 60%	3: 80%; 4: 20%	0.4
DER090	10.48	4.07	2.10	31.56	19.97	2.5: 100%	2: 20%; 3: 60%; 4: 20%	2: 60%; 3: 40%	2.6
DER096	9.17	3.37	2.33	25.22	15.38	2: 40%; 2.5: 60%	3: 100%	3: 100%	1.8
DER101	10.40	3.71	2.66	30.07	18.88	2.5: 40%; 3: 60%	2: 40%; 3: 40%; 4: 20%	2: 40%; 3: 60%	2.0
DER102	8.48	3.66	1.77	24.70	15.90	2: 60%; 2.5: 40%	1: 20%; 3: 80%	2: 20%; 3: 40%; 4: 40%	1.4
DER103	7.67	3.54	1.49	23.72	13.07	2: 40%; 2.5: 20%; 3: 40%	2: 40%; 3: 60%	2: 20%; 3: 40%; 4: 40%	2.4

[a]For details refer to Table 1.

Table S2. Mean values and coefficients of variation (CV, %) for the content in ascorbic acid (mg $100g^{-1}$ FW), total phenolics (mg CAE $100g^{-1}$ FW) and nitrates (mg $100g^{-1}$ FW).

	AA		TP		Nitrates	
Population	**Mean**	**CV (%)**	**Mean**	**CV (%)**	**Mean**	**CV (%)**
BGUPM1235-67	30.97	22.0	112.49	7.6	768.67	5.7
BGUPM1549-68	52.51	9.1	107.50	3.7	1200.93	5.7
DER001	70.51	4.0	130.01	5.5	754.22	3.7
DER003	42.70	8.0	107.62	6.1	747.33	5.8
DER006	39.66	21.8	109.05	4.0	1094.33	3.8
DER008	55.72	22.8	111.49	2.0	895.18	3.2
DER013	56.20	10.9	108.87	7.4	963.58	6.1
DER014	53.75	21.9	110.78	1.9	861.59	8.1
DER017	48.92	23.9	118.20	3.4	818.67	6.9
DER018	59.33	21.4	110.58	1.8	769.04	7.8
DER021	58.73	23.5	110.96	2.8	1016.93	4.9
DER023	51.07	17.4	107.96	4.3	1012.86	6.2
DER031	36.78	32.0	108.57	2.9	1037.90	7.0
DER039	52.83	17.5	119.20	8.4	863.85	4.7
DER040	36.36	21.7	114.18	0.2	1064.75	7.7
DER041	44.51	12.3	108.54	7.3	1050.46	3.0
DER045	55.33	19.9	130.44	1.1	886.06	9.4
DER051	60.71	6.8	104.76	4.7	818.92	5.0
DER052	56.42	20.1	119.83	3.9	850.30	10.2
DER055	68.51	21.8	127.63	0.6	873.01	8.6
DER056	47.96	22.2	124.22	4.7	925.32	7.0
DER057	60.66	13.6	123.83	5.5	749.45	2.6
DER058	59.52	6.9	124.86	8.4	869.78	2.0
DER060	49.95	14.8	108.30	5.0	941.22	4.1
DER061	69.07	1.9	117.88	5.6	770.59	3.1
DER062	62.48	9.1	119.80	5.2	913.50	2.2
DER064	86.72	1.9	116.83	4.8	856.90	7.6
DER067	51.21	8.7	122.27	4.0	706.75	10.0
DER068	44.97	7.9	108.40	4.8	921.16	4.3
DER069	39.17	15.4	117.16	2.8	668.65	3.1
DER071	58.46	13.0	109.35	0.8	878.79	6.0

DER073	36.09	14.1	114.52	3.2	929.51	5.4
DER075	59.82	3.8	109.56	3.0	1034.23	6.0
DER077	52.55	20.5	116.58	6.9	943.29	6.3
DER080	49.78	6.9	113.49	4.1	1006.96	6.5
DER081	47.88	6.3	118.23	3.5	720.86	2.8
DER082	53.73	17.9	117.91	2.8	953.97	6.5
DER085	66.04	2.6	124.10	2.5	728.92	2.4
DER087	50.83	15.5	119.91	4.1	939.14	2.8
DER089	53.99	20.2	127.67	6.0	794.27	2.8
DER090	44.76	15.9	114.78	4.9	972.38	5.2
DER096	53.16	7.0	123.04	1.6	882.26	6.8
DER101	69.08	12.4	118.90	3.5	980.05	1.4
DER102	40.74	3.9	122.35	1.4	868.49	7.7
DER103	57.36	12.9	121.51	1.8	755.29	7.8
Total	*53.28*	*23.5*	116.31	6.9	892.43	*13.8*
P-value	<0.001		<0.001		<0.001	

2.2. Main glucosinolates and volatile compounds determined in wall rocket (*Diplotaxis erucoides* (L.) DC.)

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Keywords: Allyl isothiocyanate; glucosinolates; sinigrin; volatile organic compounds; wall rocket

Abstract

Wall rocket (*Diplotaxis erucoides* (L.) DC.) is a wild vegetable rich in glucosinolates and sulphur volatiles. Information of this vegetable is scarce, especially regarding the volatile profile. This work was aimed at analysing the content in sinigrin and describing the main volatile compounds (VOCs) identified in wall rocket. Twenty-four populations were evaluated. Sinigrin was analysed by high performance liquid chromatography, and volatiles were extracted by the head space-solid phase microextraction technique, and analysed by gas chromatography. Sinigrin was the main glucosinolate identified, while its degradation product allyl isothiocyanate was the main VOC (73.6% of total identified GC-area). This result suggests that the hydrolysis of sinigrin in wall rocket mainly derives in allyl isothiocyanate. Other isothiocyanates were also determined, suggesting the presence of other glucosinolates apart from sinigrin. Esters were also of relevance in the volatile profile, presumably responsible of the green notes in the aroma. Esters were positive correlated with each other, and c*is*-3-hexenyl valerate/isovalerate highlighted in particular for the negative correlation with isothiocyanates, mainly allyl isothiocyanate. The present work describes the volatile profile of wall rocket for the first time, thus increasing the knowledge in the aroma and taste of this species. Moreover, it provides a basis for future breeding programmes; in particular, the correlations established which could be exploited in the development of differential mild to hard-pungent varieties.

Introduction

Wall rocket (*Diplotaxis erucoides* (L.) DC. subsp. *erucoides*) is a wild edible plant widespread along the Mediterranean region (Martínez-Laborde, 1997). It is taxonomically related to rocket crops, salad rocket (*Eruca sativa* Mill.) and wild rocket (*D. tenuifolia* (L.) DC.). Wall rocket is appreciated for the tender leaves that can be eaten in salads or as condiment for other dishes (e.g., pasta, soups), and has potential as crop. As part of the family *Brassicaceae*, wall rocket is rich in glucosinolates as secondary metabolites, in particular sinigrin (Di Gioia *et al.*, 2018). Glucosinolates are known to

provide the characteristic aroma and taste in crops of this family. This is consequence of the enzymatic hydrolysis into other volatile compounds (VOCs) after tissue damage (Bell *et al.*, 2018). However, there is a lack of information regarding the volatile profile of wall rocket. Information in this respect can be useful in breeding programmes aimed at enhancing the organoleptic quality of wall rocket as new crop. In this context, the present work studied the content in sinigrin and the main volatile constituents of wall rocket. Comparison of the isothiocyanates fraction with literature for rocket crops was used as well in an attempt to highlight the similarities and differences among the wild vegetable and the related crops.

Materials and methods

Twenty-four populations of wall rocket were evaluated for the content in sinigrin and the profile in volatiles constituents. Populations were grown under greenhouse during the late spring season, and harvested before reaching the flowering stage. Three replicates per population were evaluated. Sinigrin was extracted from freeze-dried material and analysed by high performance liquid chromatography (HPLC) as described in Grosser and van Dam (2017). Results were expressed as mg of sinigrin in 100 g of fresh weight. Volatile organic compounds (VOCs) were extracted from fresh leaves by the head space-solid phase microextraction (HS–SPME) technique, and analysed by gas chromatography–mass spectrometry (GC–MS). Details of the analysis are provided in Guijarro-Real *et al.* (2019). Compounds were tentatively identified and semi-quantified based on the integration of peak areas.

Results and discussion

Sinigrin was the main glucosinolate determined in the current chromatograms. The average content was 7.76 ± 0.57 mg 100 g^{-1} FW, with values ranging between 1.35 ± 0.06 and 18.18 ± 0.47 mg SIN 100 g^{-1} FW (Table 1). In agreement with our results, sinigrin has been previously described as the main glucosinolate in wall rocket (D'Antuono, Elementi, & Neri, 2008; Di Gioia *et al.*, 2018). This aliphatic compound is also of great relevance in other *Brassicaceae* crops such as kale, horseradish or mustard (Agneta *et al.*, 2014; Hwang, Park, Dang, Kim, & Seo, 2019). When it is

enzymatic hydrolysed by myrosinase, sinigrin can release different volatile compounds including allyl isothiocyanate (Bell *et al.*, 2018).

Samples of wall rocket analysed in the current study displayed lower values compared to the results of Di Gioia *et al.* (2018). Apart from genotype, the accumulation of glucosinolates can be influenced by environmental conditions. Several authors have highlighted the effect of the growing environment (e.g., cultivation system, fertilization, or even growing period) in the content of glucosinolates determined in different organs (Ferioli *et al.*, 2013; Kovacic *et al.*, 2015; Bonasia *et al.*, 2017). Moreover, the content can be also influenced by the phenological stage and harvest maturity (Agneta *et al.*, 2014; Bell and Wagstaff, 2017). These factors may explain the differences between our results and the work of Di Gioia *et al.* (2018).

Regarding the volatile profile, the main VOCs identified corresponded to the groups of isothiocyanates and ester compounds (Table 1). Seven different isothiocyanates were identified in the former group. Allyl isothiocyanate was the main representative VOC. Its GC-peak area represented an average relative abundance of 96.7% against the total isothiocyanates, and 73.6% of the total VOCs identified. There was a 6-fold difference between the lowest and the greatest absolute values (GC-peak area). These results are in concordance with the analysis of glucosinolates, and suggest that the enzymatic hydrolysis of sinigrin in fresh tissue of wall rocket derives mainly in allyl isothiocyanate. In contraposition, Hanschen and Schreiner (2017) determined that the glucosinolates hydrolysis in *Brassica oleracea* varieties derived mainly in nitriles instead of isothiocyanates. Degradation into different groups of VOCs is determined by several factors including the pH, presence of determined ions (e.g., Fe^{2+}) or presence of specific proteins (Hanschen and Schreiner, 2017).

Allyl isothiocyanate provides a pungent, lachrymose and mustard-like flavour (Bell *et al.*, 2018), and its high abundance may be great responsible of the taste and aroma of this vegetable. In addition, this VOC has also potential anticarcinogenic properties (Sávio *et al.*, 2015); thus, its presence increases the potential of wall rocket as functional vegetable.

Table 1. Mean value and range (in parenthesis) of sinigrin and main VOCs tentatively identified in the materials of wall rocket (n = 24).

Glucosinolates (mg 100 g^{-1} FW)					
Sinigrin	7.76	(1.3-18.2)			
Volatile organic compounds (GC-peak area, x10^{6})					
Total VOCs	879.463	(405.6-1240.4)	Total isothiocyanates	703.7	(186.2-1102.5)
Total esters	116.3	(60.3-248.2)	Total others	59.5	(37.1-117.3)
Isothiocyanates					
allyl ITC	680.6	(182.8-1069.8)	hexyl ITC	0.5	(nd-0.8)
phenylethyl ITC	1.5	(nd-4.5)	phenylmethyl ITC	2.9	(nd. 8.2)
3-butenyl ITC	6.6	(1.5-11.7)	3-methylbutyl ITC	11.6	(1.8-20.8)
pentyl ITC	tr				
Esters					
cis-3-hexenyl propionate	10.3	(5.3-26.2)	cis-3-hexenyl sovalerate	51.4	(7.8-146.6)
cis-3-hexenyl butyrate	36.5	(0.8-10.2)	cis-3-hexenyl valerate	7.0	(1.9-16.2)
hexyl butyrate	0.9	(0.2-1.7)			
Others					
decanal	0.2	(0.2-0.4)	tetradecane	2.3	(0.26-6.3)
cis-3-hexen-1-ol	56.4	(30.4-112.6)	β-ionone	0.6	(0.0-2.3)

nd = not detected; tr = traces.

Six more isothiocyanates were found in the volatile profile. Due to the relationship of different isothiocyanates with bitter taste (Bell *et al.*, 2017), its presence would contribute as well to the pungent and bitter flavour of wall rocket. Moreover, since different isothiocyanates have different glucosinolate precursors (Bell *et al.*, 2018), our results suggest that other glucosinolates may be found in wall rocket tissues.

The isothiocyanates fraction of wall rocket differed from the profiles previously determined for wild rocket (Mastrandrea *et al.*, 2017) and salad rocket (Bell *et al.*, 2016) crops (Table 2). Wall rocket has been often considered as germplasm related to rocket crops in nutritional and even sensorial acceptance studies (Bennett *et al.*, 2006; D'Antuono *et al.*, 2008; D'Antuono *et al.*, 2009; Di Gioia *et al.*, 2018). However, no previous studies have described the volatile profile of this wild vegetable. Our results suggest that despite the taxonomical linkage among these species, it does not correspond to a similar isothiocyanates profile, and probably to the glucosinolates profile.

Table 2. Comparison of the isothiocyanates fraction determined for wall rocket (current study), wild rocket (Mastrandrea *et al.*, 2017) and salad rocket (Bell *et al.*, 2016).

	Wall rocket	Wild rocket	Salad rocket
Compound			
Allyl isothiocyanate	*		
Hexyl isothiocyanate	*		*
Phenylethyl isothiocyanate	*		
Phenylmethyl isothiocyanate	*		
Pentyl isothiocyanate	*	*	*
3-Butenyl isothiocyanate	*	*	*
3-Methylbutyl isothiocyanate	*		*
4-Methylpentyl isothiocyanate		*	*
4-Methylthiobutyl isothiocyanate		*	

The main isothiocyanate is marked in grey.

On the other hand, six esters were identified, accounting for a mean relative abundance (GC-peak area) of 16.9% from the total identified VOCs (Table 1). Two compounds, identified as c*is*-3-hexenyl butyrate and *cis*-3-hexenyl isovalerate, added a relative abundance above 80% for the esters fraction (40.1% and 44.2% on average, respectively). Esters are included in the group of ‘‘green leaf volatiles’’ (GLV) (López-Gresa *et al.*, 2017), and would be in part responsible of the green notes found in the leaves.

Finally, both phenotypic and environmental correlations were determined. Correlations were positive among esters, with high coefficients determined among different esters (Fig. 1). This correlation may be related to the biosynthesis pathway of these compounds (López-Gresa *et al.*, 2017). On the other hand, valerate and isovalerate esters highlighted for their negative correlations with isothiocyanates, especially with allyl-isothiocyanate (Fig. 1). Considering that esters are GLV, the balance between esters and isothiocyanates can determine the pungency of the material. Thus, the negative correlations among them could be exploited in order to develop varieties of high pungency or other ones with lower pungency and greater green notes.

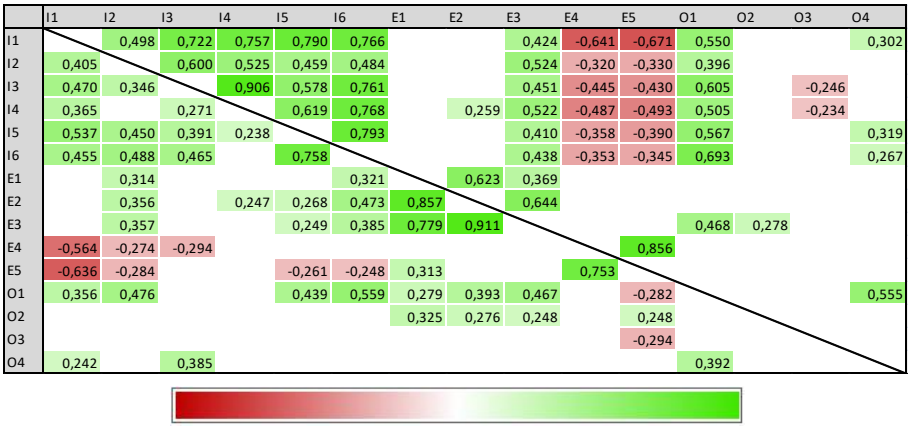

Fig. 1. Phenotypic (above the axis) and environmental (below the axis) correlations among the volatile organic compounds identified. Only the significant correlations ($P < 0.05$) are represented. I1: Allyl ITC; I2: hexyl ITC: I3: phenylmethyl ITC; I4: phenylethyl ITC; I5: 3-butenyl ITC; I6: 3-methylbutyl ITC; E1: *cis*-3-hexenyl propionate; E2: *cis*-3-hexenyl butyrate; E3: hexyl butyrate;

E4: *cis*-3-hexenyl isovalerate; E5: *cis*-3-hexenyl valerate; O1: *cis*-3-hexen-1-ol; O2: decanal; O3: tetradecane; O4: β-ionone. Colours determined according to the scale: -1 (left edge) to +1 (right edge).

Conclusion

The glucosinolate profile of wall rocket is rich in sinigrin. Its enzymatic hydrolysis under normal conditions mainly releases allyl isothiocyanate. This VOC is in fact the main volatile determined in the profile of wall rocket, great responsible of its pungent, characteristic flavour. Ohter isothiocyanates were determined as well, indicating the co-occurrence of different glucosinolates not previously described in sinigrin-rich materials. Esters were also an important fraction in the volatile profile, mainly *cis*-3-hexenyl butyrate and *cis*-3-hexenyl isovalerate. Different correlations were established among volatiles. This work increases the knowledge in the aroma and taste of wall rocket, and should be considered for the development of mild to hard-pungent varieties by exploitation of the correlations among VOCs.

Acknowledgements

C. Guijarro-Real is grateful to the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for the predoctoral FPU grant (FPU14-06798).

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2.3. Stability of sinigrin, carotenoids, chlorophylls and total reducing capacity of wall rocket after a simulated *in vitro* digestion

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Keywords: β-carotene, chlorophyll *b*, digestive stability, *Diplotaxis erucoides*, glucosinolates, micellarization efficiency, reducing capacity

Abstract

Wall rocket (*Diplotaxis erucoides*) is a wild vegetable traditionally consumed along the Mediterranean. Wall rocket is rich in sinigrin, chlorophylls and carotenoids, and has high reducing capacity. However, digestion can affect the bioaccessibility of those compounds. In this work, a three-step *in vitro* digestion was performed to evaluate the effect on these traits. Sinigrin was completely degraded during the digestion, probably associated to an accumulation of isothiocyanates and other hydrolysis compounds. Chlorophylls had also low stability during the digestion (0-17%), while carotenoids were highly stable (74-94%). However, the micellarization efficiencies were in any case below 7%. These low efficiencies may correspond to the high liposolubility of these compounds. Finally, the reducing capacity increased after digestion, probably as consequence of the acidic hydrolysis of phenolic compounds. In addition, the micellarization efficiency was almost 90%, suggesting that most of the molecules with reducing capacity remained in the aqueous phase. According to these results, the digestion of wall rocket may have a high, positive effect on the bioaccessibility of molecules with great reducing capacity, and presumably on the bioaccessibility of isothiocyanates by degradation of sinigrin. This study increases the knowledge regarding the effect of digestion on these bioactive compounds present in wall rocket.

Introduction

Wall rocket (*Diplotaxis erucoides* (L.) DC. subsp. *erucoides*) is a wild, annual plant widespread along the Mediterranean region and traditionally eaten in different countries (Guarrera and Savo, 2016). Its aroma and taste has certain resemblance to products as mustard and wasabi. As other *Brassicaceae*, the species is rich in glucosinolates, mainly sinigrin (Di Gioia *et al*., 2018). The hydrolysis-products of glucosinolates, especially the isothiocyanates, have been studied for their antimicrobial and anticarcinogenic activities (Girgin and El, 2015; Sávio *et al.*, 2015). As other leafy vegetables, wall rocket is also rich in chlorophyll and carotenoids

(Salvatore *et al.*, 2005), photosynthetic compounds with bioactive properties. In addition, wall rocket has great reducing capacity (Disciglio *et al*., 2017).

Several works have studied the bioactive properties of these compounds using *in vitro* (e.g., tumorous cells) and *in vivo* (e.g., mammals) models (Sávio *et al*., 2015; Ramos-Bueno *et al.*, 2016; Zhou *et al.*, 2016). However, information on the digestion and bioaccessibility of bioactive compounds is often scarce, and no studies exist on this issue for wall rocket. In this respect, only the bioaccessible fraction, i.e., the fraction released in the digestive tract from the food matrix that is available for absorption (Fernández-León *et al.*, 2017), has the potential to exert the protective activity. As a consequence, complementary studies based on digestion and bioaccessibility of compounds should be considered in epidemiological studies in order to increase the understanding on their protective effects. In this work, we study the bioaccessibility of sinigrin, carotenoids and chlorophylls, and the effect of the digestion on the total reducing capacity in wall rocket. The information obtained may be relevant for determining the bioactive properties of wall rocket after the process of digestion.

Materials and Methods

Wall rocket plants were grown during the late spring season under greenhouse. Before reaching the flowering stage, plants were harvested and clean leaves were freeze-dried. Four biological replicates were processed. The *in vitro* digestion protocol was adapted from Girgin and El (2015), and an oral digestion step was added. Three extracts were analysed: raw material, digesta fraction (after intestinal digestion) and aqueous fraction (after centrifugation). Sinigrin was analysed according to Grosser and van Dam (2017). Carotenoids and chlorophylls in raw material were extracted with ethanol (Guzman *et al.*, 2012), or with petroleum ether: acetone (3:1) in the digesta and aqueous fractions, and analysed by HPLC (Ferruzzi *et al.*, 2001; Richins *et al.*, 2014). Total reducing capacity was determined spectrophotometrically by the Folin-Ciocalteu procedure (Plazas *et al*., 2014), after extraction with methanol: acidic water (4:1) for 1 h.

Digestive stability (DS) and micellarization efficiency (ME) were determined as: DS=100*(DG/RAW), ME=100*(AQ/DG), where RAW, DG

and AQ are the content of each compound or reducing capacity in the raw material, total digesta and aqueous fraction, respectively. Determinations were based on four independent replicates.

Results and discussion

The average concentration of sinigrin determined for the raw material was 9.8 mg 100 g^{-1} FW. After the *in vitro* digestion of these materials, the sinigrin was not detected either in the aqueous fraction or in the digesta (Fig. 1). The ingestion of *Brassicaceae* products with activated myrosinase (i.e., not cooked vegetables or exposed to other treatments for enzyme inactivation) has shown to increase the hydrolysis of GSLs (Girgin and El, 2015). Consequently, the content of intact GSLs after digestion can significantly decrease (Fernández-León *et al.*, 2017). Nevertheless, this enzymatic hydrolysis during the digestion may be beneficial, considering that the breakdown products, mainly isothiocyanates, possess great bioactive properties (Girgin and El, 2015). However, complementary studies would be needed in order to evaluate the transformation into isothiocyanates and absorption rates.

Wall rocket materials were rich in chlorophylls *a* and *b* (16.4 and 8.9 mg 100 g^{-1} FW, respectively), while the contents in carotenoids β-carotene and lutein were more than 10-fold lower (0.3 and 1.5 mg 100 g^{-1} FW, respectively) (Fig. 1, Table 1). Qualitative similar results have been determined in other green vegetables, where high amounts of chlorophylls are biosynthesised as photosynthetic pigments (Guzman *et al.*, 2012). However, the digestion process basically degraded the chlorophylls, especially chlorophyll *a*; chlorophyll *b* had a stability percentage of 16.7% (Table 1). On the contrary, lutein and β-carotene were more stable during the digestion process (93.8% and 74.1%, respectively), although the initial contents were more than 10-fold lower with respect to the chlorophylls (Fig. 1, Table 1). On the other hand, the micellarization efficiency determined for carotenoids and chlorophyll *b* was very low, between 1.0% (chlorophyll *b*) to 6.5% (β-carotene). Carotenoids and chlorophylls are lipid-soluble antioxidants (Guzman *et al.*, 2012), thus explaining the low micellarization efficiency or retention in the aqueous phase.

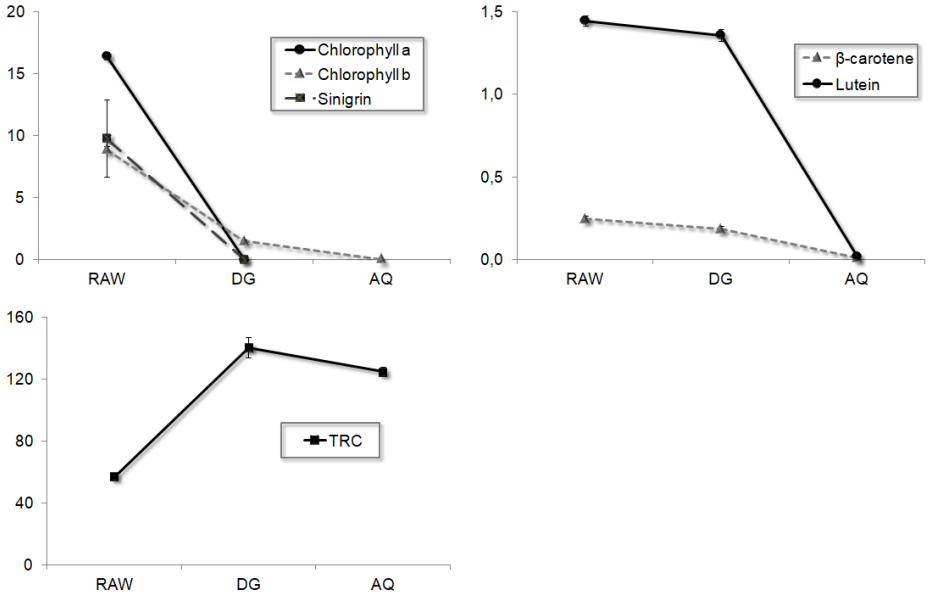

Fig. 1. Mean content in sinigrin, chlorophylls (chlorophyll *a*, chlorophyll *b*), carotenoids (β-carotene and lutein) and total reducing capacity (TRC) in the raw material (RAW), digesta (DG) and aqueous (AQ) fraction of wall rocket leaves. Results are expressed as mg 100 g^{-1} of fresh material –the TRC is expressed as equivalents of gallic acid, GAE–. Standard error bars are also represented.

Table 1. Mean content ± SE of the compounds evaluated, and percentage of digestive stability and micellarization efficiency in wall rocket leaves.

	Raw material (mg 100 g^{-1} FW)	**Digestive stability (%)**	**Micellarization efficiency (%)**
Sinigrin	9.77 ± 3.10	0	-
Chlorophyll a	16.42 ± 0.30	0	-
Chlorophyll b	8.88 ± 0.27	16.71	1.02
β-carotene	0.25 ± 0.02	74.14	6.47
Lutein	1.45 ± 0.03	93.81	1.52
TRC[a]	57.06 ± 2.53	245.85	88.84

[a]Expressed as mg of gallic acid equivalents

Finally, the total reducing capacity in the raw materials had an average value of 57.1 mg GAE 100 g^{-1} FW. This activity increased 145.8% with the digestion process (Fig. 1, Table 1). A similar situation was observed by Sengul *et al.* (2014) in pomegranate. The total reducing capacity measured with the Folin-Ciocalteu procedure is commonly associated to the content in total phenolic compounds (Stagnari *et al*., 2015). Phenolic compounds are commonly conjugated with other molecules such as sugars. Under acid conditions, as in the gastric phase, these compounds are hydrolysed and the phenolic fraction (aglycon) released, with a consequent increase in the reducing capacity (Cartea *et al.*, 2011). On the other hand, the total reducing capacity in the aqueous phase was slightly lower than the digesta, with a determined micellarization efficiency of 88.8% (Table 1). This result suggests that most of the molecules with reducing capacity that reach the digesta are bioaccessible for absorption. Nevertheless, studies analysing the degradation process of individual phenolics might complement the results obtained in this work regarding the reducing capacity measured.

Conclusion

All compounds evaluated in wall rocket experienced a decrease in their content as consequence of the digestion process. Sinigrin and chlorophyll *a* were the most affected compounds, with a complete degradation. However, increasing the content in the raw material may increase as well the digesta product (Fernández-León *et al*., 2017). Moreover, degradation of sinigrin may be positive for the formation of isothiocyanates. Carotenoids were more stable than chlorophyll *b*, although the micellarization efficiency was below 7% in all cases. These results may correspond to the lipophilic character of these molecules. Finally, the total reducing capacity increased with the digestion process, probably as consequence of the acid hydrolysis of phenolic compounds. These results increase the knowledge regarding the bioactive properties of wall rocket after digestion, and are a base for a better understanding on its consideration as functional food.

Acknowledgements

C. Guijarro-Real is grateful to the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for the predoctoral FPU grant (FPU14-06798).

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Capítulo 3. Adaptación de la rabaniza como nuevo cultivo

3.1. Development of a germination protocol for wall rocket and effects on baby-leaf quality traits

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Keywords: *Diplotaxis erucoides*; germination; morphology; nutritional quality; secondary dormancy

Submitted to Scientia Horticulturae

Abstract

Wall rocket (*Diplotaxis erucoides*) is a wild vegetable with potential as a crop. The seeds present secondary dormancy mechanisms that can become essential for the survival of wall rocket as a weed or in the wild. However, adaptation to crop conditions requires high and synchronised germination. The present work was aimed at studying whether different treatments improve the germination success of wall rocket seeds, and the effects on subsequent crop quality (morphology, yield, ascorbic acid and phenolics). By using a L_8 orthogonal array design, the main effects of soaking the seeds, scarification with sodium hypochlorite (NaClO), gibberellic acid (GA_3), potassium nitrate (KNO_3), cold, and heat treatments on germination traits of wall rocket were evaluated. NaClO scarification was the most efficient treatment and significantly increased the early and final germination, the germination rate and the vigour index. The best germination results were obtained when the NaClO scarification was followed by application of GA_3. Thus, a protocol consisting on scarification with 2.5% NaClO for 5 min followed by treatment with 150 ppm GA_3 for 24 h is proposed to improve wall rocket germination success. In addition, the germination treatments did not greatly affect the morphoagronomic characters of baby-leaf plants. Scarification with NaClO reduced the days to harvest but did not affect the yield, so its use could have commercial benefits. Moreover, the content in ascorbic acid increased in treatments using NaClO, which may increase the added value of the potential crop. Overall, this study contributes to the domestication of wall rocket by providing a simple germination method that in addition has potential beneficial effects for crop quality.

Introduction

The term wild edible plants (WEPs) refers to species that are directly gathered from the wild for its consumption (Shin *et al.*, 2018). These species may contribute to the diet with macro- and micronutrients like minerals and vitamins, and also represent an opportunity for adding new flavours and textures to the diet (e.g., Grivetti and Ogle, 2000; Molina *et al.*, 2014;

Morales *et al.*, 2014; Guijarro-Real *et al.*, 2019a, 2019b). For these reasons, the use, marketing and domestication of WEPs have been promoted during the last decades, as an alternative for improving human diet quality. This is the case, for example, of watercress (*Nasturtium officinale*) and wild and salad rocket (*Diplotaxis tenuifolia* and *Eruca sativa*) that were established as crops (Molina *et al.*, 2016) and have become usual ingredients on modern salads. However, there are still many other WEPs that can be considered as a source of new potential crops.

Wall rocket (*Diplotaxis erucoides* (L.) DC.) is an annual species belonging to the *Brassicaceae* family, traditionally gathered and consumed in different Mediterranean countries like Spain and Italy (Guarrera and Savo, 2016; Parada *et al.*, 2011). The plant is consumed by the leaves and tender shoots, appreciated by its characteristic, mild pungent flavour resembling the aroma and taste of other *Brassicaceae* crops like mustard. The culinary use of wall rocket is mainly fresh as a complement for salads, or cooked in preparations like pasta, soups or mixtures of cooked vegetables (Guarrera and Savo, 2016). In addition, the small, white flowers can be also considered as a decorative component in high cuisine (Guijarro-Real *et al.*, 2018). As food markets are increasing efforts in offering new and distinctive products, the particular taste and pungency of wall rocket make it a good candidate for its domestication and introduction into cultivation.

The introduction and commercial exploitation of WEPs possess, however, a series of critical points that must be evaluated, including germination traits and cultivation conditions (Ceccanti *et al.*, 2018). One of the main problems for the domestication of wall rocket is the presence of secondary dormancy in the seeds (Martínez-Laborde *et al.*, 2007) and the consequent discontinuous germination. Artificial selection in cultivated species has led to a reduction of seed dormancy, thus allowing a rapid and synchronised germination for adaptation to crop systems (Née *et al.*, 2017). By contrast, secondary dormancy and irregular germination can become essential for the survival of weeds (Darmency *et al.*, 2017). In the case of wall rocket, Martínez-Laborde *et al.* (2007) proposed that fresh seeds of wall rocket are presumably non dormant. However, mechanisms of secondary dormancy would be activated in those fresh seeds that did not germinate,

thus remaining in the soil as part of the soil seed bank. This irregular germination over time has been previously proposed as an adaptive strategy to control the demographic populations of wall rocket (Sans and Masalles, 1994), thus decreasing the competition for water and nutrients for increasing the population's survival.

The presence of secondary dormancy in the seeds hampers the breeding programmes and commercial cultivation of wall rocket, since a fast and uniform germination is required in both cases. The release of secondary dormancy is controlled by various regulators including phytohormones, mainly abscisic acid and gibberellins, and other specific proteins, and it is highly influenced by environmental factors such as temperature, light, water potential or content in nitrates in the soil (Finch-Savage and Footitt, 2017). Treatments changing or modifying those factors have demonstrated to be useful for breaking dormancy in several wild and cultivated species, and can be used routinely (Hellier, 2018).

In addition, this potential new crop is aimed at being consumed mainly as baby-leaf, before the appearance of the flower bud. Due to its short life cycle, this stage can be reached in one to two months, depending on the cultivation conditions, so the application of specific dormancy-breakdown treatments may affect the quality of the final product. This type of changes in the products has been reported elsewhere. For example, Handa *et al.* (2017) optimized the soaking and germination conditions of horsegram (*Macrotyloma uniflorum*) seeds to decrease the antinutritional factors but maintaining the nutritional properties. In the same way, Tavares *et al.* (2014) evaluated the treatment of rice seeds with salicylic acid, and the effect on produced seed quality and yield.

Therefore, the main objective of this work was to obtain a highly efficient germination protocol for wall rocket without impairing the baby-leaf quality. For that reason, the main effects of six factors were evaluated. The experimental design consisted in an orthogonal array and was adapted from Ranil *et al.* (2015), whom used it for evaluating up to seven factors for developing a germination protocol in *Solanum torvum*. In addition, the effect of the different treatments in selected morphoagonomic and nutritional traits of the baby-leaves was evaluated. The results of this work will be useful for

ensuring a quick, synchronised germination in breeding programmes, thus facilitating the domestication of this WEP. Finally, studying the effect on different morphoagronomic and nutritional traits can be relevant for selecting the most adequate germination protocol for ensuring the quality of wall rocket as a commercial baby-leaf vegetable.

Materials and methods

Plant material

Mature seeds from a wild population of wall rocket were collected in the spring of 2015 in Teulada, Alicante, Spain (coordinates 38º 43' 15" N; 0º 05' 06" E). Once in the laboratory, the collected seeds were manually cleaned from the siliques and other vegetable organs, and dehydrated for two weeks at room temperature. The dehydrated, mature seeds were then placed in a plastic bag and stored at 4 ºC in a hermetic jar until use, using silica gel for control of humidity. The germination assay was performed during the next spring.

Germination assay

The germination assay was performed in Petri dishes (9.0 × 2.5 cm; Phoenix Biomedical, Mississauga, Ontario, Canada) filled with 1.5 cm of moistened commercial Neuhaus Humin-substrat N3 nursery growing substrate (Klasmann-Deilmann Gmbh, Geeste, Germany). In each Petri dish, twenty-five seeds, previously treated according to the specific treatment, were placed on top of the substrate. Seven replicates were used, with a total of 175 seeds evaluated in each treatment. Petri dishes were placed in a climatic chamber, organizing the experiment in order to ensure that the application of all treatments finished the same day, considered as day 0 or starting day for the germination evaluation (Table 1). The environmental conditions in the climatic chamber remained constant during the germination assay, with a photoperiod of 16 h light / 8 h dark at 25 ºC (Martínez-Laborde *et al.*, 2007), and maintaining the relative humidity at 50–60%. The substrate was watered as needed in order to keep adequate moisture.

Table 1. Orthogonal matrix L_8 (2^6) indicating the eight treatments applied for testing the germination of wall rocket seeds. The six factors evaluated (soaking, NaClO, GA_3, KNO_3, cold and heat application) were applied at two possible levels (-, no application; +, application) in each of the eight treatments.

Treatment	Starting day[a]	Factors					
		Soaking	NaClO	GA_3	KNO_3	Cold	Heat
Control	0	-	-	-	-	-	-
T1	-8	-	-	-	+	+	+
T2	-2	-	+	+	-	-	+
T3	-8	-	+	+	+	+	-
T4	-9	+	-	+	-	+	-
T5	-3	+	-	+	+	-	+
T6	-9	+	+	-	-	+	+
T7	-1	+	+	-	+	-	-

[a]The starting day of application of treatment was adjusted in order to synchronize the day 0 (starting day of the germination evaluation) for the eight treatments.

The germination assay was designed according to the work of Ranil *et al.* (2015), with slight modifications. The effect of six factors on seed germination was evaluated: soaking, sodium hypochlorite (NaClO), gibberellic acid (GA_3), potassium nitrate (KNO_3), cold and heat. The presence/absence of light was not evaluated as a factor, and seeds were placed on the top of the substrate in all treatments.

The effect of each factor was evaluated at two levels: a) level -, if the factor was not applied; or b) level +, if the factor was applied. Details of each factor were:

- Soaking: immersion of seeds in distilled water for 24 h, prior to sown.

- NaClO: immersion of seeds in 2.5% commercial NaClO for 5 min, followed by three rinses with distilled water, 10 min each. The scarification was performed prior to sown.

- GA_3: treatment of seeds with 150 ppm GA_3 (Duchefa Biochemie, Haarlem, The Netherlands) by immersion for 24 h, with a final rinse with distilled water. Treatment with GA_3 was performed prior to sown.

- KNO_3: application of 1000 ppm KNO_3 (Panreac, Montcada i Reixac, Spain) in the plate for moistening the peat.

- Cold: stratification of seeds for seven days at 4 ºC, after being sown.

- Heat: incubation of seeds for 24 h at 37 ºC, after being sown.

In order to analyse the effect of these factors, a L_8 orthogonal array matrix design (2^6) was followed. Eight different treatments were tested, using specific combinations of factors in order to ensure that all of them were applied in four of the treatments (Ranil *et al.*, 2015). The resulting treatments are summarized in Table 1, in which factors were applied observing the following order: soaking, NaClO, GA_3, KNO_3, cold and heat application.

Evaluation of traits in the germination assay

At day 0 all treatments and replicates were placed in a climatic chamber in order to evaluate the germination. Evaluation started at day 3 and followed during seven consecutive days, with a final evaluation at day 11. Seeds were considered as germinated when the radicle emerged. However, sprouts with erratic germination, it is, with failures in the subsequent radicle development, were not considered as viable and removed from the count.

The germination traits evaluated were: a) early germination, considered as percentage of seeds germinated at day 3; b) final germination, considered as percentage of germinated seed at day 11; c) germination rate, in percentage (%), calculated as $(S_1 \cdot t_1 + S_2 \cdot t_2 + ... + S_n \cdot t_n)/(t_1 + t_2 + ... + t_n)$, where S_n is the cumulative percentage of germinated seeds at day n and t_n is the number of days from day 0 at which the count was performed; and d) vigour index, calculated as $(S_1/t_1)+(S_2/t_2)+\ldots+(S_n/t_n)$ (Ranil *et al.*, 2015). Germination rate determines the potential for a high final germination combined with a rapid germination, and vigour index determines the

potential for a rapid germination. In addition, the hypocotyl length (cm) in the sprouts was measured. Measurement was performed when the first true leaf reached a size of one third the size of cotyledons.

Growing conditions and evaluation of baby-leaf plants

Germinated sprouts were individually transplanted into 7x7x8 cm plastic pots filled with the same commercial substrate used in the germination assay. Thirteen to thirty-six plants of each treatment, depending on germination success, were transplanted. Transplanted plants were adapted for one day to room temperature conditions and then moved to a glasshouse equipped with a cooler system. Plants were placed following a completely randomized design and grown until the appearance of the first flower bud, before stem elongation. During the growing period, water was supplied regularly to maintain the substrate moistened, with no addition of fertilizers.

Once the baby-leaf plants reached the defined developmental stage, the aerial part was harvested, transported to the laboratory in sealed bags for avoiding excessive loss of moisture and stored at 4 ºC until analysis. The time elapsed between harvesting plants and placing under cooling conditions was less than one hour.

Morphoagronomic characterization

Characterization was performed within the next 24 h. Seven morphoagronomic traits were evaluated: total height, in cm; stem length between the cotyledons and the first leaf, in mm; length of the first and second internodes, in mm; length of the largest leaf, in cm; total number of leaves per plant; and earliness, measured as the number of days needed for the appearance of the flower bud.

Determination of nutritional parameters

Weight of plants prior to the freeze-drying process was recorded as fresh weight (FW, g). Plants from each treatment were then used for analysing the content in ascorbic acid (AA) and total phenolics (TP). Three replicates were performed for each analysis.

Content in AA was determined as described in Cano and Bermejo (2011), with slight modifications. Briefly, 1.0 g of fresh leaf tissue was homogenized with 5 ml of 3.0% (w/v) cold *meta*-phosphoric acid (Sigma-Aldrich; Saint Louis, MO, USA) for 1 min in a mortar, filtered and centrifuged at 2,500 rpm for 10 min at 5 ºC. The supernatant was filtered through a 0.22 µm PVDF filter (Teknokroma, San Cugat del Vallès, Spain) and analysed by HPLC in a 1220 Infinity HPLC (Agilent Technologies; Santa Clara, CA, USA). A Brisa C_{18} column (150mm × 4.6 mm id, 3µm) (Teknokroma, San Cugat del Vallès, Spain) was used, with an isocratic phase of methanol: 1.0% acetic acid (5:95) for 15 min, an injection volume of 5 µL and a flow rate of 1 mL min^{-1}. Quantification was performed at 254 nm using an external standard calibration of ascorbic acid (Sigma-Aldrich), and results expressed as mg AA 100 g^{-1} FW.

Content in TP was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) as indicated in (Plazas *et al.*, 2014). Briefly, 0.125 g of freeze-dried, finely ground material was extracted with 70% acetone (v/v) containing 0.5% glacial acetic acid (v/v) solution. An aliquot of 65 µl was incubated with 500 µl of diluted Folin-Ciocalteu reagent (1:10; Scharlab SL, Sentmenat, Barcelona, Spain) for 5 min, plus 500 µl of sodium carbonate solution (60 g/L) for other 90 minutes. Absorbance was measured at 750 nm in an iMark™ Microplate Reader (Bio-Rad; Hercules, CA, USA). Chlorogenic acid (Sigma-Aldrich) was used as standard and results were expressed as mg of chlorogenic acid equivalents in each 100 g of freeze-dried material (mg CAE 100 g^{-1} DW).

Statistical analysis

Data from the germination, morphoagronomic and nutritional traits were submitted to a one-way analysis of variance (ANOVA). Data expressed as percentage (for early germination, final germination and germination rate) or as the proportion of the maximum possible value (for vigour index) were arcsine-transformed prior to the analysis (McDonald, 2014). Normality was tested as well for morphoagronomic and nutritional data, and log-transformed if needed. Differences between treatments were studied with the Duncan multiple range test at $P = 0.05$. Significance of the six factors effects was tested by partitioning the degrees of freedom and sums of squares of the

ANOVA, and the magnitude was measured as the difference between treatments in which the factor was applied (level +) and those ones in which it was not applied (level -) (Ranil *et al.*, 2015). Finally, Pearson pairwise comparisons were performed in order to evaluate the correlation between AA and TP.

Results

Germination assay

The treatment effect was highly significant for the four germination parameters evaluated (Table 2), with significant differences among means (Fig. 1). Early germination was high for treatments T2, T3, T6 and T7, with values ranging between 49.7% (T7) to 79.4% (T3). By contrast, control, T1, T4 and T5 displayed low germination percentages, ranging from 2.3% (T1) to 22.3% (T4). This large difference between treatments displaying high and low response was also obtained for final germination, germination rate, and vigour index (Fig. 1). Treatments T2 and T3 displayed the greatest percentage for final germination (80.0% and 91.4%, respectively), whereas the percentage of germinated seeds in control and low-response treatments was below 35%. In the same way, T2 and T3 displayed the highest germination rates (86.0% and 76.5%, respectively), while this rate was < 30.0% for low-response treatments with T1 giving the lowest value (4.8%). Finally, T2, T3 and T6 showed the best vigour index values, close to 200 (Fig. 1).

The effect of each factor was analysed as the difference between average values when the factor was applied (level +) or not (level -) (Table 2). Scarification with NaClO was the only factor having a highly significant effect on the four germination traits, with a positive effect when it was applied (Table 3). Moreover, this factor had the highest positive effect, and its application increased the early germination, final germination and germination rate values in more than 50.0%, and the vigour index in 147.6 units. In addition, the effect of treating with GA_3 was significant ($P < 0.05$) for final germination and highly significant ($P < 0.01$) for the other germination traits (Table 2). GA_3 application induced an increase between 7.5% (final germination) and 14.3% (early germination), and an increase of

39.0 units in the vigour index (Table 3). For the rest of factors evaluated, the effect was non-significant, or when significant, negative for the germination traits (Table 2, Table 3).

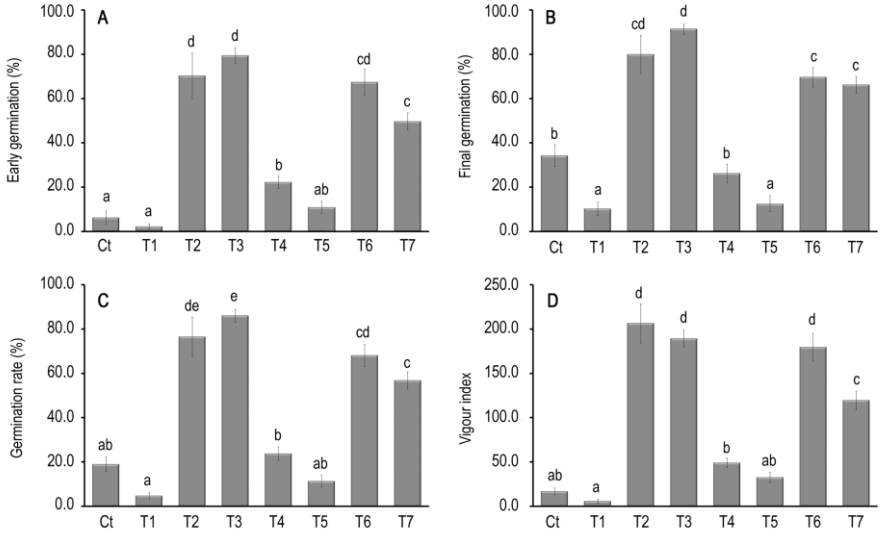

Fig. 1. Mean values ± SE in the eight treatments (control, Ct, or treatments T1 to T7) tested using a L_8 orthogonal array design, for the four germination traits evaluated in seeds of wall rocket. A) Percentage of early germination (at day 3 after sown). B) Percentage of final germination (at day 11 after sown). C) Percentage of the germination rate. D) Value of the vigour index. Means separated by different letters are significantly different according to the Duncan multiple range test (P = 0.05).

Table 2. Degrees of freedom (*Df*), F-ratio and *P*-value for the treatment effect, and for the orthogonal comparisons between the two levels tested for each factor in the germination test for wall rocket seeds. The germinations traits evaluated included early germination (at day 3), final germination (at day 11), germination rate and vigour index.

		Early germination		**Final germination**		**Germination rate**		**Vigour index**	
	Df	**F-ratio**	***P*-value**	**F-ratio**	***P*-value**	**F-ratio**	***P*-value**	**F-ratio**	***P*-value**
Treatment	7	32.9	<0.001	32.6	<0.001	44.3	<0.001	55.3	<0.001
Factor									
Soaking	1	0.2	0.6261	9.2	0.0040	3.0	0.0917	1.4	0.2406
NaClO	1	201.6	<0.001	192.7	<0.001	271.2	<0.001	344.7	<0.001
GA_3	1	20.3	<0.001	6.9	0.0115	17.3	<0.001	24.0	<0.001
KNO_3	1	3.8	0.0565	5.3	0.0255	8.2	0.0061	10.8	0.0019
Cold	1	3.6	0.0641	0.1	0.8206	0.8	0.3888	2.3	0.1353
Heat	1	0.5	0.4756	11.5	0.0014	6.4	0.0150	2.5	0.1227

Table 3. Average values for the germination traits analysed in wall rocket seeds, when each germination factor (soaking, NaClO, GA3, KNO3, cold and heat) was applied at each level (- no application; + application). Difference between the two levels is also indicated (Δ +/-).

	Early germination (day 3, %)			Final germination (day 11, %)			Germination rate (%)			Vigour index		
Factor	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**
Soaking	39.6	37.6	-2.0^{ns}	54.0	43.7	-10.3^{**}	46.6	40.0	-6.6^{ns}	104.6	95.2	-9.4^{ns}
NaClO	10.4	66.7	56.3^{***}	20.9	76.9	56.0^{***}	14.7	71.9	57.1^{***}	26.1	173.7	147.6^{***}
GA_3	31.4	45.7	14.3^{***}	45.1	52.6	7.5^{*}	37.2	49.4	12.3^{***}	80.5	119.4	39.0^{***}
KNO_3	41.6	35.6	-6.0^{ns}	52.6	45.1	-7.5^{*}	46.8	39.8	-7.1^{**}	113.0	86.9	-26.1^{**}
Cold	34.3	42.9	8.6^{ns}	48.3	49.4	1.1^{ns}	40.9	45.7	4.8^{ns}	93.9	106.0	12.1^{ns}
Heat	39.4	37.7	-1.7^{ns}	54.6	43.1	-11.4^{**}	46.4	40.2	-6.2^{*}	93.7	106.2	12.5^{ns}

ns, *, ** and *** indicate non significant, or significant at P = 0.05, 0.01 and 0.001, respectively.

Morphoagronomic characterization and nutritional value

For the morphoagronomic characterization, hypocotyl length of the sprouts, and earliness, total height, number of leaves per plant, stem length from cotyledons to 1st leaf, length of 1st and 2nd internodes and length of the largest leaf in the pre-flowering stage were measured. The hypocotyl length of sprouts had no differences among treatments, with an average value of 1.3 cm (Table S1). In the pre-flowering stage, no significant differences were determined among treatments for the number of leaves per plant, first internode and leaf length. The mean values for these traits were, respectively, 8.8 leaves, 3.7 mm and 8.7 cm (Table S1). On the other hand, differences were determined among treatments for total height, length from cotyledons to 1st leaf and 2nd internode (Fig. 2). Plant height was on average 10.8 cm. Treatments T4 and T7 developed plants between 1.0 and 2.0 cm higher than the control. For the cotyledon-to-1st leaf trait, the mean value was 2.0 mm. Plants from T1 displayed the lowest value (1.0 mm), similar to the control, while T2 developed plants with the greatest values (3.7 mm). On the contrary, plants from T2 developed the shortest 2nd internode (2.8 mm) while treatment T1 developed plants with the longest values (8.6 mm). Thus, addition of cotyledon-to-1st leaf, 1st internode and 2nd internode measurements resulted in a loss of significance among treatments ($P = 0.368$), so factor effects were not analysed. Finally, earliness was analysed (Fig. 2). The days needed to develop the flower bud was, on average, 21.4 days, with values ranging between 18.9 (T3) and 23.8 (T5) days. Treatments T2, T3, T6 and T7 gave the lowest values, with around 20 days needed for appearance of the flower bud. These treatments had in common the scarification of seeds with NaClO. By contrast, T1, T4 and T5 needed around three days more for reaching that developmental stage.

Effect of the individual factors used in the germination test were analysed for plant height and earliness (Table 4). The only germination factor with a significant effect on plant height was soaking the seeds prior to the sown. Treatments that included this factor developed plants on average 0.87 cm higher than those ones that did not include the soaking step (Table 5). For earliness, factors with significant effect included soaking the seeds, the treatment with NaClO and the application of heat (Table 4). While

soaking the seeds and applying heat resulted in an increase of 1.1 and 0.3 days, respectively, treatment with NaClO was the only factor that reduced the time needed for the appearance of the flower bud, in almost 3 days (Table 5).

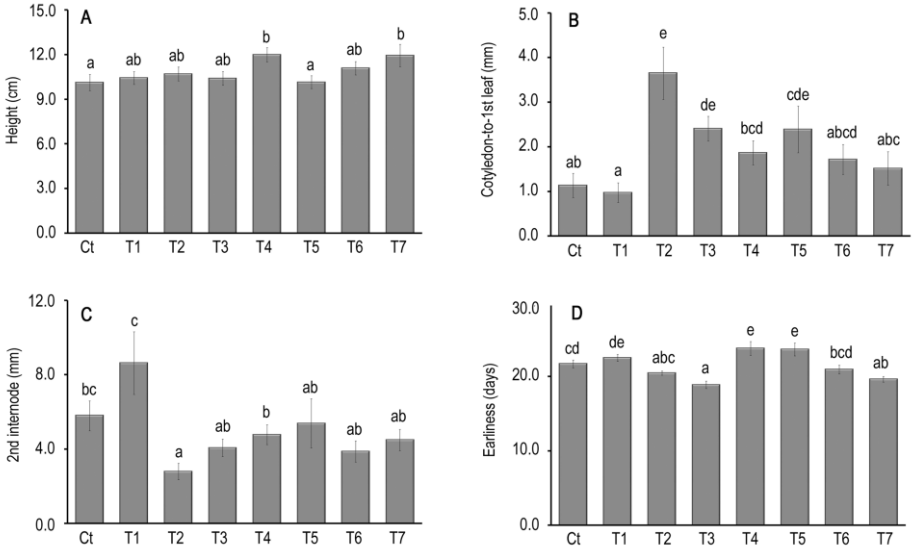

Fig. 2. Mean values ± SE of morphoagronomic traits for baby-leaf plants of wall rocket germinated with the different germination treatments (control, Ct, or treatments T1 to T7). Only the morphoagronomic traits displaying differences among treatments are included. A) Plant height (cm). B) Cotyledon-to-1st leaf length (mm). C) 2nd internode length (mm). D) Earliness or days needed for the appearance of the flower bud. Means separated by different letters are significantly different according to the Duncan test ($P = 0.05$).

Table 4. Degrees of freedom (*Df*), F-ratio and *P*-value for the effect of germination treatment on morphoagronomic and nutritional traits in baby-leaf plants of wall rocket, and for the orthogonal comparisons between the two levels tested for each factor in the germination test. Only traits with significant differences among germination treatments are considered: plant height, earliness, content in ascorbic acid (AA) and content in total phenolics (TP).

		Plant height		**Earliness**		**AA content**		**TP content**	
	Df	**F-ratio**	***P*-value**	**F-ratio**	***P*-value**	**F-ratio**	***P*-value**	**F-ratio**	***P*-value**
Treatment	7	2.1	0.0473	8.9	<0.001	3.7	0.0141	6.9	<0.001
Factor									
Soaking	1	5.8	0.0182	6.8	0.0100	0.0	0.8941	7.2	0.0175
NaClO	1	1.0	0.3232	47.5	<0.001	18.1	0.0006	11.2	0.0042
GA_3	1	0.1	0.8144	1.3	0.2556	0.1	0.7299	4.5	0.0450
KNO_3	1	0.5	0.4962	1.7	0.1997	2.6	0.1236	9.5	0.0082
Cold	1	0.5	0.4962	0.2	0.6860	1.3	0.2674	13.9	0.0023
Heat	1	2.1	0.1530	4.4	0.0378	3.8	0.0691	0.1	0.7860

Table 5. Average values for the morphoagronomic and nutritional traits in baby-leaf plants of wall rocket, when each germination factor (soaking, NaClO, GA3, KNO3, cold and heat) was applied at each level (- no application; + application). Difference between the two levels is also indicated (Δ +/-) during the germination test. Only traits with significant differences among germination treatments are considered: plant height, earliness, content in ascorbic acid (AA) and content in total phenolics (TP).

	Plant height (cm)			Earliness (days)			AA content (mg AA $100g^{-1}$ FW)			TP content (mg CAE $100g^{-1}$ DW)[a]		
Factor	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**
Soaking	10.4	11.3	0.9^{*}	20.9	22.0	1.1^{*}	79.0	79.7	0.7^{ns}	1291.1	1328.6	37.6^{*}
NaClO	10.7	11.0	0.4^{ns}	22.9	20.0	-2.9^{***}	68.8	89.8	21.0^{***}	1286.2	1333.5	47.4^{**}
GA_3	10.9	10.8	-0.1^{ns}	21.2	21.7	0.5^{ns}	78.5	80.2	1.7^{ns}	1325.3	1294.4	-30.9^{*}
KNO_3	11.0	10.7	-0.2^{ns}	21.7	21.2	-0.5^{ns}	83.4	75.3	-8.0^{ns}	1331.3	1288.4	-42.8^{**}
Cold	11.1	10.6	-0.5^{ns}	21.0	21.9	0.9^{ns}	74.5	84.2	9.6^{ns}	1311.8	1307.9	-3.9^{**}
Heat	10.4	11.3	0.8^{ns}	21.3	21.6	0.3^{*}	79.5	79.2	-0.3^{ns}	1319.4	1300.3	-19.1^{ns}

ns, *, ** and *** indicate non significant, or significant at $P = 0.05$, 0.01 and 0.001, respectively. [a]CAE: equivalents of chlorogenic acid.

On the other hand, the fresh weight, content in AA and content in TP were analysed. No significant differences were determined for the fresh weight, with an average value of 1.5 g (Table S1). By contrast, significant differences between germination treatments were detected for the content in AA and TP (Table 4). Application of the different germination treatments did not negatively affected the content in AA with respect to the control (Fig. 3). The greatest difference was detected for plants germinated with treatments T2 (96.2 mg AA $100g^{-1}$ FW) and T6 (101.1 mg AA $100g^{-1}$ FW) compared to the control (64.16 mg AA $100g^{-1}$ FW). The only germination factor with a significant effect in the AA content was scarification of the seeds with NaClO (Table 4). Thus, treatments including scarification with NaClO had on average an increase of 21.0 mg AA $100g^{-1}$ FW with respect to the no application (Table 5).

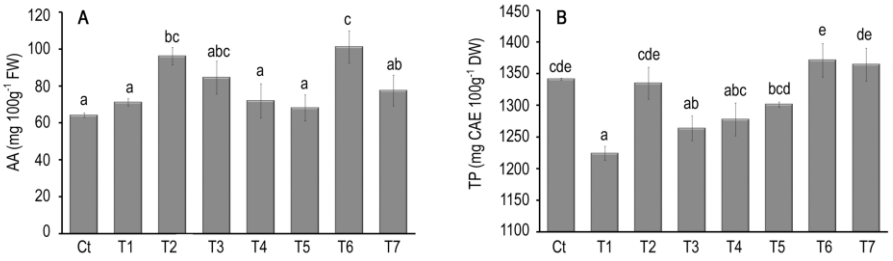

Fig. 3. Mean values ± SE of nutritional traits for baby-leaf plants of wall rocket germinated with the different germination treatments (control, Ct, or treatments T1 to T7). A). Content in ascorbic acid (AA, mg $100g^{-1}$ FW). B) Content in total phenolics (TP, mg of chlorogenic acid equivalents $100g^{-1}$ DW). Means separated by different letters are significantly different according to the Duncan test ($P = 0.05$).

In addition, plants germinated with different treatments also displayed significant differences for the content in TP (Fig. 3). The average content was 1,309.8 mg CAE $100g^{-1}$ DW, with values ranging between 1,224.2 (T1) and 1,371.1 (T6) mg CAE $100g^{-1}$ DW. Treatments T1 and T3 (1,224.2 and 1,263.7 mg CAE $100g^{-1}$ DW, respectively) displayed values significantly lower than the content determined for the control (1,341.4 mg CAE $100g^{-1}$ DW). All treatment factors, except for heat, had a significant effect on TP

content (Table 4). The application of GA_3, KNO_3 and cold had negative effects, decreasing TP content between 3.9 (cold) and 42.8 (KNO_3) mg CAE $100g^{-1}$ DW (Table 5). By contrast, scarification with NaClO resulted in the highest increase (47.4 mg CAE $100g^{-1}$ DW). Finally, the linear correlation between the content in AA and TP in each treatment was evaluated (Fig. S1), with non significant correlations determined among these nutritional traits ($P > 0.05$).

Discussion

Effect of individual factors on germination

Wall rocket has great potential to be marketed and introduced into the diet as a baby-leaf vegetable for salads. However, its domestication and exploitation as a crop and the necessary adaptation to an agricultural large scale production requires early, vigorous, high and synchronised germination (Née *et al.*, 2017). Thus, the present study analysed the response of a wild population of wall rocket to eight germination treatments, through the evaluation of four germination traits. In addition, the effect of treatments on baby-leaf plants was also evaluated.

The best treatments for increasing the germination parameters (T2, T3, T6 and T7) had in common the scarification of seeds with NaClO. These results differ considerably from the work of Ranil *et al.* (2015), whom found a negative effect of NaClO scarification on the germination of *Solanum torvum*. On the contrary, our results are consistent with previous works, in which treatment with bleach increased the germination rates of different species (e.g., Marty and Kettenring, 2017; Wagner and Oplinger, 2017; Jones *et al.*, 2016), although the exposure times were commonly greater. Moreover, the use of NaClO is a common treatment for the scarification in tomato and tomato wild relatives seeds (Gordillo *et al.*, 2008), and it is also used for disinfection of seeds in this crop (Figàs *et al.*, 2018a, 2018b; Mehalaine *et al.*, 2017). Our results suggest that the seed coat structure may be implied in the secondary dormancy of wall rocket. Thus, using a chemical scarification as the treatment with NaClO would help to break the barrier between embryo and environment (Wagner and Oplinger, 2017), presumably by weakening the seed coat tissues and/or increasing the permeability

(Katzman *et al.*, 2001). Moreover, these results suggest that scarification with NaClO might be also useful to break dormancy in other related species from the *Brassicaceae* family, although specific studies should be conducted in order to ensure the positive effect in other species.

On the other hand, the treatment with GA_3 had also a positive effect on the seed germination of wall rocket, in line with the results of Martínez-Laborde *et al.* (2007). This effect has been also observed in other species (Née *et al.*, 2017). Moreover, GA_3 has been suggested as a dormancy-breaking treatment to be applied in seeds of the *Brassicaceae* family by genebanks (González-Benito *et al.*, 2011). GA_3 is part of the gibberellins group (GA), a group of phytohormones known for its enhancing germination effects (Graeber *et al.*, 2012; Née *et al.*, 2017). In fact, the dormancy-germination mechanisms are regulated by the balance between GA_3 and abscisic acid (Finkelstein *et al.*, 2008). While abscisic acid would be required to maintain dormancy during fruit maturation, a positive balance for GA_3 would overcome dormancy and start germination.

However, significant differences on germination were found according to the factor applied before the treatment with GA_3. Thus, when the treatment with GA_3 was preceded by soaking the seeds for 24 h (T4 and T5), values of the germination parameters were very low and close to the control. A possible explanation could be that a long soaking treatment may cause a saturation of water inside the seeds, reducing the subsequent absorption of GA_3. On the contrary, preceding the treatment with GA_3 by a scarification with NaClO (T2 and T3) significantly increased the germination traits. This increase may correspond to the synergistic effect of both factors (Hsiao, 1979). Scarification with NaClO would affect the coat, facilitating the penetration of GA_3 and therefore increasing the dormancy-breaking effect of this hormone. However, it is also possible that NaClO may act against other germination inhibitors, what would explain the great germination rates also when it is applied with no GA_3 (T6 and T7). More physiological studies should be conducted in this sense to clarify the mechanisms activated in wall rocket dormancy, and how the NaClO scarification can break them.

The rest of the factors had no effect, or negatively affected the germination traits. According to these results, we suggest the use of a simplified protocol for the germination of wall rocket seeds, consisting of scarification of seeds with 2.5% NaClO for 5 min, then rinse the seeds in three changes of distilled water, 10 min each, remove the excess of water, and treat with 150 ppm GA_3 for 24 h. At this point, one short rinse with water prior to sowing would be appropriate in order to remove the excess of GA_3 from the seed surface (Small *et al.*, 2019). This protocol would ensure a proper germination of wall rocket even during the greatest dormancy period, considered after one year storage of seeds (Pérez-García *et al.*, 1995). The germination protocol described in this study has been used and validated in subsequent experiments developed by the group, providing germination rate success above 80% (unpublished results). Thus, it provides an efficient alternative that guarantees an effective, fast and uniform germination, with the need of only one day of pre-sown treatment.

Treatment effects on plant quality

Rocket crops are highly appreciated as baby-leaf products and marketed as whole leaves, for what the mechanical harvest is commonly used (Caruso *et al.*, 2018). Adaptation of wall rocket to a similar production system and marketing can promote its acceptance by consumers and also by producers. For that reason, producing plants short in height may be desirable for automatic harvesting, as the presence of long stems would require manual harvesting or subsequent manipulations for removing them. The application of GA_3 has shown to induce stem elongation in different species (e.g., Silk and Jones, 1975; Taylor and Cosgrove, 1989). In our case, no significant elongation of the hypocotyls was detected in the sprouts when seeds were treated with GA_3. In the case of the baby-leaf plant, only one of the treatments using GA_3 (T4) produced an increase in plant height with respect to the control. Thus, our results were ambiguous to declare that seed treatment with this hormone increases the plant height of wall rocket at the pre-flowering stage.

Another agronomic trait affected by the treatment was the earliness (i.e., the period needed to reach the marketing stage). Rocket species can reach the flowering stage in a short period, which varies depending on the

season, region and growing conditions (e.g., greenhouse instead of field), and plants should be commercially harvested prior to reach this stage (Bell *et al.*, 2015; Caruso *et al.*, 2018). In this respect, increasing the vegetative cycle would be of interest for producers if it derives in an increase of yield. Differences between treatments were probably consequence of a more effective and vigorous germination, which would generate more vigorous and faster growing plants. The lowest values for earliness were obtained for plants treated with NaClO. In fact, Chun *et al.* (1997) found that its application increased seedlings growth in rice. By contrast, no differences were determined for fresh weight. Consequently, our results suggest that using NaClO would not affect yield of wall rocket although the vegetative period was reduced. Moreover, this difference in days needed for crop development may have considerable impacts in reducing production costs.

Regarding the nutritional quality, the demand of healthy foods, and foods rich in bioactive compounds, have increased in the last decades as consequence of an increasing number of consumers are aware of the linkage between diet and health (Olayanju, 2018). Thus, studying the levels of bioactive molecules and the effect of germination treatments can be useful for future cultivation and marketing strategies. According to our results, applying different germination treatments did not affect the accumulation of AA in wall rocket leaves. Moreover, the use of adequate treatments may increase the content in AA, in particular if NaClO is used as scarification product. This result may be related as well with a more vigorous development of those plants. On the contrary, results were not clear for the content in TP. In fact, all factors except for heat had a significant effect on TP accumulation in wall rocket leaves. New studies focused on comparing the improved germination protocol suggested in this work, with control plants, may help to clarify whether using this protocol would increase or decrease the content in TP. Finally, a lack of correlation between AA and TP was determined. These results suggest that it would be difficult to improve both parameters by the application of germination treatments. In this sense, we suggest that future studies should especially focus on the content in AA, since this compound is of particular relevance in rocket crops (Cavaiuolo and Ferrante, 2014).

Conclusions

This study provides a germination protocol for wall rocket in order to break the secondary dormancy of this potential new vegetable crop. The proposed protocol for improving germination success consists combining the scarification of seeds using 2.5% NaClO for 5 min followed by a treatment with 150 ppm GA_3 for 24 h. This simple, short method has been validated in subsequent studies in our laboratory increasing the germination rate above 80% (unpublished results).

The germination treatments generally did not present significant differences for morphoagronomic characters at commercial level. Earliness increased with the application of NaClO, probably due to a greater vigour of plants germinated in these conditions. However, this shortage of growing period did not reduce the yield of wall rocket plants. In addition, results of AA and TP contents suggest that scarification with NaClO in wall rocket seeds could be used to increase the content in AA, increasing the marketing value of the plants. Overall, our results make an effective contribution of the domestication of wall rocket, by providing an efficient and simple method for seed germination. This method has, in addition, beneficial effects by increasing earliness and improving AA content.

Acknowledgments

C. Guijarro-Real thanks the Ministerio de Educación, Cultura y Deporte (MECD) of Spain for financial support by means of a predoctoral grant (FPU14-06798). The research did not receive any other specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Table S1. Mean values ± SE for selected morphoagronomic traits evaluated in the sprouts (n = 16) and baby-leaf plants (n = 12) of wall rocket, according to the germination treatment applied to the seeds. Only traits with no significant differences among germination treatments are included: hypocotyl length in sprouts, and number of leaves per plant, length of first internode, and length of the largest leaf in baby-leaf plants. The average ± SE for the fresh weight (FW) at the harvesting time is also included.

Treatment[a]	Hypocotyl length (cm)	Number of leaves	1st internode length (mm)	Leaf length (cm)	Fresh weight (g)
Control	1.5 ± 0.1	8.3 ± 0.5	3.9 ± 0.5	8.1 ± 0.3	1.4 ± 0.2
T1	1.1 ± 0.1	7.9 ± 0.3	4.4 ± 0.6	8.2 ± 0.3	1.4 ± 0.1
T2	1.4 ± 0.1	8.8 ± 0.4	3.5 ± 0.4	8.7 ± 0.2	1.5 ± 0.1
T3	1.4 ± 0.1	9.3 ± 0.5	3.4 ± 0.3	8.9 ± 0.3	1.6 ± 0.1
T4	1.2 ± 0.1	9.3 ± 0.4	3.7 ± 0.6	9.3 ± 0.3	1.8 ± 0.2
T5	1.1 ± 0.2	8.9 ± 0.4	3.1 ± 0.6	8.2 ± 0.3	1.3 ± 0.2
T6	1.3 ± 0.1	8.8 ± 0.3	3.5 ± 0.7	8.7 ± 0.3	1.5 ± 0.1
T7	1.3 ± 1.0	9.0 ± 0.3	3.8 ± 0.5	9.1 ± 0.4	1.6 ± 0.1
Mean	*1.3 ± 0.0*	*8.8 ± 0.1*	*3.7 ± 0.2*	*8.7 ± 0.1*	*1.5 ± 0.0*

[a]Details of treatments are described in Table 1.

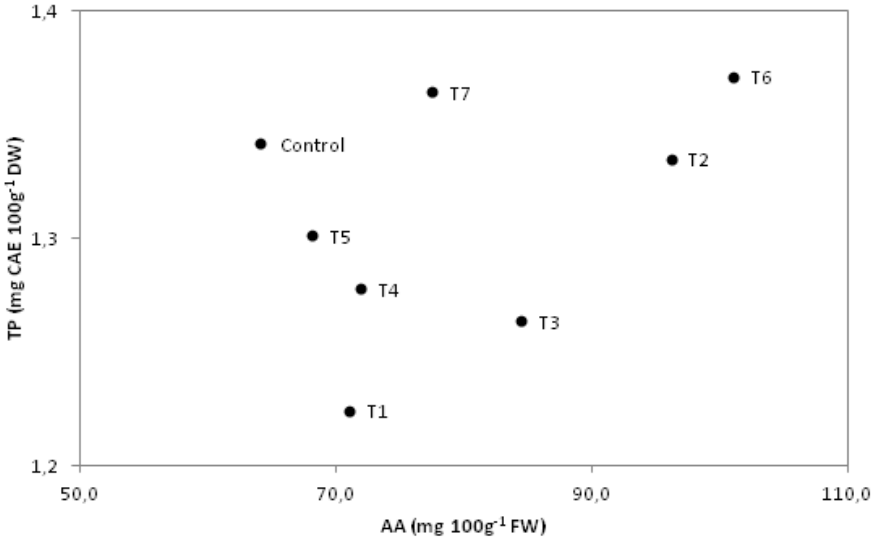

Fig. S1. Phenotypic correlation between the content in ascorbic acid (AA, mg 100g-1 FW) and total phenolics (TP, g CAE 100g-1 DW) for baby-leaf plants of wall rocket germinated with the different treatments[a] tested (control, T1 to T7). Details of treatments are described in Table 1.

3.2. Potential of wall rocket *(Diplotaxis erucoides*) as a new crop: Influence of the growing conditions on the visual quality of the final product

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Keywords: *Diplotaxis erucoides*; field cultivation; greenhouse cultivation; leaf colour; leaf morphology; new crops

Guijarro-Real, C., Prohens, J., Rodríguez-Burruezo, A., Fita, A. 2019. Potential of wall rocket (Diplotaxis erucoides) as a nre crop: Influence of the growing conditions on the visual quality of the final product. Scientia Horticulturae, 258, 108778

DOI: 10.1016/j.scienta.2019.108778

Abstract

Wild edible plants can be used for developing new crops and diversifying food markets. Wall rocket (*Diplotaxis erucoides*) is an annual weed with potential as a new crop. The present study aims at evaluating the effects of different growing conditions in the visual quality of this potential new crop. We evaluated eleven accessions of wall rocket, together with commercial rocket accessions (*Eruca sativa* and *D. tenuifolia*). Experiments were simultaneously conducted under field and greenhouse systems, and performed during two seasons. Fifteen descriptors related to leaf size, colour and shape were evaluated. Analysis of variance detected significant differences in size and shape among the three species studied, revealing the distinctiveness of wall rocket from the other rocket crops. This distinctiveness may enhance its establishment as a new crop. Comparison between the wall rocket accessions was also performed. There was relatively low morphological diversity among them. By contrast, the growing conditions had a high effect on the visual quality, especially for colour related traits and intensity of lobation, and also in the flowering time. As a consequence, the heritability estimates were low to moderate. The principal component analysis (PCA) clustered accessions according to the growing conditions, thus reinforcing the importance of environment in the morphology of wall rocket. The most promising quality of the leaves was obtained under field conditions, where the bright green colour and intensity of lobation were enhanced. In particular, accession DER006-1 was identified as a good candidate for developing a new cultivar. These results establish a basis for the management of wall rocket as a new crop. At the same time, results regarding the low diversity registered for morphology in the accessions evaluated have important implications for future breeding programmes of wall rocket.

Introduction

Rocket crops are minor vegetables from the family *Brassicaceae* characterized by the distinctive pungent taste and aroma of their leaves (Bell and Wagstaff, 2014). This common name includes different species, from which only two are economically important as crops: *Diplotaxis tenuifolia* (L.) DC. (wild rocket), and *Eruca vesicaria* (L.) Cav. subsp. *sativa* (Miller) Thell., also known as *E. sativa* Mill. (salad rocket) (Tripodi *et al.*, 2017). Although known since Antiquity, these two species are a perfect model of modern domestication for becoming cultivated crops (D'Antuono *et al.*, 2009; Molina *et al.*, 2016). Salad rocket is appreciated and widely cultivated in the Middle East and Southern Asia, while wild rocket has gained much popularity in European countries (Cavaiuolo and Ferrante, 2014). However, other related species from these genera are also edible and have the potential of becoming new crops, although nowadays they remain underutilized (D'Antuono *et al.*, 2009; Di Gioia *et al.*, 2018). Among them, wall rocket (*Diplotaxis erucoides* (L.) DC. subsp. *erucoides*) is an edible species of potential interest.

Wall rocket is an annual wild and weedy plant widespread around the Mediterranean regions of Europe and Africa, Central Europe and Western Asia, but also naturalized in America (Martínez-Laborde, 1990; Pignone and Martínez-Laborde, 2011). As a wild vegetable, wall rocket has been traditionally gathered in different countries such as Italy, Spain or France, for being consumed raw in salads, or added to other dishes like pasta, soups and omelettes (Couplan, 2015; D'Antuono *et al.*, 2009; Guarrera and Savo, 2016). The edible part of this species is represented by the tender leaves, which are mainly gathered during the vegetative stage of the plant. They are appreciated by the pungent, slightly bitter flavour which resembles the characteristic spicy, even burning flavour of some *Brassicaceae* crops such as mustard or wasabi. The flowers can be used as an edible, decorating component as well (Bianco *et al.*, 1998), and present the same characteristic flavour of leaves, but at lighter intensity. On the contrary, the flavour clearly differs from the common rocket crops (D'Antuono *et al.*, 2009). This distinctive character can be a key feature for promoting its exploitation as a new crop.Wall rocket can reach the flowering stage in a short period, which

varies depending on the season and region. The species should be harvested prior to the appearance of the floral bud, as it is common in rocket crops (Bell *et al.*, 2015; Caruso *et al.*, 2018; D'Antuono *et al.*, 2009). This condition, together with the staggered sowing commonly used in the management of rocket crops, allows the establishment of several commercial cycles during the year. This means that the crop would have to be grown in different seasons. However, there is a lack of information regarding the influence of season on the leaf morphological traits of wall rocket. Environmental conditions such as the quantity or quality of light received, together with the temperature ranges, determine the duration of the vegetative cycle and can also affect different morphological traits related to the quality of the final product (Hatfield and Prueger, 2015; Stagnari *et al.*, 2018). Moreover, although these conditions are dependent on the season, the use of protective systems such as greenhouses can modify them. For this reason, in the current study we have evaluated the field system and an alternative protected system under heated greenhouse. The aim of this study is to establish a base for the establishment of wall rocket as a new crop. Two independent experiments were carried out in two consecutive growing cycles in which environmental conditions differed, so that an indirect effect of the time of sowing could be also considered. We consider that a better understanding on the management of wall rocket as a crop may contribute to its enhancement. Other studies have analyzed the effect of cultivation practices (e.g., soilless cultivation) on other species with potential as crops (e.g., Egea-Gilabert *et al.*, 2013; Egea-Gilabert *et al.*, 2014); even for wall rocket, the effect of cultivation management on nutritional traits have been tested (Di Gioia *et al.*, 2018). However, we have not found works analyzing the effect on visual quality. On the other hand, the study was developed with pre-selected germplasm of wall rocket from the domestication programme that is being developed at the Universitat Politècnica de València (UPV, Valencia, Spain), in order to evaluate the effect of season and growing system in the visual quality of the crop. The use of local germplasm adapted to Mediterranean conditions may be more adequate for its future establishment as a new crop in countries from this region.

Material and methods

Plant material and cultivation

Ten pre-selected accessions of wall rocket, corresponding to the first generation seedlings of ten different wild populations collected in the Valencian Community (Spain), were used in the current study (Table S1). These local populations are conserved at the UPV. In addition, four commercial cultivars of rocket species from Shamrock Seed Co. (Salinas, CA, USA) were included: var. SSC2402, and var. Wild rocket, both belonging to *D. tenuifolia*; var. S. Rocket SSC2965 from *E. sativa*; and var. Wasabi corresponding to *D. erucoides*. The latter is, to our knowledge, the only commercial variety available of this species.

Plants were simultaneously grown in two different conditions at the UPV experimental stations: in a heated glass greenhouse with plants growing in trays (39º 29' 0" N, 0º 20' 26" W), and in field under anti-pest mesh (39º 28' 56" N, 0º 20' 11" W). Two independent experiments were carried out during two consecutive growing cycles, the late autumn-winter season (from now on, "winter season") and late winter-early spring season (from now on, "spring season"), with the same experimental design followed in the four conditions. Thus, for each condition the fourteen accessions (with independence of the species) were distributed according to a randomised block design, consisting of five blocks, one replicate per accession and block, and thirty plants per replicate (Fig. 1).

Seeds were treated with 2.5% sodium hypochlorite for 5 min followed by 100 ppm gibberellic acid solution for 24 h. The treatment was applied in order to break possible secondary dormancy and ensure a high, synchronised germination (Martínez-Laborde *et al.*, 2007). Treated seeds were sown in commercial Neuhaus Humin-substrat N3 substrate (Klasmann-Deilmann Gmbh, Geeste, Germany), placed for two days in a growing chamber with long day conditions (16/8 h, 25 ºC) to promote the fast germination of seeds, and then moved to a heated greenhouse. Thirty plants per replicate were used. Plants used for the greenhouse system were directly sown in 40 x 25 cm^2 trays and remained in the heated greenhouse during all the experiment. Plants for the field system were firstly sown in seedling trays with the

commercial substrate, placed into a greenhouse until the second true leaf appeared and then thirty plants per replicate were transplanted to the field, using the same plant density as in the greenhouse system.

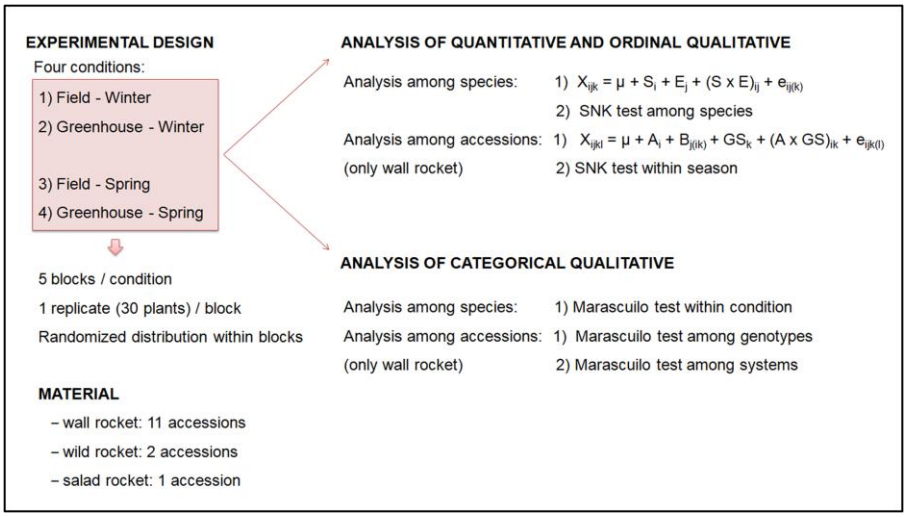

Fig. 1. Experimental layout and statistical treatment of data.

Morphological and agronomic traits

A total of eleven quantitative and four qualitative traits were evaluated. Many traits were adapted from the normalized descriptors for salad rocket (*Eruca spp.*) (IPGRI, 1999), considering the diversity among the three species. In addition, other traits that we considered of relevance were also included (Table 1). The fourth leaf of five plants per accession and replicate were analyzed when fully expanded and before the elongation of the floral stem. The relative chlorophyll content was measured with a chlorophyll meter (SPAD-502 Plus, Konica-Minolta, Tokio, Japan), and results were expressed as SPAD units. The rest of quantitative traits were measured using the Tomato Analyzer v3.0 software (Rodríguez *et al.*, 2010). Qualitative traits were measured using predetermined values (categorical) or scales (ordinal), as indicated in Table 1. Finally, the days to flowering were calculated as days after sowing needed to ensure that the floral bud was visible in at least five plants per accession and replicate, before the floral

stem elongation. This trait was only measured in the accessions of wall rocket.

Table 1. Descriptors used for the leaf characterization of the wall rocket, wild rocket and salad rocket accessions.

Descriptor	Code	Units/scale
Quantitative		
Days to flowering[a]	FLW-Time	days
Leaf length[b]	LL	cm
Leaf width	LW	cm
LL/LW ratio[b]	LL/LW	$cm \cdot cm^{-1}$
Leaf perimeter[b]	LP	cm
Leaf area[b]	LA	cm^2
Lamina colour lightness	L*	0 = black; 100 = white
Lamina colour hue angle	HUE	0º = red; 90º = yellow; 180º = green; 270º = blue
Lamina colour chroma	CHROMA	0 = completely unsaturated; 100 = fully saturated
Relative chlorophyll content	SPAD	SPAD units
Number of lobes	LOB-Num	-
Qualitative categorical		
Leaf blade shape	SHAPE	1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate
Terminal lobe shape	T-SHAPE	1 = lanceolate, wild rocket type; 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type
Margin shape	M-SHAPE	1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate
Qualitative ordinal		
Intensity of lobation	LOB-Int	0-5 (0 absent, 5 deep lobation)

[a]Trait measured for wall rocket accession, considered as days after sowing needed for developing a visible floral bud in at least five plants per accession, block and system. [b]Trait measured including petiole.

Data analysis

Statistical treatment of data was different depending on the nature of the traits (i.e., quantitative and ordinal qualitative traits, or categorical qualitative traits). Two analyses were performed in both cases: 1) for comparison among species; and 2) for comparison among accessions of wall rocket (Fig. 1).

For quantitative and ordinal qualitative data, data were subjected to fixed effects model analysis of variance (Gomez and Gomez, 1984). The analysis among species was performed using the average values for each accession across the five blocks as data. Average data were submitted to a multivariate analysis of variance (ANOVA) in order to test the effects of species (S, with three levels: wall rocket, wild rocket and salad rocket), environment (E, with four environments: field-winter, greenhouse-winter, field-spring, greenhouse-spring) and S x E interaction. The linear model used was: $X_{ijk} = \mu + S_i + E_j + (S \times E)_{ij} + e_{ij(k)}$, where X_{ijk} is the value for accession k of species i and environment j, μ is the general mean, S_i is the effect of the species i, E_j is the effect of the environment j, $(S \times E)_{ij}$ is the effect of the interaction between species i and environment j, and $e_{ij(k)}$ is the residual error of the accession k. Mean values of the three species were obtained and significant differences were analyzed using the Student-Newman-Keuls multiple range test ($P = 0.05$). The second analysis only included the accessions of wall rocket. The effects of accession (A, eleven accessions), growing system (GS, field or greenhouse) and A x GS interaction for each season were tested by means of a multivariate ANOVA, using the values of the five replicates (blocks) for each accession. The linear model adopted in this case was: $X_{ijkl} = \mu + A_i + B_{j(ik)} + GS_k + (A \times GS)_{ik} + e_{ijk(l)}$, where X_{ijkl} is the value for replicate l of accession i in block j and growing system k, μ is the general mean, A_i is the effect of the genotype i, $B_{j(ik)}$ is the effect of block j for accession i and system k, GS_j is the effect of the growing system j, $(A \times GS)_{ik}$ is the effect of the interaction between accession i and system k, and $e_{ijk(l)}$ is the residual error of the replicate l. Study of the differences was performed using a Student-Newman-Keuls test ($P = 0.05$). Broad-sense heritabilities (H^2) were calculated according to Wrickle and Weber (1986). H^2 for each specific condition was calculated by

the formula: $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2)$, and for each system was calculated by the formula: $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{GE}^2 + \sigma_E^2)$, where σ_G^2, σ_E^2 and σ_{GE}^2 are the estimates of genotype, environment, and genotype x environment variances, respectively.

Categorical qualitative data were expressed as percentage of each category against the total for each descriptor. Signification of differences were studied by means of the Marascuilo test ($P = 0.05$). A first analysis was performed among the three species for eahc specific environment. The second analysis compared traits among the eleven accessions of wall rocket and also among environments.

Finally, a Principal Component Analysis (PCA) was performed using the Clustvis tool (Metsalu and Vilo, 2015) for the accessions of wall rocket. Both quantitative and qualitative data were used in the PCA. Data were ln-transformed, centred and vector scaling was applied to rows prior to analysis. The category corresponding to "entire margin shape" was not included in the analysis since the category was only present in one accession and specific condition.

Results

Variation among the three species

The contribution of the species, environment and S x E interaction effects to the total sum of squares varied among traits. The species had great effect in most parameters related to size (up to 67.8%, for leaf area), and in the number of lobes and intensity of lobation (51.4% and 39.5%, respectively), while the effect of the environment was mainly no significant (Table S2). In addition, there was a significant effect of the S x E interaction for all traits except for the leaf length/width ratio; the contribution to the total sum of squares for those traits ranged between 8.1% (lightness) and 46.0% (perimeter).

Table 2 shows the mean values for the three species for the different traits. Compared to the other rocket crops, leaves of wall rocket were short in length and had a medium width value (Fig. 2); the leaf area was intermediate

between wild and salad rocket. In addition, this species developed an intermediate lobation considering both number of lobes and intensity (Table 2); wild rocket displayed the greatest lobation characters, while salad rocket developed leaves more entire.

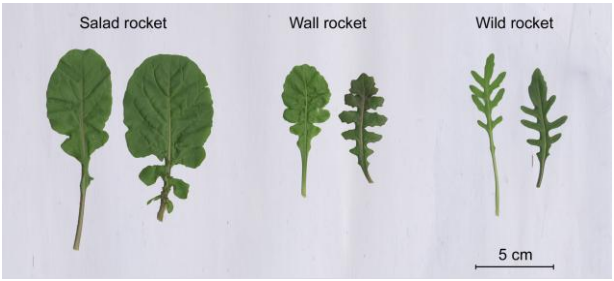

Fig. 2. Leaves of salad rocket, wall and wild rocket derived from plants cultivated under greenhouse (left) or field (right) systems.

Table 2. Mean values of wall rocket (WallR), wild rocket (WildR) and salad rocket (SaladR) for the quantitative and ordinal qualitative traits evaluated.

Descriptor[a]	WallR	WildR	SaladR
LL	8.24[a]	8.66[b]	10.74[c]
LW	3.01[b]	2.80[a]	3.87[c]
LL/LW	2.78[a]	3.14[b]	2.83[a]
LP	27.07[a]	29.41[ab]	30.44[b]
LA	11.86[b]	8.02[a]	21.40[c]
L*	44.13[b]	44.57[b]	40.96[a]
Hue	128.60[b]	127.74[a]	128.80[b]
Chroma	31.75[a]	31.46[a]	30.97[a]
SPAD	38.60[a]	39.85[a]	40.02[a]
LOB-Num	6.57[b]	8.29[c]	3.10[a]
LOB-Intens	2.92[b]	3.99[c]	1.91[a]

Means within traits with different letters indicate significant differences among species (P = 0.05) according to the Student-Newman-Keuls multiple range test. [a]LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

Wall rocket also presented differences for qualitative traits (Fig. 2). On average, 61% of leaves presented spatulated shape both in field and greenhouse, together with obovate in greenhouse (33.8%) or obovate and elliptic in field (21.0% and 17.3%, respectively) (Fig. 3, Table S3). By contrast, wild rocket developed leaves mainly lanceolated but also elliptic (60.1% and 35.4% on average, respectively), while leaves of salad rocket were mainly obovate (77.5% on average). According to the shape of the terminal lobe, both wall rocket and salad rocket displayed salad rocket type, mainly rounded, but also acute in the former and broadly rounded in the latter (Fig. 3, Table S3). Finally, the margin shape of salad rocket and wild rocket was mainly entire, while in wall rocket the main shape was crenate-dentate.

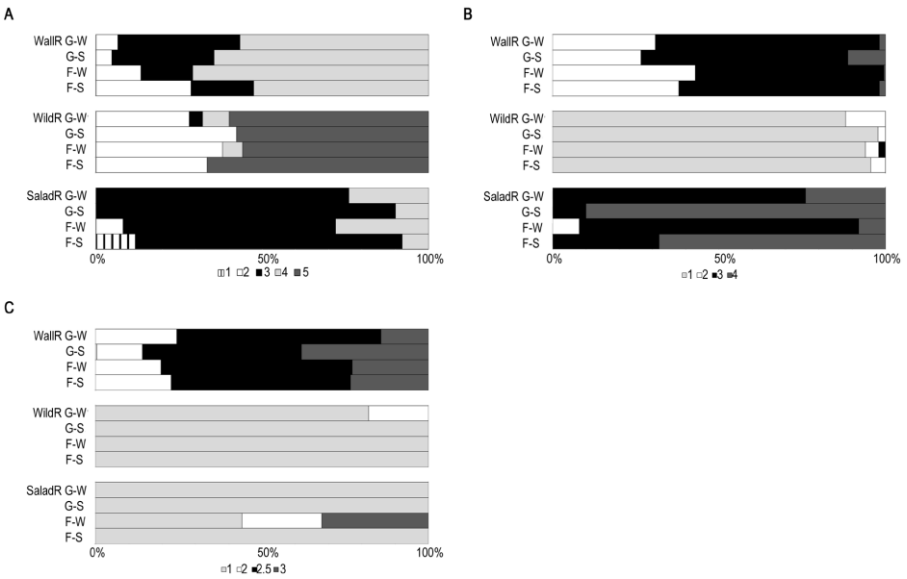

Fig. 3. Percentage of categorical descriptors analyzed in leaves of wall rocket (WallR), wild rocket (WildR) and salad rocket (SaladR), in the four environments described: greenhouse-winter season (G-W), greenhouse-spring season (G-S), field-winter season (F-W), field-spring season (F-S). A) Categories for the leaf shape: 1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate. B) Categories for the shape of terminal lobe: 1 = lanceolate to acute, wild rocket type; 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type. C) Categories for the shape of margin: 1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate.

Variation among wall rocket accessions

Effect of accession and environment in the quantitative traits

Significant differences among the eleven accessions were determined for most quantitative traits and intensity of lobation, in both seasons (Table 3). However, differences were no significant for leaf length and area, but also for leaf width and colour hue angle in winter, and relative chlorophyll content in spring. In any case, the contribution of the accession effect to the total sum of squares was low, ranging between 1.7% (flowering time) and 25.0% (leaf length/width ratio) in winter, and between 5.2% (flowering time) and 28.1% (lightness) in spring (Table 3).

The effect of the growing system was also highly significant for most traits, especially during the winter season (Table 3). The contribution to the total sum of squares ranged from 0.0% (leaf width) to 90.4% (flowering time) in winter. Eight of the twelve traits presented percentages > 35%. Specifically, the system was the greatest contributor for the flowering time, leaf colour lightness and chroma, relative chlorophyll content and intensity of lobation (Table 3). In spring, this contribution was commonly lower, and ranged from 0.2% (flowering time) to 58.6% (number of lobes). Only three traits presented values > 35%: colour hue angle, number of lobes and intensity of lobation, being the greatest contributor in the latter two. Surprisingly, effects on the flowering time, leaf colour lightness and chroma were no significant and accounted for < 7% (Table 3).

On the other hand, A x GS interaction was only significant for three traits in spring (flowering time, leaf length/width ratio and intensity of lobation), and five in winter (leaf length/width ratio, colour lightness and chroma, relative chlorophyll content and intensity of lobation) (Table 3). Nevertheless, the contributions to the total sum of squares were, in any case, < 17%.

Table 3. Sum of squares (in percentage, %) for effects of accession (A), growing system (GS), A x GS interaction, block (B), and residuals (R) for the quantitative and qualitative ordinal descriptors evaluated in the eleven accessions of wall rocket during the winter and spring seasons.

	Winter				
Descriptor[a]	**A**	**GS**	**A x G**	**B**	**R**
FLW-Time	1.7**	90.4***	0.4^{ns}	3.3	4.1
LL	6.0^{ns}	37.4***	3.5^{ns}	6.8	46.3
LW	15.1^{ns}	0.0^{ns}	8.5^{ns}	8.1	68.3
LL/LW	25.0***	43.4***	5.7*	3.6	22.2
LP	23.3**	0.8^{ns}	5.9^{ns}	9.0	60.9
LA	4.0^{ns}	11.5**	5.7^{ns}	7.1	71.7
L*	8.5***	70.3***	3.8**	6.5	10.9
HUE	10.0^{ns}	21.7**	14.5*	6.8	47.0
CHROMA	6.9***	64.2***	3.4^{ns}	9.9	15.5
SPAD	4.7**	73.0***	3.2*	5.9	13.1
LOB-Num	23.2***	43.9***	2.4^{ns}	7.6	23.0
LOB-Intens	16.8***	54.1***	4.3*	8.2	16.6
	Spring				
Descriptor[a]	**A**	**GS**	**A x G**	**B**	**R**
FLW-Time	5.2**	0.2^{ns}	4.2*	77.6	12.8
LL	8.3^{ns}	0.6^{ns}	12.6^{ns}	20.6	57.9
LW	11.9*	19.5**	7.3^{ns}	17.2	44.1
LL/LW	21.7***	34.4***	16.4***	3.3	24.2
LP	18.0***	31.0**	4.5^{ns}	17.8	28.8
LA	10.6^{ns}	10.0^{ns}	8.5^{ns}	18.7	52.2
L*	28.1***	6.5^{ns}	4.5^{ns}	22.7	38.1
HUE	6.3*	37.6*	2.5^{ns}	30.1	23.5
CHROMA	13.5**	5.3^{ns}	5.2^{ns}	38.1	37.9
SPAD	5.5^{ns}	27.9*	3.7^{ns}	36.0	26.9
LOB-Num	10.1***	58.6***	5.1^{ns}	4.2	22.0
LOB-Intens	14.8***	50.9***	7.2**	6.3	20.7

[a]LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio (cm·cm^{-1}), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

Table 4. Broad sense heritability (H^2) of the quantitative and ordinal qualitative descriptors evaluated in the eleven accessions of wall rocket under each growing condition, and global heritability in each system.

	Greenhouse			Field		
Descriptor[a]	**Winter**	**Spring**	**Total**	**Winter**	**Spring**	**Total**
FLW-Time	0.12	0.35	0.22	0.22	0.05	0.18
LL	0.00	0.05	0.06	0.20	0.11	0.05
LW	0.00	0.02	0.01	0.35	0.25	0.30
LL/LW	0.54	0.52	0.43	0.37	0.42	0.23
LP	0.04	0.16	0.17	0.41	0.39	0.36
LA	0.00	0.00	0.00	0.16	0.28	0.15
L*	0.31	0.40	0.36	0.59	0.16	0.39
HUE	0.06	0.20	0.17	0.31	0.00	0.13
CHROMA	0.15	0.22	0.31	0.42	0.00	0.19
SPAD	0.23	0.12	0.14	0.21	0.00	0.18
LOB-Num	0.44	0.27	0.36	0.33	0.13	0.18
LOB-Intens	0.45	0.43	0.43	0.45	0.03	0.24

[a]FLW-Time: flowering time (days), L: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

Heritability of quantitative traits

Heritability was low (< 30%) to moderate (< 70%) in all cases (Table 4). Moreover, heritability estimates of 0 were obtained for some traits under specific environments, like for some size related traits in greenhouse-winter condition, or colour related traits in field-spring condition.

In the greenhouse system, traits including leaf length/width ratio, colour lightness, number of lobes and intensity of lobation had moderate values in each specific season and in the system (Table 4). Moderate values were maintained under field conditions for leaf length/width ratio and colour lightness, but also for leaf width and area. Surprisingly, flowering time, colour related traits, chlorophyll content and lobation traits presented great

differences between seasons in the field (Table 4). Thus, values were moderate or almost moderate for winter (0.21-0.59), while they decrease to very low (< 0.16) or even 0.0 during spring.

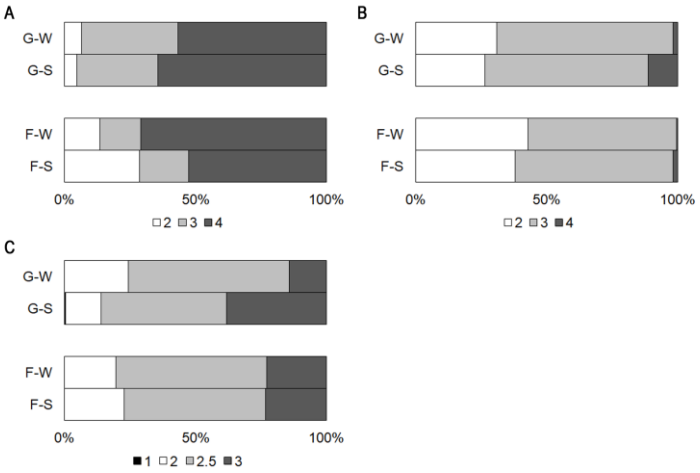

Fig. 4. Percentage of categorical descriptors analyzed in the eleven accessions of wall grown under the four environments described: greenhouse-winter season (G-W), greenhouse-spring season (G-S), field-winter season (F-W), field-spring season (F-S). A) Categories for the leaf shape: 2 = elliptic; 3 = obovate; 4 = spatulate. B) Categories for the shape of terminal lobe: 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type. C) Categories for the shape of margin: 1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate.

Variation registered for the qualitative traits

No great differences were determined among accessions for the qualitative traits (data not shown). Thus, all the accessions were analyzed together and only the effect of the environment was considered (Fig. 4, Table S4). The spatulate shape was predominant in both systems and seasons (> 52%), representing up to 70.9% of total under field-winter conditions. Obovate shape represented between 30.9% (spring) and 36.7% (winter) of leaves in the greenhouse system, while this percentage decreased to 17.3%

on average in the field. By contrast, percentage of elliptic leaves in the latter increased up to 28.5% (spring) (Fig. 4, Table S4).

The terminal lobe was mainly rounded, with values between 56.7% (field-winter) and 67.3% (greenhouse-winter). The second main category corresponded to the acute shape, especially in the field, representing up to 42.9% of total leaves in winter (Fig. 4, Table S4). Finally, the margin was mainly an intermediate crenate-dentate shape. Interestingly, greenhouse-spring conditions increased the percentage of dentate margins to 38.3%.

Principal Component Analysis

A PCA was performed using both quantitative and qualitative descriptors. The first and second component explained, respectively, 27.4% and 21.5% of the total variance (Fig. 5), which increased to 61.8% when the third component was considered. The first component had strong, positive correlation with colour related traits, leaf length and length/width ratio, obovate shape of leaves and rounded terminal lobe (Fig. 5a). It was negatively correlated with leaf perimeter, relative chlorophyll content, number of lobes and intensity of lobation, elliptic shape of leaves and acute terminal lobe, and flowering time. The second component had positive correlations with flowering time, leaf length/width ratio, colour lightness and chroma, and crenate-dentate margin shape (Fig. 5a). It was negative correlated with leaf width, perimeter and area, colour hue angle and broadly rounded terminal lobe.

The projection of accessions in the PCA score plot confirmed that samples were mainly separated according to the specific conditions in which they were grown (Fig. 5b). The first component separated between samples from the greenhouse system, with positive values, and the field system, which had negative values. The second component separated samples between seasons (Fig. 5b). Samples grown in the winter season had positive values in this axis, while material from the spring season generally presented negative values. This distribution corresponded to differences in the mean values among conditions (Fig. 4, Table 5). For instance, plants in spring needed on average 34 days to reach the flowering stage. However, this time increased from 16 to 33 days in winter (greenhouse and field, respectively).

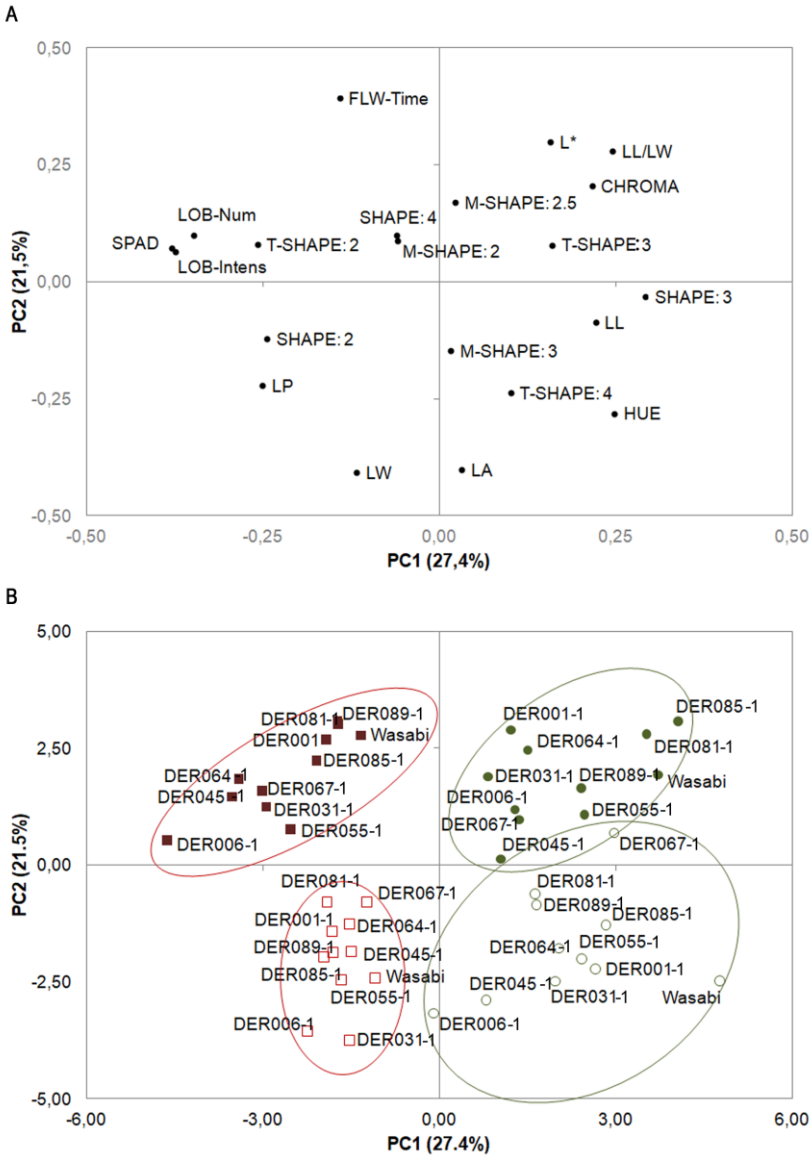

Fig. 5. Principal Component Analysis of the eleven accessions of wall rocket evaluated under the four growing conditions. A) PCA loading plot for the first (PC1) and second (PC2) component analysis. Descriptors correspond to: LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation, SHAPE: leaf blade shape (2 = elliptic; 3 = obovate; 4 = spatulate; 5 =

lanceolate), T-SHAPE: terminal lobe shape (2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type), M-SHAPE: margin shape (2 = crenate; 2.5 = crenate-dentate; 3 = dentate). B) PCA score plot for the first (PC1) and second (PC2) components, with identification of the specific environmental conditions in which plants were grown: greenhouse-winter season (*coloured circle*), greenhouse-spring season (*open circle*), field-winter season (*coloured square*), and field-spring season (*open square*).

Plants growing in the field increased the relative content in chlorophyll, especially in winter, as well as the number of lobes and intensity of lobation (Table 5). This system also increased the percentage of elliptic leaves (Fig. 4). By contrast, the greatest values of leaf colour lightness and chroma were found in plants grown in the greenhouse, winter condition (Table 5).

Finally, some accessions plotted in similar positions within each PCA graph (Fig. 5b). Thus, DER006-1 was mainly placed in the lower left extreme of the plot, opposite to the commercial var. Wasabi. By contrast, accessions such as DER001-1, DER081-1, DER085-1 or DER089-1 mainly plotted close between them, but also close the commercial variety. Accessions from the greenhouse-spring season, however, formed a more compact plot that affected comparisons.

Table 5. Mean values and range of the quantitative and ordinal qualitative descriptors evaluated in the eleven accessions of wall rocket in the greenhouse and field systems, during the winter and spring season.

	Winter			
	Greenhouse		**Field**	
Descriptor[a]	**Mean**	***Range***	**Mean**	***Range***
FLW-Time	50.47[a]	(48.60; 52.60)	66.98[b]	(65.40; 70.00)
LL	8.87[b]	(7.92; 9.25)	7.45[a]	(6.90; 8.03)
LW	2.82[a]	(2.54; 3.13)	2.83[a]	(2.56; 3.12)
LL/LW	3.20[b]	(2.75; 3.70)	2.68[a]	(2.50; 3.01)
LP	25.83[a]	(21.82; 29.77)	26.50[a]	(22.73; 30.71)
LA	11.23[b]	(10.04; 12.45)	9.76[a]	(8.58; 10.82)
L*	48.24[b]	(46.90; 50.46)	43.19[a]	(40.88; 44.20)
HUE	128.52[b]	(127.54; 129.13)	127.49[a]	(126.27; 128.77)
CHROMA	37.02[b]	(34.79; 40.19)	29.41[a]	(26.31; 31.72)
SPAD	34.43[a]	(31.14; 37.24)	45.04[b]	(42.58; 48.13)
LOB-Num	6.00[a]	(4.44; 7.52)	8.28[b]	(7.40; 9.20)
LOB-Intens	2.43[a]	(1.68 - 3.16	3.83[b]	(2.96 - 4.40
	Spring			
	Greenhouse		**Field**	
Descriptor[a]	**Mean**	***Range***	**Mean**	***Range***
FLW-Time	34.18[a]	(32.40; 36.60)	34.45[a]	(33.80; 35.75)
LL	8.41[a]	(7.57; 9.13)	8.25[a]	(7.55; 8.93)
LW	3.04[a]	(2.78; 3.26)	3.37[b]	(3.17; 3.70)
LL/LW	2.78[b]	(2.39; 3.19)	2.46[a]	(2.20; 2.65)
LP	25.13[a]	(21.85; 27.91)	30.84[b]	(26.79; 37.40)
LA	12.39[a]	(10.76; 14.01)	14.06[a]	(12.56; 16.51)
L*	42.10[a]	(40.35; 45.05)	43.00[a]	(41.76; 44.52)
HUE	129.89[b]	(129.15; 130.57)	128.50[a]	(128.06; 128.99)
CHROMA	29.61[a]	(26.32; 32.32)	30.94[a]	(29.71; 31.98)
SPAD	34.82[a]	(32.00; 37.76)	40.09[b]	(37.87; 42.74)
LOB-Num	4.21[a]	(1.60; 6.48)	7.80[b]	(7.08; 8.52)
LOB-Intens	1.97[a]	(0.67 - 3.28	3.45[b]	(3.05 - 3.72)

Means within rows for each cycle with different letters are significantly different at $P = 0.05$ according to the Student-Newman-Keuls multiple range test. [a]FLW-Time: flowering time (days), L: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf

length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

Discussion

Wall rocket is broadly considered as a weed (e.g., Araj and Wratten, 2015; Martínez-Laborde *et al.*, 2007; Pignone and Martínez-Laborde, 2011). However, our research is pioneering on the study of this species as a crop, with the aim of developing new commercial cultivars. The development of materials adapted to cultivated conditions and with distinctive traits increases the chances of the establishment of wall rocket as a new crop, by encouraging the acceptance of producers and consumers.

In particular, the present work was focused on analyzing the effect that greenhouse and field cultivations have on morphological traits of interest in the final product. These two systems present great differences in terms of temperature, light intensity, wind, or air humidity, among others, factors that can affect growth and development of plants (Figàs *et al.*, 2018b). Moreover, for vegetable crops with short cycle, differences in the month of sowing determine the environmental conditions during the growth period, and this seasonal climate variability can affect visual quality as well (Bonasia *et al.*, 2017).

Commercial materials of salad and wild rocket were included in the analyses in order to compare with our materials. The three species have been previously compared in terms of nutritional characteristics (e.g., D'Antuono *et al.*, 2008; Di Gioia *et al.*, 2018). However, there is little information regarding parameters of visual quality in these commercial crops (e.g., Bonasia *et al.*, 2017; Egea-Gilabert *et al.*, 2009; Taranto *et al.*, 2016). Our results showed that the three species were clearly differentiated in leaf size and shape. This indicates that the leaf traits chosen give a good result for comparing and describing the three species. Thus, they can be used as a basis for the future development of wild and wall rocket descriptors, since current normalized descriptors are specifically developed for *Eruca* spp (IPGRI,

1999). Furthermore, our results indicate that, even when different species can be considered together as rocket crops, they are distinct enough to be presented as different commercial products. In the particular case of wall rocket, this distinctiveness can play a key role in the commercial success of the new crop. In fact, food markets are continually looking for new products for diversification, and in some cases the opportunity derives from the domestication of wild edible plants (Egea-Gilabert *et al.*, 2013).

When the eleven accessions of wall rocket were compared, results indicated the presence of genotypic differences. However, this variation was not of big magnitude, and its effect was low compared to the effect of the growing system. The lack of wide variation has been previously reported in other species from the same genus, in particular for wild rocket, in contrast to the greater variation registered for the salad rocket (Taranto *et al.*, 2016). This relatively low diversity must be considered in breeding programmes, since breeders exploit genetic variability for developing materials of interest (Voss-Fels and Snowdon, 2016). Thus, our results suggest that a limit number of morphological different varieties may be developed with our materials. Nevertheless, according to the wide distribution described for the species (Pignone and Martínez-Laborde, 2011), we do not discard that materials from other regions may increase the morphological diversity.

By contrast, we found a great influence of the environmental conditions in most morphological traits. Modification of morphology by plants is a common adaptation to environmental conditions (Stagnari *et al.*, 2018). Thus, our results support the idea that the evaluation of genotypes across different environments should be a critical determinant when new crop cultivars are being developed (Stommel *et al.*, 2015). In addition, when the crop management to be implemented is unclear, as it happens with new crops, the evaluation of materials among different environments may help to identify the best environmental conditions in order to obtain a desirable quality.

The combined inter-population low genetic diversity and high influence of the environmental conditions, together with the intra-population diversity registered, affected heritability estimates. Unlike our results, in an ideal situation, heritability of morphological traits should be high (Figàs *et*

al., 2018a), thus increasing the success of the breeding selection. In addition, estimates varied among growing conditions for many traits, which is described as a general behaviour for broad-sense heritability (Hoffmann and Merilä, 1999). Thus, estimates should be performed as independent for different conditions, and materials selected for those specific conditions (Rodríguez-Burruezo *et al.*, 2002).

The PCA did not cluster materials by genotype or origin. Figàs *et al.* (2018b) found similar results when clustering materials of tomato corresponding to the same varietal type that are genotypically close among them. On another study, Egea-Gilabert *et al.* (2009) found that, even for accessions of *Eruca* sp. genotypically different, leaf morphological traits were not strong enough to group materials by origin. Thus, our clustering results reinforce the hypothesis that the materials had relative poor morphological variation among them. On the contrary, leaf morphology was strongly conditioned, not only by the growing environment but also the season. In this way, field conditions increased the number and intensity of lobation. Bell *et al.* (2017) found that marked leaf lobation in salad rocket increased acceptance in European consumers as it is the expected shape of rocket crops. In a similar way, growing wall rocket under conditions that increase the lobation may promote the acceptance of the crop. Colour of leafy vegetables plays also a decisive role in the acceptance by consumers (Colonna *et al.*, 2016). In this respect, plants growing under field increased the relative chlorophyll content, main determinant of the colour in green vegetables (Roshanak *et al.*, 2016), while the greenhouse-winter combination increased the chroma and lightness. Since bright green colour of leaves is desirable for markets, our results indicate that field conditions will provide a final product with better colour-related traits. However, winter in the field promoted the accumulation of anthocyanins, visible as purple spots along the surface (data not shown). This accumulation, induced by low temperatures and related to stress tolerance (D'Amelia *et al.*, 2018), implied loss of quality in the final product, and may indicate that the harsh winter is not adequate for the crop. However, the use of climate crop cover nets may reduce this negative aspect and other related to the very cold conditions, but new experiments should be conducted for assessing its effect.

On the other hand, leaves were smaller in winter, especially under field conditions. Reducing size of leaves is common when plants grow under cold temperatures (Buitrago Acevedo *et al.*, 2017). During this season, plants also increased the vegetative stage period, especially under field conditions. This result matches expectations, since increasing the day length and temperature results in a higher development of rocket crops (Bonasia *et al.*, 2017).

Finally, despite the low effect of genotype, distribution of accessions within each graph may be an indicator of the presence of some global similarities among specific materials. As a promising result, the pre-selected accession DER006-1 seems a good candidate for the breeding programme, with the goal of obtaining a new, distinct variety from the existing commercial Wasabi.

Conclusions

This work is a basis for the determination of the proper conditions for growing wall rocket as a new crop. In agreement with our findings, field conditions would be an adequate option for its development. Under these conditions, plants developed leaves with a higher number of lobes and also a great intensity of lobation, which may increase its acceptance in markets due to similarity with wild rocket. However, wall rocket was distinct enough to be considered as a new vegetable. Field conditions also positively affected the colour quality of the product. Nevertheless, the presence of anthocyanins in the late autumn-winter season may damage this visual quality. Thus, the harsh winter conditions should be presumably avoided for growing this new crop in the field, unless protected.

On the other hand, results indicated the presence of low morphological variation among materials, and also low-moderate heritability of the traits evaluated. This lack of diversity must be considered for future breeding programmes. Nevertheless, multivariate principal component analysis was useful for defining the accession DER006-1 as a good candidate for the development of a new variety, distinct from the commercial var. Wasabi.

Acknowledgements

C. Guijarro-Real is grateful to the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for the predoctoral FPU grant (FPU14-06798). Authors also thank Dr. A. M. Adalid-Martínez, Ms. K. Aguirre, and Ms. S. Benická for helping in the field tasks.

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Table S1. Geographical location of the original ten wild populations of wall rocket from which the pre-selected accessions derive.

Accession	Location	Province	Coordinates	
			Latitude	Longitude
DER089-1	Cabanes	Castellón	40º 11' 06" N	0º 10' 17" E
DER085-1	Castellón de la Plana	Castellón	39º 59' 42" N	0º 03' 36" W
DER001-1	Alfara del Patriarca	Valencia	39º 32' 21" N	0º 23' 06" W
DER006-1	Oliva	Valencia	38º 54' 42" N	0º 06' 49" W
DER055-1	San Isidro de Benagéber	Valencia	39º 34' 03" N	0º 23' 49" W
DER064-1	Casinos	Valencia	39º 41' 49" N	0º 42' 49" W
DER067-1	Losa del Obispo	Valencia	39º 41' 48" N	0º 53' 18" W
DER081-1	Benavites	Valencia	39º 43' 49" N	0º 14' 25" W
DER031-1	Montroy	Alicante	39º 21' 02" N	0º 38' 05" W
DER045-1	Jijona	Alicante	38º 38' 30" N	0º 28' 37" W

Table S2. Sum of squares (in percentage, %) for the effects of species (S, wall rocket, wild rocket and salad rocket), environment (E), S x E interaction and residuals (R) for the quantitative and qualitative ordinal descriptors evaluated in the three species.

Descriptor[a]	S	E[b]	S x E interaction	R
LL	39.9^{***}	19.5^{ns}	20.6^{***}	19.9
LW	43.3^{***}	24.1^{ns}	12.9^{***}	19.7
LL/LW	15.9^{***}	41.2^{**}	8.2^{ns}	34.6
LP	9.9^{***}	8.4^{ns}	46.0^{***}	35.7
LA	67.8^{***}	15.4^{ns}	9.5^{***}	7.3
L*	17.5^{***}	52.5^{**}	8.1^{*}	21.9
HUE	18.3^{***}	10.7^{ns}	39.2^{***}	31.8
CHROMA	0.8^{ns}	45.4^{ns}	22.1^{***}	31.7
SPAD	1.8^{ns}	54.9^{ns}	28.7^{***}	14.5
LOB-Num	51.4^{***}	9.3^{ns}	12.6^{**}	26.8
LOB-Intens	39.5^{***}	9.8^{ns}	15.9^{***}	34.8

^{ns}, *, ** and *** mean no significant, or significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. [a]LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation. [b]Four environments were considered in the analysis, corresponding to: 1) greenhouse system in winter cycle; 2) field system in winter cycle; 3) greenhouse system in spring cycle; and 4) field system in spring cycle

Table S3. Percentage of categorical descriptors (%) analyzed in leaves of wall rocket (WallR), wild rocket (WildR) and salad rocket (SaladR), in the four environments described: greenhouse-winter season (G-W), greenhouse-spring season (G-S), field-winter season (F-W), field-spring season (F-S).

	G-W			G-S			F-W			F-S		
Descriptor[a]	**WallR**	**WildR**	**SaladR**	**WallR**	**WildR**	**SaladR**	**WallR**	**WildR**	**SaladR**	**WallR**	**WildR**	**SaladR**
SHAPE												
1	-	-	-	-	-	-	-	-	-	-	-	12.0[a]
2	6.5[a]	28.0[b]	-	4.7[a]	42.2[b]	-	13.5[a]	38.0[b]	8.0[a]	28.5[a]	33.3[a]	-
3	36.7[b]	4.0[a]	76.0[c]	30.9[a]	-	90.0[b]	15.6[a]	-	64.0[b]	18.9[a]	-	80.0[b]
4	56.7[b]	8.0[a]	24.0[a]	64.5[b]	-	10.0[a]	70.9[b]	6.0[a]	28.0[a]	52.6[b]	-	8.0[a]
5	-	60.0[a]	-	-	57.8[a]	-	-	56.0[a]	-	-	66.7[a]	-
T-SHAPE												
1	-	88.0[a]	-	-	97.8[a]	-	-	94.0[a]	-	-	95.6[a]	-
2	30.9[b]	12.0[a]	-	26.5[b]	2.2[a]	-	42.9[b]	4.0[a]	8.0[a]	37.9[b]	4.4[a]	-
3	67.3[a]	-	76.0[b]	62.3[b]	-	10.0[a]	56.7[b]	2.0[a]	84.0[c]	60.2[b]	-	32.0[a]
4	1.8[a]	-	24.0[b]	11.3[a]	-	90.0[b]	0.4[a]	-	8.0[a]	1.9[a]	-	68.0[b]
M-SHAPE												
1	-	82.0[a]	100.0[b]	0.4[a]	100.0[b]	100.0[b]	-	100.0[b]	44.0[a]	-	100.0[a]	100.0[a]
2	24.4[a]	18.0[a]	-	13.6[a]	-	-	19.6[a]	-	22.4[a]	22.7[a]	-	-
2.5	61.5[a]	-	-	47.9[a]	-	-	57.5[a]	-	-	53.9[a]	-	-
3	14.2[a]	-	-	38.1[a]	-	-	22.9[a]	-	32.0[a]	23.4[a]	-	-

Different letters within row and environment indicate significant differences ($P = 0.05$) according to the Marascuilo procedure. [a]SHAPE: leaf blade shape (1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate); T-SHAPE: terminal lobe shape (1 = lanceolate, wild rocket type; 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type); M-SHAPE: margin shape (1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate)

Table S4. Percentage of categorical descriptors (%) analyzed in the eleven accessions of wall rocket grown under greenhouse or field conditions, during the winter and spring seasons.

	Winter		Spring	
Descriptor[a]	**Greenhouse**	**Field**	**Greenhouse**	**Field**
SHAPE				
2	6.5[a]	13.5[b]	4.7[a]	28.5[b]
3	36.7[b]	15.6[a]	30.9[b]	18.9[a]
4	56.7[a]	70.9[b]	64.5[b]	52.6[a]
T-SHAPE				
2	30.9[a]	42.9[b]	26.5[a]	37.9[b]
3	67.3[b]	56.7[a]	62.3[a]	60.2[a]
4	1.8[a]	0.4[a]	11.3[b]	1.9[a]
M-SHAPE				
1	-	-	0.4[a]	-
2	24.4[a]	19.6[a]	13.6[a]	22.7[b]
2.5	61.5[a]	57.5[a]	47.9[a]	53.9[a]
3	14.2[a]	22.9[b]	38.1[b]	23.4[a]

Different letters within row and environment indicate significant differences (P = 0.05) according to the Marascuilo procedure. [a]SHAPE: leaf blade shape (2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate); T-SHAPE: terminal lobe shape (2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type); M-SHAPE: margin shape (1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate)

3.3. Influence of the growing conditions in the content of vitamin C in *Diplotaxis erucoides*

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Keywords: Ascorbic acid, dehydroascorbic acid, field, greenhouse, wall rocket

Guijarro-Real, C., Rodríguez-Burruezo, A., Prohens, J, Adalid-Martínez, A.M., Fita, A. 2017. Influence of the growing conditions in the content of vitamin C in Diplotaxis erucoides. Bulletin UASVM Horticulture, 74 (2), 144–145

DOI: 10.15835/buasvmcn-hort:0011

Abstract

Diplotaxis erucoides is an edible plant with potential for marketing. Here, we analysed the influence of the growing conditions in this species, *D. tenuifolia* and *Eruca sativa*, and studied the relation among the ascorbic (AA) and dehydroascorbic (DHA) acid forms. Plants were grown in the late winter-spring season under two conditions, greenhouse and field. The contents in AA, DHA and vitamin C (VC) were analysed by HPLC. The content of VC and AA were, in general, remarkable higher in the plants grown in the field. On the other hand, the mean percentage of DHA was less than 11%, being in this case higher for plants grown in the greenhouse. Thus, growing this potential crop in the field seems a better option in order to increase the content in VC, being AA the main form present at the moment of gathering.

Introduction

Traditionally, Mediterranean cultures have included wild edible plants as part of their traditional cuisine, being wall rocket an example of this tradition. Wall rocket (*Diplotaxis erucoides*) is an annual plant with lobated, edible leaves, widespread in the Mediterranean region (Martínez-Laborde, 1997). It can be considered as a good source of vitamin C. However, few studies have been done with this species (D'Antuono *et al.*, 2008) and there is a lack of information about the agronomical conditions for its growth. Thus, considering that VC is an important trait for its quality and due to the influence that culture conditions have in the content of this compound, it is necessary to establish the optimum conditions for increasing its accumulation. Here we analysed the influence of the growing conditions in the content of VC, ascorbic acid (AA) and dehydroascorbic acid (DHA) in wall rocket and related rocket crops.

Materials and Methods

Five populations of wall rocket were evaluated and compared with the commercial *Eruca sativa* cv. SSC 2965 and *D. tenuifolia* cv. SSC 2402, both

from Shamrock Seed CO (Table 1). Plants were grown under two growing conditions, greenhouse and field. Five replicates per sample and condition were grown, with two measurements per sample. The content in AA and VC were analysed by HPLC, after the reduction of the DHA to AA with tris(2-carboxy ethyl)phosphine hydrochloride (Chebrolu *et al.*, 2012). DHA was calculated as the difference between VC and AA.

Results and discussion

The three species presented remarkable values of VC (Table 1). This content was, in general, higher for plants grown in the field, with the exception of salad rocket cv. 2965 that remained stable. The mainly form detected was AA in the both systems, with mean values of 44.9±1.9 and 74.5±1.9 mg/100 g FW for greenhouse and field, respectively. Thus, both total VC and AA form presented the same trend between culture conditions and also among accessions. On the other hand, although the content in DHA was higher for the field conditions, differences between the systems were not as remarkable as those found for the AA. Due to this fact, the calculated percentage of DHA was higher for plants grown in the greenhouse, representing in any case less than 11% of the total VC.

According to our results, wall rocket could be considered as a leafy vegetable with high content in VC as it happens with their related rocket crops (Colonna *et al.*, 2016). Moreover, choosing the correct culture system may also contribute to the accumulation of this compound since the growing conditions can affect the grade of stress and response in plants. As our results suggest, growing this crop in the field is more interesting for this goal. On the other hand, as it happens in many horticultural crops (Lee and Kader, 2000), the main form of the vitamin C present in this leafy vegetable at the moment of gathering was the AA, a powerful antioxidant with high interest for human health.

Conclusion

Wall rocket has interest as a new crop with high content in vitamin C, mostly in the form of AA. Growing this vegetable in the field would be a

good option for its commercial exploitation, since this kind of culture seems to enhance the content in vitamin C.

Acknowledgements

Carla Guijarro-Real thanks the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for a predoctoral grant (BOE-A-2014-13539).

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Table 1. Mean values and standard errors in vitamin C (VC), ascorbic (AA) and dehydroascorbic acid (DHA) (mg/ 100g FW), and percentage of DHA in the total VC for greenhouse (G) and field (F).

Accession	VC		AA		DHA		% DHA	
	G	F	G	F	G	F	G	F
DER001-1	55.56±4.69^{b}	93.22±6.90^{cd}	50.54±4.27^{bc}	86.17±6.29^{de}	5.02±0.55^{a}	7.04±0.65^{bc}	9.09±0.62^{ab}	7.50±0.27^{a}
DER045-1	44.06±6.22^{ab}	89.95±7.65^{cd}	38.72±5.70^{ab}	82.70±6.77^{cde}	5.34±0.74^{a}	7.25±1.02^{bc}	12.80±11.23^{c}	7.85±0.59^{a}
DER055-1	60.27±8.48^{b}	80.64±3.92^{bcd}	54.79±7.71^{c}	75.25±4.06^{cd}	5.48±0.98^{a}	5.40±0.72^{ab}	9.30 ±1.13^{ab}	6.92±1.04^{a}
DER064-1	50.62±4.19^{b}	94.58±3.50^{d}	45.61±3.97^{bc}	88.81±3.22^{e}	5.01±0.45^{a}	5.77±0.46^{b}	10.15±0.86^{bc}	6.06±0.43^{a}
DER081-1	50.43±3.90^{b}	78.83±4.36^{bc}	45.53±3.34^{bc}	72.56±4.10^{bc}	4.90±0.69^{a}	6.26±0.65^{b}	9.43±0.91^{abc}	8.03±0.76^{a}
cv. SSC2402	33.65±5.46^{a}	70.93±3.22^{ab}	27.44±4.76^{a}	61.96±3.07^{ab}	6.21±0.91^{a}	8.97±1.14^{c}	20.11±1.98d	12.67±1.59^{b}
cv. SSC2965	54.74±7.99^{b}	57.85±3.18^{a}	51.65±7.65^{bc}	54.44±2.84^{a}	3.09±0.40^{a}	3.41±0.44^{a}	5.86±0.50^{a}	5.74±0.60^{a}
Average	*49.9 ±2.09*	*80.85±2.07**	*44.90±1.91*	*74.55±1.90**	*5.0±0.28*	*6.30±0.28**	*10.97±0.40**	*7.82±0.40*

Different letters indicate differences between accessions (Student-Newman-Keuls test, $P < 0.05$) and * means significant between conditions (LSD, $P < 0.05$).

3.4. Growing conditions affect the functional quality of the edible wall rocket (*Diplotaxis erucoides* (L.) DC.)

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Keywords: Ascorbic acid, field, greenhouse, nitrates, sinigrin, total phenolics, wall rocket

Abstract

Wall rocket (*Diplotaxis erucoides* (L.) DC.) is a wild edible vegetable widespread along the Mediterranean, with potential to become a functional food. The species is rich in ascorbic acid (AA) and phenolic compounds (TP), and sinigrin (SIN) as main glucosinolate. As other leafy vegetables, it also accumulates nitrates (NO_3^-) as antinutrients. Considering that these compounds are affected by the environmental conditions, identifying the proper cultivation conditions for an enhanced quality can promote this vegetable. In this work, eleven accessions of wall rocket were evaluated under field and greenhouse as model systems, and compared to cultivated rocket crops (*Eruca sativa* and *Diplotaxis tenuifolia*). Experiments were performed in two independent cycles (winter and early spring) as an indirect measurement of the effect of sowing date. Wall rocket did not differ greatly from the cultivated rocket crops, except for the presence in SIN that was unique for wall rocket. This distinctive character, affecting the flavour, can promote the commercial production as a new crop. The within-species analysis did not show a significant accession effect in general terms. On the contrary, it revealed a significant effect of the growing system for many traits, mainly in winter cycle. Field conditions increased the accumulation of AA and TP and also decreased the NO_3^- levels; significant correlations were established among the accumulation of these compounds. As a counterpart, the relative water content significantly decreased in the field-winter environment (87%), making the product to seem less fresh. These results are of relevance for the potential adaptation of wall rocket into crop conditions, suggesting that cultivation under field conditions enhance the functional quality of wall rocket. The lack of differences among accessions also provides relevant information for breeding programmes, suggesting that the selection strategy should consider other aspects with greater variability.

Introduction

Modern societies have become increasingly aware of the importance of diet as part of a healty lifetyle. Thus, many consumers look for additional health benefits obtained from specific foods, known as functional foods

(Nutrition Society, 1999; Pinela *et al.*, 2017). These demands offer an opportunity for the revalorization of wild edible plants (WEPs). In fact, several WEPs have enhanced bioactive properties and may be considered as functional foods (Guarrera and Savo, 2013; Ceccanti *et al.*, 2018). A promising strategy for such revalorization is the domestication and adaptation into cultivation systems aimed at its production as crops.

Mediterranean cultures have a rich ethnobotanic knowledge and tradition in the consumption of WEPs, as compiled in many works (e.g., Parada et al., 2011; Guarrera and Savo, 2016; Licata *et al.*, 2016). These reports show a great diversity of WEPs that have potential as new crops, including the edible Diplotaxis erucoides (L.) (wall rocket). Wall rocket (Diplotaxis erucoides (L.) DC. subsp. erucoides) is an annual plant from the Brassicaceae family broadly distributed along the Mediterranean regions (Martínez-Laborde, 1997). Considered as a weed for many crops, the species is also appreciated as a wild vegetable for its characteristic, mild pungent flavour. The tender leaves of wall rocket can be eaten fresh or cooked, in salads, soups, pasta dishes or even fried in omelettes (Guarrera and Savo, 2016; Licata *et al.*, 2016). One commercial cultivar of wall rocket is currently available (cv. 'Wasabi', Shamrock Seed Company, Inc.), but as far as we know it is not extensively cultivated.

Wall rocket is taxonomically related to the popular rocket crops *Diplotaxis tenuifolia* (L.) DC. (wild rocket) and *Eruca sativa* Mill. (salad rocket). These crops accumulate great contents of biocompounds, including vitamin C, phenolic compounds and glucosinolates (Spadafora *et al.*, 2016), thus displaying an added value in terms of functional quality. Both vitamin C and phenolic compounds are potent antioxidants against oxidative stress (Procházková *et al.*, 2011; Adikwu and Deo, 2013; Ashor *et al.*, 2016; Panche *et al.*, 2016) –vitamin C is, in addition, an essential microelement with antiscorbutic activity (Mandl *et al.*, 2009)–. Glucosinolates (GSLs) are secondary metabolites from the *Brassicaceae* and other families within the *Capparales* order (Gols *et al.*, 2018). Their hydrolysis products are responsible of the characteristic flavour in the *Brassicaceae* (Bell *et al.*, 2015), and are also studied as indirect antioxidants (Vig *et al.*, 2009). The main glucosinolate determined in wall rocket is sinigrin (D'Antuono *et al.*,

2008; Di Gioia *et al.*, 2018), which provides a highly characteristic pungent, sulphurous flavour, described as "mustard like" or "horseradish like" (Bell *et al.*, 2018). By contraposition, rocket crops also accumulate high amounts of nitrates (Schiattone *et al.*, 2018), considered as antinutrients with potential health risks (Habermeyer *et al.*, 2015). As consequence, its accumulation in foodstuff (mainly leafy vegetables) is controlled, and European maximum levels are established for the commercial production of specific vegetables including rocket crops (European Comission, 2011).

Rocket crops are cultivated under field and greenhouse conditions (Di Gioia *et al.*, 2018), and can be grown in the Mediterranean regions for the greater part of the year. Thus, these crops are developed under variable agronomic and environmental factors that include location, temperature, hours and incidence of sunlight or time to harvest, among others. These environmental changes can affect the accumulation of bioactive compounds (Durazzo *et al.*, 2013). For instance, the content in nitrates can decrease as light intensity and photoperiod increase (Cavaiuolo and Ferrante, 2014). Stresses such as exposure of plants to heat shock, chilling or high light conditions activate the accumulation of protective phytochemicals like ascorbic acid or phenolic compounds (Oh *et al.*, 2009). Moreover, in the particular case of *Brassicaceae,* stresses such as growing under non-optimal temperature conditions can increase the content in glucosinolates as well (Björkman *et al.*, 2011).

Although these are general behaviours, information related to the effect of cultivation on wall rocket is scarce (Di Gioia *et al.*, 2018). Ceccanti *et al.* (2018) suggested that, as part of the breeding programmes of wild edible plants into new crops, it is important to study the proper cultivation practices to allow a large-scale production with high yields; such cultivation should ensure, at the same time, a good quality product including nutritional quality. The current study was aimed at analysing the effect of two model systems (greenhouse and field) on the functional quality of pre-selected accessions derived from local germplasm. The work is part of an established breeding programme (Universitat Politècnica de València (UPV), Valencia, Spain). The use of local germplasm correspond to the ‘‘Focused Identification of Germplasm Strategy’’ (FIGS), which can be successful for

the development of cultivars adapted to our Mediterranean conditions (Street *et al.*, 2016; Sogbohossou *et al.*, 2018). Two independent experiments were performed in two consecutive cycles aimed at considering an indirect effect of date of sowing. Comparison under different environments allows identifying the most adequate growing conditions for wall rocket as a crop. Thus, the current study is a basis for the future cultivation of this emerging crop under our local conditions, with a high added value and a reduced content in nitrates. Moreover, the study provides a general insight into the behaviour of this species under cultivation, which can be also adapted to other regions.

Materials and methods

Plant material and cultivation

Ten pre-selected accessions of wall rocket, and four commercial cultivars of rocket species, were evaluated in the experiment. The pre-selected accessions correspond to the first generation seedling from wild populations collected in the Valencian Community (Spain) and conserved at the UPV. The commercial cultivars (Shamrock Seed Co.; Salinas, CA, USA) included the species *D. tenuifolia* (var. SSC2402 and var. Wild Rocket), *E. Sativa* (var. S. Rocket SSC2965), and the commercial cultivar of *D. erucoides* that is available (var. Wasabi).

The experiment was performed at the UPV during two independent growing cycles: the late autumn-winter season (from now on, winter season) and late winter-early spring season (from now on, spring season). In each cycle, assays were simultaneously carried out under two cultivation systems selected as model for wall rocket cultivation: a heated glasshouse (39º 29' 0" N, 0º 20' 26" W) and an experimental field under anti-pest mesh (39º 28' 56" N, 0º 20' 11" W). The same experimental design was followed in the four environments, as described: a randomized block design with five blocks, each block including one replicate of thirty plants per accession –the fourteen accessions, corresponding to the three species, were completely randomized in each block–.

Seeds were treated with commercial sodium hypochlorite 2.5% (v/v) plus gibberellic acid 100 ppm (Duchefa Biochemie, Haarlem, The Netherlands) (Guijarro-Real *et al.*, 2018), sown in seedling trays with commercial Neuhaus Humin-substrat N3 substrate containing NPK fertilizer 1.3 g l^{-1} (Klasmann-Deilmann Gmbh, Geeste, Germany), and trays placed in a growing chamber with long day conditions (16/8 h, 25 ºC). Two days later, trays were moved to a glasshouse. Plants used for the greenhouse system were sown in 40 x 25 cm2 trays and remained in trays during all the experiment, while plants used for the field system were removed from the greenhouse and transplanted when the second true leaf appeared. The content in nitrogen in the field system was 0.1 %.

Preparation of samples

Except for some failures during growth, five replicates per accession, growing system and season were harvested. All plants in each replicate were harvested together, except for plants with visible growing damages (e.g., very small size compared to the average of the block) that were discarded. Samples were processed the same day of harvesting. One fresh sub-sample was destined for the analysis of ascorbic acid, and the remaining material was frozen at -80ºC and then freeze-dried. Difference between the weight before and after freeze-drying was used to calculate the water content. The freeze-dried material was powdered with a commercial grinder and stored in darkness until the analysis of total phenolics, sinigrin and nitrates. All results were expressed per each 100 g of fresh material (FW).

Traits measured

The content in ascorbic acid (AA) was measured according to Cano and Bermejo (2011) with slight modifications. Briefly, 1.0 g of fresh material was homogenized with 5 ml of 3.0% (w/v) cold *meta*-phosphoric acid (Sigma-Aldrich; Saint Louis, MO, USA) for 1 min using a mortar. The aqueous phase was filtered trough a 0.22 µm PVDF filter (Millipre-Merck, Darmstadt, Germany) and analysed on a HPLC 1220 Infinity LC System (Agilent Technologies; Santa Clara, CA, USA) using a BRISA C_{18} column (150 mm x 4.6 mm i.d., 3 µm particle size; Teknokroma; Barcelona, Spain). The mobile phase consisted on methanol: 1% acetic acid (5:95) for 15 min at

a flow rate of 1 ml min^{-1}. The injection volume was 5 µl, and quantification was performed at 254 nm using an external standard calibration of *L*-ascorbic acid (Sigma-Aldrich).

The content in total phenolics (TP) was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) as described in Guijarro-Real *et al.* (2019). 0.125 g of were extracted with 70% acetone containing 0.5% acetic acid for 24 h. Aliquots of 65 µl reacted with 500 µl of diluted Folin-Ciocalteu (1:10) for 5 min, plus 500 µl of 60 g L^{-1} sodium carbonate for 90 min. Quantification was performed at 765 nm using chlorogenic acid as external standard, and results were expressed as mg of chlorogenic acid equivalents (mg CAE 100 g^{-1} FW).

The content in sinigrin (SIN) was determined as described by Grosser and van Dam (2017) with slight modifications. Firstly, 0.1 g of powdered samples were heated for 2 min at 75ºC using a Termoblock TD150 P2 (Falc Instruments, Treviglio, Italy) for myrosinase inactivation (Pasini et al., 2011). Extraction was then performed using 1 ml plus 1 ml of methanol 70% (v/v) for 30 min at 75ºC. After centrifugation, the supernatant was injected into a SPE column containing a DEAE Sephadex anion exchanger (A-25, Sigma-Aldrich) activated with sodium acetate buffer 20 mM (pH 5.5), and incubated with 20 µl of diluted sulfatase overnight. Desulphonated sinigrin was eluted with 500 µl plus 500 µl of milliQ water and analysed using the same HPLC apparatus as for AA analysis and a Luna® Omega C_{18} column (150 mm x 4.6 mm i.d., 3 µm particle size; Phenomenex, Torrance, CA, USA). The mobile phases consisted of acetonitrile (A) and water (B), with the following gradient: from 98% A to 65% A in 35 min, then equilibrated for 5 min to the initial conditions. The injection volume was 10 µl and the flow rate, 0.75 ml min^{-1}. Quantification was performed at 229 nm using desulphoned sinigrin hydrate (PhytoPlan, Heidelberg, Germany) as external standard.

Finally, the content in nitrates was determined using a nitrate-selective ion (Crison Instruments S.A., Alella, Barcelona, Spain), with an extraction protocol adapted from Egea-Gilabert *et al.* (2014). Nitrates (NO_3^-) from 0.1 g were extracted with 50 mL of distilled water for 15 min in continuous

stirring, and stabilised with 1 mL of 2 M diammonium sulfate ($(NH_4)_2\ SO_4$) buffer in the moment of measurement using the nitrate-selective ion.

Data analysis

Data were subjected to fixed effects model analysis of variance (Gomez and Gomez, 1984) using the Statgraphics Centurion XVII v.17.2 (Statpoint Technologies, Inc., Warrenton, VA, USA). Two different analyses were performed: 1) comparison among species and 2) comparison among accessions of wall rocket. For the analysis of species, the average values for accession considering the five replicates per environment were used as data. Data were then submitted to a multivariate analysis of variance (ANOVA) and the effects of species (S, corresponding to three levels: wall rocket, wild rocket, salad rocket), environment (E, four levels: greenhouse in winter, field in winter, greenhouse in spring, field in spring) and the S x E interaction were tested. The linear model applied was: $X_{ijk} = \mu + S_i + E_j + (S \times E)_{ij} + e_{ij(k)}$, where X_{ijk} is the value for accession k of species i and environment j, μ is the general mean, S_i is the effect of the species i, E_j is the effect of the environment j, $(S \times E)_{ij}$ is the effect of the interaction between species i and environment j, and $e_{ij(k)}$ is the residual error of the accession k. Mean values and standard error were obtained for the three species and significant differences determined using the Student-Newman-Keuls multiple range test ($P = 0.05$).

The analysis of wall rocket was aimed at studying the presence of differences among accessions and/or among systems, considering each growing cycle independently. Thus, individual data were submitted to a multivariate analysis of variance (ANOVA) and the effects of accession (A, eleven accessions), growing system (GS, two levels: greenhouse, field) and the A x GS interaction were tested. The linear model applied was: $X_{ijkl} = \mu + A_i + B_{j(ik)} + GS_k + (A \times GS)_{ik} + e_{ijk(l)}$, where X_{ijkl} is the value for replicate l of accession i in block j and growing system k, μ is the general mean, A_i is the effect of the genotype i, $B_{j(ik)}$ is the effect of block j for accession i and system k, GS_j is the effect of the growing system j, $(A \times GS)_{ik}$ is the effect of the interaction between accession i and system k, and $e_{ijk(l)}$ is the residual error of the replicate l. Mean values and standard errors were obtained, and significant differences determined according to the LSD test ($P = 0.05$, for

comparison among systems) or Student-Newman-Keuls multiple range test (P = 0.05, for comparison among accessions). Finally, the Spearman rank coefficients of correlation (ρ) were calculated for phenotypic (n = 44) and environmental (n = 213) correlations.

Results

Differences among species

The three species were compared in terms of content in water, AA, TP and NO_3^-; differences for the content in SIN were not analysed because this compound is only present in materials of wall rocket. A significant effect of the environment was determined in the four traits evaluated (Table 1). This factor was the main contributor to the total sum of squares in all cases, with values ranging between 52.8% (NO_3^-) and 72.6% (TP). On the contrary, the species factor was only significant for the relative content in water and the content in AA. The contribution to the total sum of squares was, in any case, lower than 10.5% (Table 1). In addition, a significant S x E interaction was determined for all traits except for the content in AA. For those traits, the interaction was greater contributor than the species, accounting for up to 17.7% (water content) of the total sum of squares.

Table 1. Percentage of sum of squares for the effects of species (S, three levels: wall rocket, wild rocket, salad rocket), environment (E, four levels: greenhouse in winter, field in winter, greenhouse in spring, field in spring), S x E interaction and residuals for the content in water, ascorbic acid (AA), total phenolics (TP) and nitrates (NO_3^-).

Parameter	S	E	S x E	Residual
Water content	4.21^{*}	56.22^{*}	17.69^{***}	21.88
AA	10.22^{***}	59.10^{**}	5.57^{ns}	25.12
TP	0.83^{ns}	72.61^{**}	7.74^{*}	18.82
NO3	3.86^{ns}	52.78^{*}	13.98^{**}	29.38

^{ns}, *, ** and *** indicate no significant or significant at P < 0.05, 0.01 and 0.001, respectively

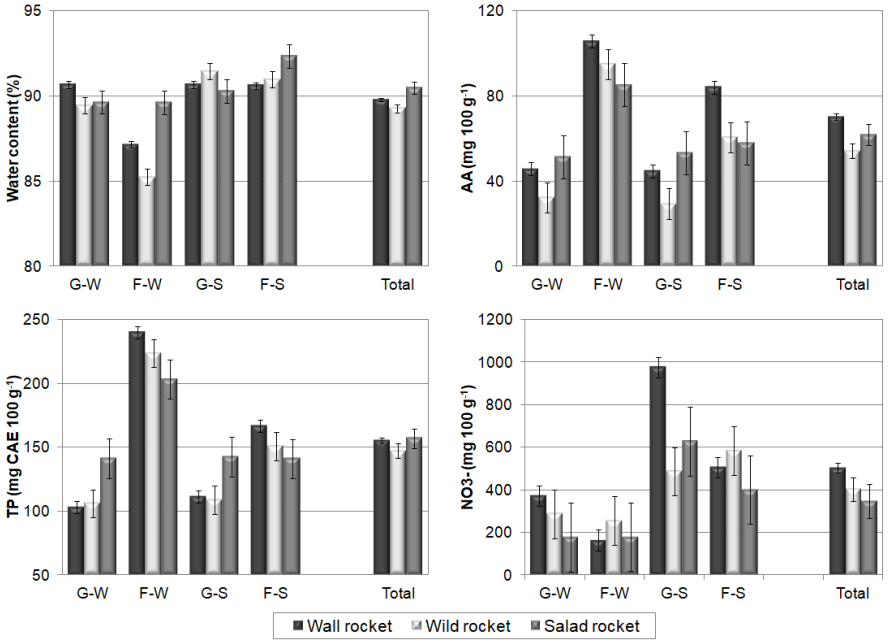

Fig. 1. Mean values ± SE for the content in water, ascorbic acid (AA), total phenolics (TP) and nitrates (NO_3^-) determined for wall rocket, wild rocket and salad rocket in the four environments tested: G-W = greenhouse-winter, F-W = field-winter, G-S = greenhouse-spring, F-S = field-spring; and global average value ± SE for the three species. CAE: chlorogenic acid equivalents

The mean values for each trait are summarised in Fig. 1. The average content in water was close to 90.0% for the three species, with wild rocket displaying on average the lowest values. Differences among species were mainly remarkable for plants growing in the field. The field-winter environment especially affected the relative content in water for wall rocket and wild rocket, significantly decreasing with respect to the other environments. The content in AA was highly affected by the cultivation system in all species. Wall rocket accumulated the highest content in AA, being on average 70.25 mg 100 g^{-1} FW. This value was 1.3-fold greater than the accumulation in wild rocket materials. This global situation was individually observed for the field but not the greenhouse system (Fig. 1). On the other hand, wall rocket accumulated the greatest values in TP for the field but not the greenhouse system. However, differences between species

were not determined for the global values. In the same way, non significant differences were determined for the content in NO_3^-. However, wall rocket showed a greater accumulation under greenhouse conditions (372.3 and 974.0 mg 100 g^{-1} FW for winter and spring, respectively) compared to the other rocket species (Fig. 1).

Variation among wall rocket accessions

Effects of accession, environment and interaction

The effects of accession (A, eleven accessions), growing system (GS, two levels: field or greenhouse) and A x GS interaction were analysed in each growing cycle (Table 2). The effect of the growing system during the winter cycle was highly significant for all traits except for the content in NO_3^-. The contribution to the total sum of squares ranged between 16.5% (NO_3^-) and 81.1% (TP); moreover, this factor was the greatest contributor for the content in water, AA and TP (>50% of the total sum of squares). On the contrary, the contribution of the growing system to the total sum of squares was lower during the spring cycle for all traits. Values significantly decreased, ranging in this case between 0.1% (content in water) and 57.3% (TP) (Table 2). During this cycle, the effect of the system was only significant for the contents in AA and TP. As in the winter cycle, this factor remained as the main contributor to the total sum of squares for both AA and TP, accounting for 52.8% and 57.3%, respectively.

On the other hand, non significant effects for the accession factor were determined for most of the traits in any of the cycles (Table 2). This factor only affected the content in AA and SIN during the winter cycle. However, the contribution to the total sum of squares was low in both cases (3.8% and 10.0%, respectively). During the spring cycle, the effect of accession contributed in less than 11.7% to the sum of squares. Finally, the A x GS interaction effects were mostly no significant (Table 2). As exception, an interaction effect was determined during the spring cycle for the contents in AA and TP. However, this effect only accounted for 3.5% and 7.0% to the sum of squares.

Table 2. Sum of squares (in percentage) for the effects of accession (A, eleven accessions), growing system (GS, field or greenhouse), A x GS interaction, block and residuals for the content in water, ascorbic acid (AA), total phenolics (TP), sinigrin (SIN) and nitrates (NO_3^-) evaluated in the eleven accessions of wall rocket during the winter and spring seasons.

Season	Parameter	A	GS	A x GS	Block	Residual
Winter	Water	3.63^{ns}	60.62^{***}	2.92^{ns}	6.36	26.47
	AA	3.81	63.37^{***}	3.26^{ns}	14.92	14.64
	TP	1.21^{ns}	81.11^{***}	1.33^{ns}	5.42	10.93
	SIN	10.00	35.89^{**}	4.19^{ns}	11.44	38.48
	NO_3^-	2.84^{ns}	16.55^{ns}	3.04^{ns}	34.22	43.35
Spring	Water	1.72^{ns}	0.07^{ns}	6.14^{ns}	47.30	44.76
	AA	2.75^{ns}	52.85^{***}	7.00^{*}	13.63	23.77
	TP	2.97^{ns}	57.35^{**}	3.54^{*}	22.76	13.38
	SIN	11.63^{ns}	5.10^{ns}	14.96^{ns}	9.06	59.25
	NO_3^-	9.53^{ns}	5.04^{ns}	9.49^{ns}	32.99	42.96

^{ns}, *, ** and *** indicate no significant or significant at $P < 0.05$, 0.01 and 0.001, respectively

Differences between growing conditions

Mean values ± standard error for the different traits and environments are summarised in Table 3. During the winter cycle, plants growing under field conditions accumulated less water in their tissues compared to the greenhouse system (87.1% vs. 90.9%). On the contrary, the contents in AA, TP and SIN significantly increased in the former system. Thus, values obtained in the field system were more than 2–fold greater with respect to the greenhouse (Table 3). By contrast, differences were reduced during the spring cycle. Plants from the greenhouse and field systems only differed in the accumulation of AA and TP. As in the winter cycle, the field system provided the greatest contents for both compounds (Table 3). In this case, the differences were between 1.5 (TP) and 1.9–fold times (AA).

Table 3. Mean values ± SE (n = 11) for the content in water, ascorbic acid (AA), total phenolics (TP), sinigrin (SIN) and nitrates (NO_3^-) for plants of wall rocket growing under greenhouse or field system, during the winter or spring season. Results are expressed as mg 100 g^{-1} FW except for the relative content in water (%). The content in TP is expressed as chlorogenic acid equivalents.

	Winter		Spring	
Trait	**Greenhouse**	**Field**	**Greenhouse**	**Field**
Water	$90.95\pm0.19^{b/-}$	$87.15\pm0.20^{a/A}$	$90.67\pm0.13^{-/-}$	$90.59\pm0.10^{-/B}$
AA	$45.72\pm2.92^{a/-}$	$105.54\pm4.19^{b/B}$	$44.76\pm2.55^{a/-}$	$84.07\pm2.82^{b/A}$
TP	$100.76\pm2.72^{a/-}$	$239.93\pm6.15^{b/B}$	$108.77\pm2.58^{a/-}$	$166.64\pm3.69^{b/A}$
SIN	$35.28\pm4.37^{a/A}$	$73.02\pm4.25^{b/B}$	$47.88\pm4.93^{-/B}$	$35.36\pm2.44^{-/A}$
NO_3^-	$372.36\pm23.00^{-/A}$	$163.67\pm5.91^{-/A}$	$973.99\pm96.35^{-/B}$	$504.83\pm5.65^{-/B}$

Different letters within rows indicate significant differences between growing systems for each season (lower case letters) or between seasons for each growing system (upper case letters)

The indirect effect of the growing period was also evaluated. Thus, the nutraceutical parameters were compared between winter and spring, for both the greenhouse and the field systems (Table 3). Plants grown in the greenhouse displayed the most homogeneous values between cycles. Significant differences were only determined for the levels of SIN and NO_3^-. For both traits, plants from the spring cycle accumulated the greatest contents. The accumulation of NO_3^- highlighted in this sense, with a 2.6-fold increase with respect to the winter cycle (Table 3). In fact, this environment provided the greatest accumulation of NO_3^- considering the four possible conditions (974 mg 100 g^{-1} FW). Regarding the field system, results indicated that all traits were influenced by the growing cycle (Table 3). Thus, plants developed during the winter cycle increased the accumulation of AA, TP and SIN. The greatest increase was determined for SIN, corresponding to a 2-fold increase (35.4 mg 100g^{-1} vs. 73.0 mg 100g^{-1}). On the contrary, the winter cycle reduced the content in water and also the levels in NO_3^-. This environment provided in fact the lowest accumulation (164 mg NO_3^- 100 g^{-1}) (Table 3).

Correlation between nutritional traits

All Spearman' rank phenotypic correlations were highly significant (Table 4). A positive correlation was determined between the accumulation of water and NO_3^- in the tissue (ρ = 0.498). On the contrary, both traits were negatively correlated to the nutraceutical traits (AA, TP, SIN). In the case of NO_3^-, coefficients of correlation ranged between -0.349 (SIN) and -0.554 (AA). Greater coefficients were determined for correlations with the content in water (ρ > -0.7). Regarding the nutraceutical traits, positive correlations were determined among them. The contents in AA and TP displayed the greatest coefficient value (ρ = 0.921), and both traits were moderately correlated to the content in SIN (0.455 and 0.621 for AA and TP, respectively) (Table 4).

Similar results were obtained for the analysis of environmental correlations, although the coefficients were mainly lower in this case (Table 4). The greatest decrease was determined for the correlation between NO_3^- and percentage of water, with a ρ reduction in 52% (0.237 vs. 0.498). A high reduction of the ρ coefficient was also found for the correlation between NO_3^- and AA (-0.215 vs. -0.555). Finally, a moderate environmental correlation was determined for the contents in AA and TP (ρ = 0.649) (Table 4).

Table 4. Phenotypic (above the symmetry axis, n = 44) and environmental (below the symmetry axis, n = 213) Spearman rank correlations between the content in water (Water), ascorbic acid (AA), total phenolics (TP), sinigrin (SIN), and nitrates (NO_3^-) determined in the accessions of wall rocket.

	Water	**AA**	**TP**	**SIN**	**NO_3^-**
Water		-0.7032***	-0.7832***	-0.7820***	0.4981***
AA	-0.5712***		0.9209***	0.4554**	-0.5549***
TP	-0.8546***	0.6488***		0.6211***	-0.4898**
SIN	-0.5878***	0.3670***	0.5545***		-0.3489*
NO_3^-	0.2374**	-0.2149**	-0.3252***	-0.2489***	

^{ns}, ** and *** indicate no significance or significance of the correlation at P < 0.01 and 0.001, respectively

Discussion

Wall rocket is considered a common weed in Mediterranean regions. However, it is also appreciated as a wild edible vegetable (Parada *et al.*, 2011) and therefore has the potential to become an established crop. The present work was aimed at studying the effect of different cultivation environments on the nutritional quality of selected accessions. Due to the taxonomy-linkage and similarities in terms of growth and commercial use, wall rocket can be potentially cultivated in similar conditions to the already established rocket crops. Thus, wall rocket may be produced in field or greenhouse systems, although soilless systems can be also available (Egea-Gilabert *et al.*, 2009; Di Gioia *et al.*, 2018). The greenhouse and field environments differ on several factors such as temperature, light intensity, air humidity or affection of rains, among others (Figàs *et al.*, 2018). These factors can also differ between growing cycles. Due to the importance of environmental factors on the accumulation of secondary metabolites and other compounds of nutraceutical importance (Weightman *et al.*, 2012; Colonna *et al.*, 2016; Bell and Wagstaff, 2017), the quality of the final product can be affected.

As nutritional traits, the content in AA, TP, SIN –only for wall rocket materials– and NO_3^-, together with the content in water, were evaluated. The AA instead of the total vitamin C was evaluated since we previously concluded that the AA form represented around 90% of the total vitamin C in wall rocket materials (Guijarro-Real *et al.*, 2017). Some nutritional differences, mainly the content in water and AA, were determined among the three species. However, traits were strongly affected by the growing system and significant S x E interactions were also established. Thus, the nutritional traits analysed were not useful enough to clearly identify each species. Moreover, results suggested that the environment had a similar effect for wall rocket and wild rocket materials regarding the accumulation of the different traits, unlike salad rocket. As exception, the accumulation of NO_3^- was less comparable among the species.

On the other hand, wall rocket accumulated SIN as main glucosinolate as a distinctive trait, since this compound was not determined neither in salad nor wild rocket materials. These findings are in agreement with

previous works comparing the glucosinolate profile of the three species (D'Antuono *et al.*, 2008; Di Gioia *et al.*, 2018). Nevertheless, different profiles in other wall rocket materials, characterised for the absence of SIN, have been identified as well (Bennett *et al.*, 2006; D'Antuono *et al.*, 2008). Discrepancies may correspond to differences related to the origin of materials; inter-subspecies differences, i.e., the analysis of *D. erucoides* subsp. *erucoides* or subsp. *longisiliqua* materials, as suggested by D'Antuono *et al.* (2008); or they may even correspond to inter-specific crosses.

In a second analysis, the eleven accessions of wall rocket were compared. The low contribution of the accession effect to the total sum of squares, together with a general absence of significance, were indicators of low nutritional variation among the accessions analysed. The lack of variation can correspond to the close geographic origin of materials, with exception of the commercial cultivar; or it can also correspond to low differences for wall rocket as a species in terms of nutraceutical properties. In this sense, performing new experiments with the inclusion of materials from other regions can help to clarify the overall variation for nutritional traits. On the other hand, it may also correspond to high intra-population variability considering that no homogenization efforts have been addressed, as suggested by the residual effect.

Comparison of different environments demonstrated a high effect on the final quality of the product. Plants growing in the field during the winter cycle had the most extreme environment, considering the two systems (greenhouse vs. field during the winter cycle) and the different growing periods (winter vs. spring in the field system). High adverse conditions were determined for this growing cycle, including coldness and several storm episodes, so plants were subjected to high abiotic stresses. Abiotic stress increases the levels of reactive oxygen species causing an oxidative stress in plants (Fita *et al.*, 2015). As part of the defence response to this possible oxidative damage, the content in antioxidants such as AA and phenolic compounds increase as well. Oh *et al.* (2009) determined that plants of lettuce exposed to chill-stress increased the accumulation of protective metabolites by the activation of genes involved in the biosynthesis of those

antioxidants. In the same way, it has been observed among *Brassicaceae* that plants accumulate greater content in glucosinolates when they grow under non-optimal temperatures (Björkman *et al.*, 2011), as our results suggest. In particular, a decrease in the temperature can increase the accumulation of glucosinolates according to previous authors (Kissen *et al.*, 2016; Steindal *et al.*, 2015). In contraposition, the filed-winter environment accumulated the lowest content in NO_3^-, which is also of interest for a commercial purpose. Light intensity has been positively correlated to nitrate reductase activity and a consequent lower accumulation of NO_3^- (Bonasia *et al.*, 2017). This season-dependence explains the different maxim limits established for lettuce and rocket crops in the European Union (European Comission, 2011). However, our experiment was conducted in two consecutive cycles with few differences to light exposure, and therefore it may not significantly affect the reductase activity. Thus, other physiological processes could influence this different accumulation.

The field-spring environment also provided a quality product. These plants highlighted for a great TP accumulation and also for the content in AA. It was significantly higher than the content described by Salvatore *et al.* (2005), and comparable or even greater than levels described for rocket crops by other authors (Durazzo *et al.*, 2013; Spadafora *et al.*, 2016). The accumulation of SIN, however, did not reach the levels previously described for wall rocket (Di Gioia *et al.*, 2018). In addition, the content in NO_3^- was below the maximum limit of 7,000 mg kg^{-1} imposed for rocket crops (European Comission, 2011). Finally, the increased relative water abundance was reflected in a greater visual appearance and less coriaceous aspect, traits that become essential for consumers' acceptance.

On the opposite, the greenhouse system may not be adequate for the commercial production of wall rocket, according to our results. Heated greenhouses are used to provide a more adequate and stable temperature for plants growth, compared to field conditions, but also affect other factors such as wind, air humidity, solar radiation, affection of rains and storms or crop management (Figàs *et al.*, 2018). Thus, our results suggest that growing wall rocket under greenhouse would enhance the homogenization of most quality traits, but providing a product of lower final quality. Moreover,

plants in this system accumulated high levels of NO_3^-, that in the spring cycle exceeded the limits established (European Comission, 2011) and made the product obtained not commercially acceptable.

Finally, the correlations among traits were evaluated. Phenotypic coefficients of correlations were greater than the environmental ones. These results indicated that the different factors evaluated had similar effect in materials as average; however, the residuals among those traits had a lower correlation. The high correlation between AA and TP may be explained by their antioxidant function, thus being accumulated against environmental stresses (Orsini *et al.*, 2016). However, both AA and TP were lower correlated to the content in SIN. Glucosinolates are mainly related to the defence system against pests and pathogens (Björkman *et al.*, 2011), instead of abiotic stress as the other compounds, thus explained the lower association among them. Nevertheless, the accumulation of glucosinolates is in some way affected by environmental factors as well (Björkman *et al.*, 2011; Bell and Wagstaff, 2017). On the other hand, the negative correlation between bioactive compounds and content in water corresponded to a dilution-concentration effect, i.e., when reducing the content in water, solutes are more concentrated in the tissues; and the negative correlation with NO_3^- has been also previously observed (Bonasia *et al.*, 2017), as in our results. A positive correlation between the relative water content and NO_3^- was determined, as extensively observed in many species (Cárdenas-Navarro *et al.*, 1999). There is an obligatory positive correlation between both traits as consequence of the homeostasis for NO_3^- concentration; it is, the accumulation of NO_3^- increases the capacity of plants to retain water due to the osmotic effect of this ion (Cárdenas-Navarro *et al.*, 1999; Schiattone *et al.*, 2018).

Conclusions

This work was aimed at determining the most adequate conditions for the establishment of wall rocket as a crop with enhanced quality. According to our results, growing this vegetable under greenhouse conditions would be less desirable for the functional quality of the final product. This system also increased the content in NO_3^-, even exceeding the maxim limits (European Comission, 2011). The field-winter system provided the lowest content in

NO_3^- and great antioxidant molecules. However, the lowest water content of tissues under these conditions may reduce consumers acceptance. Thus, results suggest that very stressful conditions like the harsh winter may not be adequate for a commercial production in the unprotected field. Finally, the low variability registered among populations should be considered for breeding programmes, and future selections could also consider other parameters with greater variability together with nutraceutical properties.

Acknowledgements

C. Guijarro-Real thanks the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for the financial support by means of a predoctoral FPU grant (FPU14-06798).

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Capítulo 4. Aceptación de la rabaniza por los consumidores

4.1. Volatile profile, organoleptic evaluation and consumers acceptance of wall rocket (*Diplotaxis erucoides*) as new crop

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Keywords: Affective test; consumers acceptance; *Diplotaxis erucoides*; GC-MS; HS-SPME; new crops; volatile organic compounds

Submitted to *Food Chemistry*

Abstract

Wall rocket (*Diplotaxis erucoides*) is a wild edible herb traditionally consumed in the Mediterranean regions with a characteristic, pungent flavour. However, little is known about the acceptance of wall rocket (*Diplotaxis erucoides*) as a crop, with a lack of information regarding the volatile constituents. In the present study, the volatile profile and consumer acceptance of wall rocket, and the relationship among them, was studied using three developmental stages. In addition, Dijon's mustard and wasabi paste products were used as reference for the analysis of volatiles. Most of the consumers appreciated wall rocket microgreens, while seedlings and baby-leaves were mainly appreciated by individuals that appreciate pungent tastes. The volatile profiles highlighted the levels of isothiocyanates (ITCs), being allyl-ITC the main compound identified. High levels of allyl-ITC were also determined in Dijon's mustard and wasabi, which may explain the relationship established for acceptance of the three products. Microgreens had the highest levels in ITCs, although the quantity tested did not allow its appreciation. Such levels significantly decreased in baby-leaves but they were clearly detected by consumers. Results suggest that microgreens may represent a market opportunity for general public consumption due to the low appreciation of pungency. In addition, baby-leaves may be marketed for consumers that enjoy pungent flavours.

Introduction

Consumers in modern societies look for enriching the culinary experience with new tastes and flavours. With this purpose, producers and breeders of fruits and vegetables have increased their attention in the wild edible plants (WEPs) as a source of new and attractive products, with particular flavouring and taste characteristics. An example of this renewed interest is the current commercial exploitation of different WEPs in local markets and restaurants (Evans & Irving, 2018; Łuczaj *et al.*, 2012). Moreover, there are examples of the establishment of new crops from WEPs, as they are the domestication of rocket (*Eruca sativa* and *Diplotaxis tenuifolia*) and watercress (*Nasturtium officinale*) crops (Molina, Pardo-de-

Santayana, & Tardío, 2016). However, there are still many other underutilized vegetables that represent an opportunity for the development of new crops.

Within the *Brassicaceae* family, the genera *Eruca* and *Diplotaxis* are a source of interesting WEPs for the potential development of new crops (D'Antuono, Elementi, & Neri, 2009). Among them, wall rocket (*Diplotaxis erucoides* (L.) DC. subsp. *erucoides*) represents an interesting option due to its appreciation in the traditional cuisine of different cultures. This wild has been traditionally consumed as WEP in Mediterranean countries including Spain, France or Italy (Couplan, 2015; Licata *et al.*, 2016; Parada, Carrió & Vallès, 2011). The flavour of wall rocket resembles the aroma and taste of other *Brassicaceae* crops such as mustard, horseradish and wasabi. Although a commercial cultivar exists (cv. 'Wasabi', Shamrock Seed Company, Inc.), as far as we know it is not extensively cultivated and the species remains underutilized as a WEP.

Species from the *Brassicaceae* family accumulate glucosinolates (GSLs) as secondary metabolites. When the tissue is damaged, for example with chewing, GSLs are enzymatically hydrolysed into other volatile compounds (Bell, Oloyede, Lignou, Wagstaff, & Methven, 2018). Although they are a defense mechanism against herbivores and pathogens, these hydrolysis products do not reach toxic levels for humans when are included as part of a varied diet (Angelino *et al.*, 2015). Moreover, the intake of these compounds is related to several health benefits such as the reduction of cancer risk (Fernández-León, Fernández-León, González-Gómez, Ayuso, & Bernalte, 2017).

In addition to the importance of GSLs for human health, this family of compounds is also appreciated by humans for its flavour. Different products can derive from the hydrolysis of GSLs, depending on the particular molecule, plant species and conditions in which the hydrolysis occurs (e.g., pH and presence of enzymatic co-factors) (Angelino *et al.*, 2015; Bell & Wagstaff, 2014; Hanschen & Schreiner, 2017). In rocket crops, the hydrolysis of GSLs derives in high levels of isothiocyanates (ITCs) (Bell, Spadafora, Müller, Wagstadd, & Rogers, 2016; Raffo *et al.*, 2018), which confer a characteristic bitter, hot or even burning and sulphurous flavour

(Bell, Methven, Signore, Oruna-Concha, & Wagstaff, 2017a). However, the acceptance of this characteristic flavour varies greatly between individuals. On the one hand, the degree of human sensitivity to bitter taste can increase the rejection to *Brassicaceae* crops (Bell, Methven, & Wagstaff, 2017b). On the other hand, aspects such as the profile, total content ad relative abundance of GSLs on a specific vegetable, or the presence of other compounds (e.g., sugars) can affect the flavour (Bell *et al.*, 2017a; Pasini, Verardo, Cerretani, Caboni & D'Antuono, 2011). Moreover, the developmental stage of the plant can affect the accumulation of GSLs as well (Di Gioia, Avato, Serio, & Argentieri, 2018). All these aspects can influence the acceptance of *Brassicaceae* crops.

Despite the traditional use of wall rocket, there is little information regarding its organoleptic quality. As far as we know, there are only a few works that analyse the GSLs constituents of wall rocket (Bennett, Rosa, Mellon, & Kroon, 2006; D'Antuono, Elementi, & Neri, 2008; Di Gioia *et al.*, 2018), and we have not found reports describing its volatile profile. Moreover, we have found one unique work that studied the acceptance of wall rocket, together with other *Diplotaxis* and *Eruca* germplasm materials (D'Antuono *et al.*, 2009). In this context, we addressed this experiment to evaluate the potential consumers acceptance of wall rocket when it is presented as a new, unique vegetable. The use of three different developmental stages may allow identifying if consumers prefer a specific product (microgreens, seedlings or baby-leaves). In addition, comparing the hedonic test with the analysis of volatile profiles can help to understand the degree of acceptance for the different products, by establishing a relationship between them.

Material and methods

Plant material and cultivation conditions

The present study was conducted during the months of January to March 2018 at the Universitat Politècnica de València (UPV, Valencia, Spain). Two pre-selected populations of wall rocket derived from a breeding programme were used as plant material. Seeds from both accessions

(DER001-2 and DER006-2) are kept at the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (UPV, Valencia, Spain).

Three different products were developed for the study, according to three different developmental stages of the plants. For the affective test, a randomized code of three digits was assigned to each product, as it is recommended for affective tests (O'Sullivan, 2017): P304 for microgreens, P355 for seedlings and P398 for baby-leaves (Fig. 1). Seeds were treated with 2.5% sodium hypochlorite followed by 100 ppm gibberellic acid solution, as indicated in Guijarro-Real, Rodríguez-Burruezo, Prohens, & Fita (2018). Microgreens and seedlings were obtained from accession DER006-2. Microgreens were grown in commercial Neuhaus Humin-substrat N3 substrate (Klasmann-Deilmann Gmbh, Geeste, Germany) under controlled conditions (16 h light /8 h dark; t=25ºC) for one week, until the expansion of cotyledons. Treated seeds for seedlings were directly sown in the field and grown for three weeks until the expansion of the first two true leaves. Finally, DER001-2 was used for obtaining the baby-leaf stage. Treated seeds were directly sown in the field and grown for six weeks until reaching the mature, pre-flowering stage. Sowings were performed gradually in order to ensure that the three products reached the adequate stage at the moment of the panel test. Plants in the field were grown under thermal blanket in order to protect them against the low temperatures of the winter.

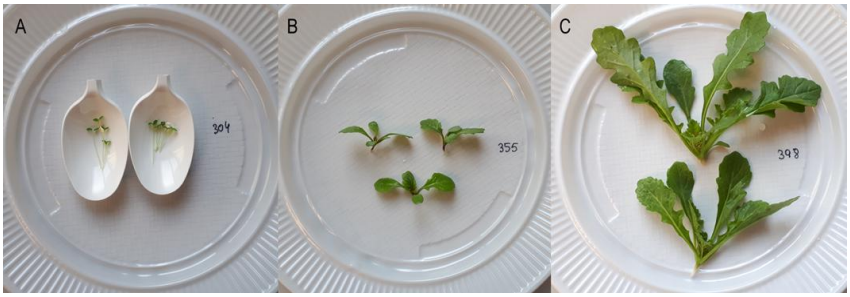

Fig. 1. Products developed for the current study. A) Microgreens, identified in the affective test as product P304. B) Seedlings, identified as product P355. C) Baby-leaves, identified as product P398. C. Guijarro-Real

Consumers acceptance test

A total of 98 untrained individuals were recruited at the UPV. As requirement, people should be older than 18 years old and sign a participation consent in which the purpose of the study and the specific use of the personal data provided were explained. The participation forms are kept at the UPV. The test was performed in one single session. For each panelist, the three products were presented in a randomised order. Products were designed for different food purposes and quantities presented in concordance. Thus, P304 was designed as a decorative element or topping, to be consumed in low amounts (i.e., 2–5 microgreens on the top of a dish), while P355 and P398 were considered to be a relevant component for dishes (the former as whole plant, the latter as cut leaves).

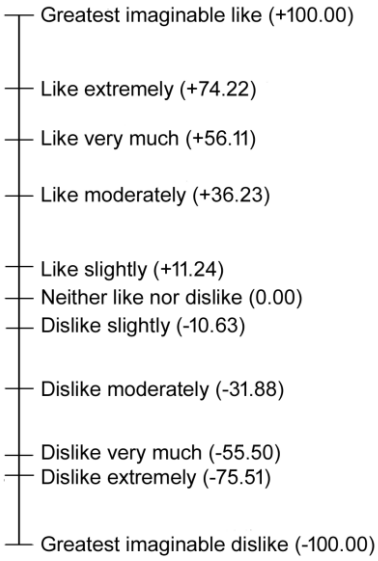

Fig. 2. Labelled Affective Magnitude (LAM) scale used in the present study. The scale includes nine points for acceptance appreciations, ranging from the "greatest imaginable dislike" to the "greatest imaginable like". Scores corresponding to each descriptor, and used for the statistical treatment of data, are provided in parentheses. Cardello & Schutz (2004).

Volunteers were asked to evaluate their preferences for the following attributes: visual appearance, texture, taste and pungency, by means of the Labelled Affective Magnitude (LAM) scale (Cardello & Schutz, 2004). The LAM scale included nine points expressing liking perceptions and ranging from the "greatest imaginable dislike" (value -100 in the scores scale) to the "greatest imaginable like" (value -100 in the scores scale) (Fig 2).

Individuals also declared their purchase intent for the products, using a 1-5 scale (1= absolutely no; 5= absolutely yes). Finally, volunteers were asked to fill a personal questionnaire (Bell *et al.*, 2017b) (Table S1). A question regarding the preference for rocket, Dijon's mustard (from now on, mustard) and wasabi paste (from now on, wasabi) was also included in the questionnaire (1= I like; 2= I don't like; 3= I've never tried).

Extraction and analysis of volatile organic compounds (VOCs)

For the analysis of VOCs, commercial Dijon's mustard (Reine de Dijon SAS, France) and wasabi paste (Kaneku Corp., Japan) were included as references. Extraction and analysis of volatile molecules was performed by means of the headspace–solid phase microextraction (HS-SPME) technique as described in Guijarro-Real, Rodríguez-Burruezo, Prohens, Raigón, & Fita (2019). Briefly, 1.5 g of fresh, finely chopped material was inserted in a sealed 20 ml vial and pre-incubated during 30 min at 40 ºC. VOCs were then adsorbed on a fibre (50/30 µm DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) during other 30 min at the same temperature, followed by a thermal desorption at 250 ºC for 30 s in the splitless mode. The fibre was conditioned for 1 h at 270 ºC prior to the analysis, and reconditioned at 250 ºC for 30 min after each sample to avoid cross-contaminations.

Compounds were analysed by gas chromatography and detected by mass spectrometry (GC–MS), using the 6890N Network GC System with autosampler coupled to a 5973 Inert Mass Selective Detector (Agilent Techonologies, Santa Clara, CA, USA) and equipped with a HP-5MS J&W silica capillary column (5% phenyl-95% methylpolysiloxane, 30 m length x 0.25 mm i.d., 0.25 µm thickness film; Agilent Technologies). A temperature ramp from 100 ºC to 250 ºC was used, increasing at a rate of 5 ºC min^{-1}, and then maintained for 10 min. The electron impact (EI) mode (70 eV

ionization energy; source temperature 225 ºC) was used for the detection by the mass spectrometer, performing the acquisition in the scanning mode (mass range *m/z* 35-350 amu).

Chromatograms were processed using the MSD ChemStation D.02.00.275 (Agilent Technologies). A tentative identification was obtained by comparison of the mass spectra with the NIST 2005 Mass Spectral Library, and also comparing the retention times and mass spectra with our customized library. A semi-quantification was obtained based on the integration of peak areas by the total ion current chromatogram (TIC) (Guijarro-Real *et al.*, 2019).

Statistical analysis

The influence of categorical data in the affective test was studied by means of the χ^2 test for heterogeneity (P = 0.05). Questionnaires were clustered for a deep analysis of results (Bell *et al.*, 2017b), and two different clustering options were established according to the panelist preference for A) Dijon's mustard and B) wasabi paste. Three groups were established in each clustering option, corresponding to the three possible answers provided (1 = like, 2 = do not like, 3 = never tried). Questionnaires that did not answer to these questions were not included in the analysis. Mean values and standard errors (SE) for visual appearance, texture, taste and pungency attributes, and for purchase intent, were calculated: 1) considering all the questionnaires as a whole (n = 98); and 2) after clustering. The purchase intent was transformed from the 5-point scale to a 3-point scale (1, 2 = low; 3 = medium; and 4, 5 = high). Data were analysed with the Kruskal-Wallis analysis, and signification of differences were calculated with the Bonferroni procedure (P = 0.05) (Dinnella, Torri, Caporale, & Monteleone, 2014). Correlations between attributes and purchase intent were studied and Spearman's rank correlation coefficient (ρ) were calculated. All statistical analyses were performed using the Statgraphics Centurion XVII software (Statpoint Technologies, Inc., Warrington, VA, USA) with the exception of the χ^2 test of heterogeneity, manually calculated on Excel sheets.

For the analysis of the volatile profiles, mean values and SE were calculated (n = 4). In addition, relative abundances were calculated as the

ratio of each individual GC area against the total GC area of compounds identified in each sample (expressed as percentage) (Guijarro-Real *et al.*, 2019). Absolute GC-peak area data were $\log_2$-transformed for normalization and subjected to a two-way factorial analysis of variance (ANOVA). Signification was evaluated by means of the Student-Newman-Keuls test ($P = 0.05$). An illustrative comparison of the profiles determined was performed by means of a Hierarchical Cluster Analysis with the distance measures based on Pearson correlations, using the ClustVis tool (Metsalu & Vilo, 2015).

Results

Profile of the untrained panellists

The personal profile of the ninety-eight volunteers recruited is given in Table S1. Five participants did not answer the personal questionnaire (5.1%), percentage that rose to 13.3% when the age was asked. Results showed a similar participation of male and female volunteers (46.9% and 48.0%, respectively), mainly European coming from Mediterranean regions (76.5%). 40.8% of participants were between 18–27 years old, while only 10.2% of the panelists were > 48 years old (Table S1).

Most of the participants appreciated rocket crops (82.7%), while preference for mustard and wasabi represented 46.9% and 37.8% of responses, respectively (Table S1). Surprisingly, the percentage of people that have never tried Dijon's mustard was higher than the percentage for wasabi paste (32.7% and 18.4%, respectively). Finally, the percentage of people that accepted or rejected wasabi paste was similar.

General perceptions of the three products tasted

Mean values for visual appearance, texture, taste, and pungency attributes were calculated. On average, positive scores were obtained for the four attributes evaluated in each product (Fig. 3A). Of all, pungency displayed the lowest values. On the other hand, the purchase intent displayed mean values > 3 for the three products, in a 1-5 scale (Fig. 3B).

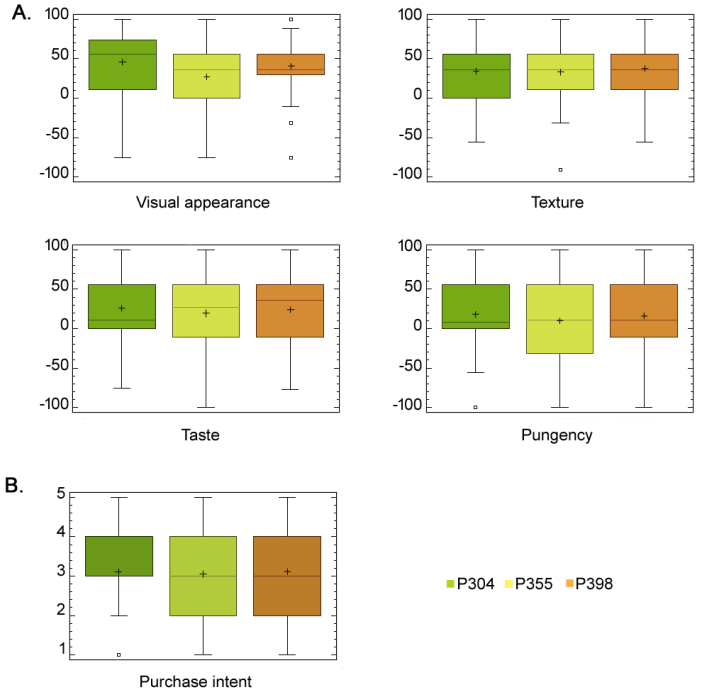

Fig. 3. Box plot chart for the attributes evaluated in P304, P355 and P398 (*n* = 98). A. Box plot chart for visual appearance, texture, taste, and pungency, with values ranging from -100 (‘‘the greatest imaginable dislike’’) to +100 (‘‘the greatest imaginable like’’). B. Box plot chart for the purchase intent, between 1 (absolutely no) and 5 (absolutely yes). The mean, median and outliers values are represented.

No significant differences were determined among the three products neither for the attributes nor for the purchase intent. As exception, the visual appearance of P355 was significantly less accepted than for the other products ($P < 0.05$) (Fig. 3A). However, all responses covered wide ranges, from -100 or the ‘‘greatest imaginable dislike’’ point to +100 or the ‘‘greatest imaginable like’’ point in the LAM scale, and from 1 (‘‘absolutely no’’) to 5 (‘‘absolutely yes’’) in the purchase intent. Thus, a deep analysis based on clustering the questionnaires was performed, as explained in the Material and Methods section. The most homogeneous groups were obtained after clustering by age and preference for mustard and wasabi (Table S1). In addition, the χ^2 test for heterogeneity revealed remarkable differences among

groups when participants were clustered according to mustard or wasabi preference (Table S2). Thus, data were deeply analysed according to these two clustering options.

Affective test by cohorts

Results for the two clustering options (A, preference for mustard; and B, preference for wasabi) are summarised in Table 1. In general, both visual appearance and texture had positive mean scores, with non significant differences. The three products had moderate to high acceptance scores according to the LAM scale (values close to +36.23 or higher). As exception, the visual appearance acceptance for P355 was lower than for P304 and P398 in group A3, as it was the case when the global means were compared (Table 1, Fig 3).

The taste and pungency of P304 was positively considered by all groups, with a slight-moderate acceptance according to the LAM scale; non significant differences were determined between clustering groups (Table 1). Some individuals indicated a lack of pungency in the microgreens as observations (data not shown). This attribute was positively perceived when panelists belonged to groups A3/B3, while it was negative for volunteers clustered in groups A1/B1. On the other hand, the preference for P355 and P398 taste/pungency depended significantly on the preference for mustard or wasabi (Table 1). Thus, both groups A1 and B1 (positive preference for mustard or wasabi, respectively) displayed the highest mean scores for taste and pungency, with values > +36.3 and corresponding to a moderate-high acceptance. Some panelists indicated that the pungency of P355 was highly intense, but it disappeared quickly and they liked it. By contrast, groups A2 and B2 (panelists that reject mustard or wasabi, respectively), and groups A3 and B3 (panelists who have never tried mustard or wasabi, respectively) had on average low scores, close to 0.0. Moreover, scores reached negative values for the pungency attribute, up to -33.6 (P355). Some panelistst in these groups indicated a rejection of pungent flavours and therefore a rejection of P355 and P398.

Table 1. Mean scores for the visual appearance, texture, taste and pungency attributes after clustering for Dijon's mustard preference (A) or wasabi paste preference (B). Groups have been established according to the acceptance/rejection expressed by the panelists for mustard and for wasabi: 1 = like; 2 = do not like; 3 = never tried. *N* indicates the number of panelists included in each group.

Group	*N*	Visual appearance			Texture			Taste			Pungency		
		P304	**P355**	**P398**	**P304**	**P355**	**P398**	**P304**	**P355**	**P398**	**P304**	**P355**	**P398**
Clustering option A: Preference for Dijon's mustard													
1	*46*	$45.8^{-/-}$	$31.5^{-/-}$	$46.8^{-/-}$	$31.5^{-/-}$	$38.8^{-/-}$	$44.7^{-/-}$	$18.2^{-/A}$	$39.9^{b/B}$	$37.7^{b/B}$	$10.7^{-/A}$	$37.2^{b/B}$	$41.2^{b/B}$
2	*15*	$40.2^{-/-}$	$19.4^{-/-}$	$28.9^{-/-}$	$28.4^{-/-}$	$18.4^{-/-}$	$27.4^{-/-}$	$29.5^{-/-}$	$-4.7^{a/-}$	$0.3^{a/-}$	$36.2^{-/B}$	$-33.6^{a/A}$	$-16.7^{a/A}$
3	*32*	$50.6^{-/B}$	$23.9^{-/A}$	$38.2^{-/AB}$	$41.6^{-/-}$	$29.7^{-/-}$	$34.0^{-/-}$	$33.9^{-/B}$	$2.2^{a/A}$	$16.0^{ab/AB}$	$15.3^{-/-}$	$-9.5^{a/-}$	$-6.1^{a/-}$
Clustering option B: Preference for Dijon's mustard													
1	*37*	$51.8^{-/-}$	$32.1^{-/-}$	$43.7^{-/-}$	$29.4^{-/-}$	$36.4^{-/-}$	$40.1^{-/-}$	$21.6^{-/-}$	$37.7^{b/-}$	$35.7^{-/-}$	$22.4^{-/-}$	$33.8^{b/-}$	$39.9^{b/-}$
2	*38*	$44.8^{-/-}$	$24.6^{-/-}$	$37.2^{-/-}$	$41.4^{-/-}$	$34.9^{-/-}$	$40.7^{-/-}$	$25.7^{-/-}$	$8.3^{a/-}$	$15.6^{-/-}$	$13.5^{-/-}$	$-11.2^{a/-}$	$2.7^{a/-}$
3	*18*	$40.0^{-/-}$	$21.1^{-/-}$	$43.1^{-/-}$	$30.5^{-/-}$	$18.6^{-/-}$	$29.3^{-/-}$	$33.0^{-/-}$	$7.2^{a/-}$	$18.7^{-/-}$	$11.6^{-/-}$	$4.4^{ab/-}$	$-7.2^{a/-}$

Different letters within columns for each clustering option (lower case), or within rows for each attribute (capital letters) indicate significant differences according to the Bonferroni procedure ($P = 0.05$). [a]*n* indicates the number of individuals in each group

Table 2. Mean scores for each attribute after grouping questionnaires by the expressed purchase intent (low, medium or high). Spearman's rank coefficient of correlation (ρ) between each attribute and purchase intent is also indicated.

	Purchase intent			
	Low	**Medium**	**High**	ρ
Visual appearance				
P304	35.3^{a}	37.0^{a}	64.2^{b}	0.335**
P355	16.7^{a}	27.2^{a}	35.6^{a}	0.193***
P398	25.1^{a}	38.8^{a}	54.5^{b}	0.367***
Texture				
P304	18.8^{a}	27.3^{a}	51.2^{b}	0.341***
P355	14.9^{a}	33.8^{ab}	47.5^{b}	0.332***
P398	24.9^{a}	35.3^{a}	49.8^{b}	0.336**
Taste				
P304	-4.2^{a}	19.9^{b}	52.9^{c}	0.651***
P355	-22.8^{a}	20.9^{b}	56.1^{c}	0.685***
P398	-22.2^{a}	27.4^{b}	55.0^{c}	0.666***
Pungency				
P304	-7.2^{a}	11.4^{a}	42.1^{b}	0.442***
P355	-37.5^{a}	8.3^{b}	53.4^{c}	0.638***
P398	-25.6^{a}	21.9^{b}	40.1^{b}	0.513***

Different letters within rows indicate significant differences according to the Bonferroni procedure (P = 0.05). ** and *** indicate significance at P = 0.01 and 0.001, respectively

A second analysis compared the preferences for P304, P355 and P398 depending on the cluster (Table 1). In this sense, the mean scores for taste and pungency of P355 and P398 were high and similar (> +36.3) in group A1 (preference for mustard), while the value for P304 was significantly lower (+11.24). On the contrary, the group A2 (rejection to mustard) provided a high mean score to P304 in terms of pungency (+36.2), but negative values for P355 and P398 (< -16.7). No significant effects were determined for the taste attribute in this group. Finally, group A3 (never tried mustard) found more pleasant the taste of P304 (+33.9) than the taste of

P355 and P398 (< +16.0). Interestingly, there were non significant differences among products when panelists were clustered by the preference for wasabi (Table 1).

Relationship between attributes and purchase intent

The purchase intent was positively correlated to all traits at a high level (Table 2). The highest Sperman's rank ρ were determined for the correlation with taste and pungency, ranging between 0.651 (P304) and 0.685 (P355) for taste, and between 0.442 (P304) and 0.638 (P355) for pungency. In addition, the correlation between taste and pungency was also studied, due to the importance of both attributes in the purchase intent. The Sperman's rank ρ were highly significant, with values of 0.377, 0.639 and 0.519 for P304, P355 and P398, respectively (data not shown).

Volatile constituents

The volatile profiles of P304, P355 and P398 were analysed by GC-MS. Important differences were found among the three products (Table 3). Nineteen compounds were identified in the volatile profile of P304. It was mainly composed of ITCs, with ten compounds accounting for 98.2% of the total relative abundance. Allyl ITC was the most representative compound, with a relative abundance of 95.7%. The volatile profile of P355 was represented mainly by isothiocyanates followed by esters (78.2% and 17.4%, respectively), with seven compounds identified in each chemical group. As in P304, allyl ITC was the main ITC identified, but it was lower in terms of absolute area and relative abundance (Table 3). Other compounds with relative abundance greater than 3.0% were *cis*-3-hexenyl butyrate (6.8%), with similar relative abundance to P398 but higher GC-peak area; *cis*-3-hexenyl isovalerate (5.4%), similar to P398 in GC-peak area; *cis*-3-hexen-1-ol (4.4%), similar to P304 in area but with greater relative abundance (4.4%); and *cis*-3-hexenyl acetate, with higher area than P398 but lower relative abundance (3.6%). Finally, P398 was very poor in volatiles, in qualitative and quantitative terms. The total GC-peak area was 0.1–fold times the area of P304 and P355. This material was rich in esters (94.1% of total relative abundance), with c*is*-3-hexenyl isovalerate (62.5%) and *cis*-3-

hexenyl valerate (13.3%) as the most representatives. Allyl ITC, the unique ITC detected, presented a relative abundance of 4.9%.

Table 3. Average GC peak areas (x10^6; ±SE) and relative abundance (%, between brackets) of individual VOCs identified in P304, P355 and P398 (n=4).

Compound	RT	P304	P355	P398
Alcohols				
cis-3-hexen-1-ol	6.15	12.6±1.2^b (1.1)	27.0±5.8^b (4.4)	0.5±0.2^a (0.7)
Aldehydes				
benzeneacetaldehyde	11.43	0.2±0.0^a (0.0)	-	-
decanal	16.13	0.15±0.0^a (0.0)	-	0.2±0.1^a (0.2)
Esters				
cis-3-hexenyl acetate	10.31	-	22.5±2.6^b (3.6)	6.4±2.2^a (10.3)
cis-3-hexenyl butyrate	15.59	5.6±2.1^a (0.5)	42.1±18.3^b (6.8)	4.4±3.3^a (7.0)
hexyl butyrate	15.74	0.1±0.0^a (0.0)	0.7±0.4^a (0.1)	-
cis-3-hexenyl propionate	13.06	-	7.5±1.1^a (1.2)	0.5±0.4^a (0.7)
cis-3-hexenyl isovalerate	16.89	0.6±0.1^a (0.1)	33.2±7.7^a (5.4)	39.0±35.2^a (62.5)
cis-3-hexenyl valerate	16.98	-	8.3±1.5^a (1.3)	11.0±2.7^a (13.3)
3-hexenyl benzoate	25.59	-	0.4±0.1^a (0.1)	0.2±0.1^a (0.4)
Ketones				
β-ionone	23.59	1.3±0.3^a (0.1)	tr	-
Isothiocyanates				
allyl isothiocyanate	6.70	1140.7±76.2^c (95.7)	462.7±47.9^b (75.0)	3.0±0.7^a (4.9)

iosbutyl isothiocyanate	8.78	0.7±0.1[a] (0.1)	-	-
3-butenyl isothiocyanate	9.62	11.0±1.9[b] (0.9)	4.3±0.7[a] (0.7)	-
3-methylbutyl isothiocyanate	11.85	6.6±0.8[a] (0.6)	5.3±0.9[a] (0.9)	-
pentyl isothiocyanate	13.04	2.3±0.1[a] (0.2)	tr	-
4-methylpentyl isothiocyanate	14.92	0.1±0.0[a] (0.0)	0.0±0.0[a] (0.0)	-
hexyl isothiocyanate	15.98	0.6±0.1[a] (0.0)	-	-
3-methylthipropyl isothiocyanate	19.12	1.0±0.4[a] (0.1)	0.5±0.3[a] (0.1)	-
benzyl isothiocyanate	20.51	3.1±0.9[a] (0.3)	0.1±0.0[a] (0.0)	-
phenylethyl isothiocyanate	23.11	4.3±1.4[a] (0.4)	2.0±1.2[a] (0.3)	-
Monoterpenes				
limonene	10.96	-	0.5±0.1[a] (0.1)	-
Nitriles				
benzenepropanenitrile	17.12	0.2±0.1[a] (0.0)	0.3±0.2[a] (0.0)	-
Sulphur compounds				
diallyl disulphide	12.47	0.2±0.1[a] (0.0)	tr	tr
Total isothiocyanates		1170.5±81.4[c] (98.2)	482.4±51.6[b] (78.2)	3.5±1.1[a] (4.9)
Total esters		6.4±2.3[a] (0.5)	107.1±19.6[b] (17.4)	58.3±45.6[ab] (94.2)
Total		1191.5±78.3[b]	617.3±76.7[b]	62.3±46.9[a]

Different letters within rows indicate significant differences for log_2-transformed data according to the Student-Newman-Keuls test (P=0.05). RT: retention time in the conditions of analysis. -: not detected. tr: traces

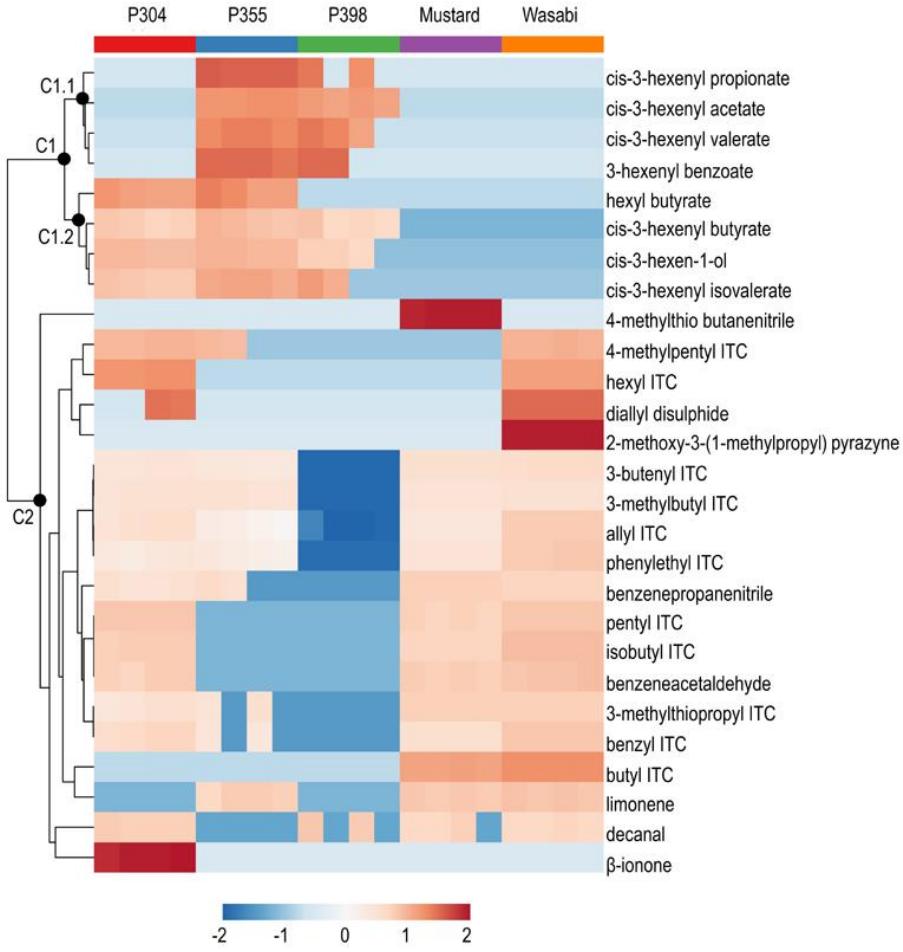

Fig. 4. Hierarchical cluster analysis of the identified VOCs considering the three samples of wall rocket (P304, P355 and P398), Dijon's mustard and wasabi paste. The analysis includes the four replicates for each material.

Differences between the three products were also reflected in the hierarchical cluster analysis (Fig. 4). In this analysis, mustard and wasabi materials were included. Compounds were grouped in two main clusters, C1 and C2. Cluster C1 included esters and alcohols that were only detected in wall rocket materials. Within this group, subcluster C1.1 included four esters that were only detected in P355 and some replicates of P398, but they were not detected in P304. On the other hand, cluster C2 was mainly determined

by GSLs breakdown products (Fig. 4). This cluster related the volatile profile of mustard and wasabi with P304 in a high degree, and to a lesser extent, with P355. Most of the compounds included in this group were determined for different materials. However, three compounds were determined as unique for certain materials. Thus, 4-methyltio butanenitrile was specific for mustard, 2-methoxy-3-(1-methylpropyl)-pyrazine for wasabi, and β-ionone for P304. Butyl ITC was the unique compound detected in both wasabi and mustard but not identified in any of the wall rocket products. Allyl ITC was also the main compound in the materials of reference, with wasabi paste displaying the greatest levels (data not shown). Finally, phenylethyl ITC was also of great relevance in the wasabi (31.3% of total abundance), displaying a GC-peak area more than 200–fold the value of P304 and almost 500–fold the value in P355.

Discussion

The most representative profile of volunteers corresponded to young to middle age Mediterranean Europeans, with no gender bias. Thus, the study provides relevant information for the potential commercialization of wall rocket in markets of Mediterranean countries. Nevertheless, new studies may be needed for other markets in order to increase the representation of potential consumers in such regions. The questionnaire revealed that most of the participants have a good acceptance for rocket crops, vegetables that are taxonomically and scientifically related to wall rocket (Bennett, Rosa, Mellon & Kroon, 2006; D'Antuono *et al.*, 2009). However, both the hedonic test and the analysis of volatile constituents revealed that wall rocket is more similar in taste and aroma to wasabi and mustard.

The results of the hedonic test differed from the work of D'Antuono *et al.* (2009), who classified the species as a vegetable with unpleasant taste. A possible explanation may be, in part, the presentation of the species. While the current work evaluated the acceptance of wall rocket as new vegetable, the study of D'Antuono *et al.* (2009) established a comparison with rocket crops and other related germplasm. However, both species have different profile of GSLs (Di Gioia *et al.*, 2018), so expecting similar taste and aromas may have negative affected the results.

Our results were analysed by clustering panellists according to the preference for mustard and wasabi. Clustering can be useful for the analysis of hedonic tests with broad ranges of responses, and it has been previously used with success (Bell *et al.*, 2017b; Dinnella *et al.*, 2014). Out of the four attributes analysed, the visual appearance is one of the most important traits for vegetables and it is usually related to freshness (Dinnella *et al.*, 2014). Moreover, visual appearance can be considered as the most important for increasing the possibility of tasting a product for the first time. The lower score of P355 could correspond to the low resemblance to common leafy vegetables. Bell *et al.* (2017b) suggested that acceptance of new vegetables can increase when they look similar to other ones that are currently commercially available. Thereby P355 would be probably the less desirable for a commercial purpose.

Microgreens have gained in popularity over the past decades, and are broadly used in restaurants as decorating components. Within the *Brassicaceae* family, species like salad rocket (*E. sativa*), radish (*Raphanus sativus* L.) or mustard (*Brassica juncea* (L.) Czern.) are commercially grown as microgreens (Xiao, Lester, Luo, & Wang, 2012), and our results suggest that wall rocket can become another commercial microgreen. The fact that those panelists who reject mustard and wasabi accepted product P304 indicates that they probably did not find an association between them, despite the high level of ITCs. A possible explanation to the discrepancy between the hedonic test and the analysis of VOCs can be related to the quantity of material tested in each case. On the other hand, the taste and pungency of products P355 and P398 were responsible for the rejection and the low purchase intent showed by a cohort of panelists. Taste and pungency in *Brassicaceae* are two attributes with positive correlation (D'Antuono *et al.*, 2009). They are highly related to the presence of GSLs (Bell *et al.*, 2018), and have a great influence in the acceptance or rejection in *Brassicaceae* crops (Engel, Baty, Le Corre, Souchon & Martin, 2002; Wieczorek, Walczak, Skrzypczak-Zielińska & Jelén, 2017; Shen, Kennedy & Methven, 2016). According to the combination of taste, pungency and visual appearance traits, our results suggest that stages of wall rocket such as baby-leaves may be considered as market opportunities addressed to consumers with preference for ‘‘mustard-like’’ flavours.

Regarding the volatile profile, wall rocket, mustard and wasabi shared allyl ITC as the main ITC. Thus, this compound would be main responsible of the relationship between the preference for wasabi, mustard and wall rocket. Allyl ITC has been previously described in the materials of reference (Bell *et al.*, 2018), as well as in other *Brassicaceae* crops such as horseradish (*Armoracia rusticana*), kale, or Brussels sprouts (*Brassica oleracea*) (Agneta, Lelario, De Maria, Möllers, Bufo & Rivelli, 2014; Ishida, Hara, Fukino, Kakizaki, & Morimitsu, 2014). However, our analysis showed other important aspects of wall rocket that have not been previously considered, to the best of our knowledge. In particular, wall rocket has been described as rich in sinigrin (D'Antuono *et al.* 2008; D'Antuono *et al.* 2009; Di Gioia *et al.* 2018), explaining thereby the high abundance of its hydrolysed product, allyl ITC (Cavaiuolo & Ferrante, 2014). However, other ITCs were determined in P304 and P355, suggesting that other GSLs can be found in wall rocket. For instance, 3-butenyl ITC is the hydrolysis product of gluconapin; 3-methylthiopropyl ITC, of iberverin; benzyl ITC, of glucotrapeolin; and phenylethyl ITC, of gluconasturtiin (Al-Gendy, Nematallah, Zaghloul, & Ayoub, 2016; Bell *et al.*, 2018; Sansom, Jones, Joyce, Smallfield, Perry & Van Klink 2015).

Another compound of great interest was diallyl disulphide, which represented < 0.1% of total abundance in P304 and was detected as traces in P355 and P398. Diallyl disulphide has been described as one of the main sulphur compounds of fresh garlic (Molina-Calle, Priego-Capote, & Luque de Castro, 2017), providing pungent and intensive garlic notes (Ma *et al.*, 2011). The compound has a very low odour threshold (Nagata, 2003), which means that it can be detected at low concentrations. Thus, its presence, even as traces, may explain the "garlic notes" described by some panelists and identified also by D'Antuono *et al.* (2009).

Finally, wall rocket materials had also high relative abundance in esters, especially P398. Those esters derive from the enzymatic degradation of polyunsaturated fatty acids. They are part of the called "green leafy volatiles" (including aldehydes, alcohols and esters) which are responsible of the green notes detected in many vegetables (Raffo, Masci, Moneta, Nicoli, Sánchez del Pulgar & Paoletti, 2018; Ruther, 2000). However, the volatile

profile of P398 was unexpected due to the lack of ITCs and high abundance in esters. Plants of P398 were exposed to colder growing conditions, which could affect the biosynthesis and accumulation of GSLs. Thus, new experiments in different growing conditions and locations should be repeated in order to better understand the variations in taste that wall rocket can suffer, which is primordial for crop quality homogenization.

Conclusions

To our knowledge, this is the first report evaluating the volatile profile of wall rocket. Microgreens and seedlings were rich in ITCs, especially the former. Baby-leaves displayed very low levels, while the relative abundance of esters increased to the highest in this stage. As in wasabi and mustard, allyl ITC was the main ITC identified in wall rocket, presumably influencing their flavour similarities. Moreover, other ITCs were detected, suggesting that other GSLs apart from sinigrin are synthesized in the species.

This is also the first report in developing a hedonic test for wall rocket using a large quantity of panellists. Results indicated that acceptance of the products was mainly related to the acceptance of their taste and pungency. Microgreens were well accepted by panellists although this stage displayed the highest levels of allyl ITC, probably due to the low quantity tasted. Seedlings and baby-leaves were also accepted by consumers that enjoy these characteristic pungent flavours. Thus, the study suggest that both microgreens and baby-leaves of wall rocket could be considered as good market opportunities, the former for the general public, and the latter for a more specific cohort. Seedlings would be the less interesting stage due to its lower visual appearance.

Acknowledgements

C. Guijarro-Real thanks the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for its financial support by means of a predoctoral FPU grant (FPU14-06798). Authors also thank Dr. A.M. Adalid-Martínez and Dr. C.K. Pires for supporting during the tasting session, and Ms. E. Moreno for her support in the GC-MS analysis.

Conflict of interest

Authors declare no conflicts of interest.

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Table S1. Personal questionnaire. Data are expressed as percentage against the total of participants (n = 98).

Individuals' profile (%)					
Gender		**Age**		**Origin**	
Female	48.0	18-27	40.8	Mediterranean-European	76.5
Male	46.9	28-37	14.3	South American	10.2
NA[†]	5.1	38-47	21.4	Asiatic	4.1
		48-57	7.1	Nordic/Central European	4.1
		58-76	3.1	NA	5.1
		NA	13.3		
Do you like...? (%)					
Rocket crops		**Dijon's mustard**		**Wasabi paste**	
Yes	82.7	Yes	46.9	Yes	37.8
No	9.2	No	15.3	No	38.8
Never tried	3.1	Never tried	32.7	Never tried	18.4
NA	5.1	NA	5.1	NA	5.1

[†]NA= not answered

Table S2. *P*-values of the χ^2 test for heterogeneity between the personal questionnaire and the affective evaluation of attributes and purchase intent. Those significant ($P < 0.05$) are marked in bold.

	Visual appearance			**Texture**			**Taste**			**Pungency**		
Personal questions	**304**	**355**	**398**	**304**	**355**	**398**	**304**	**355**	**398**	**304**	**355**	**398**
Origin	0.9787	0.5108	0.4481	0.2306	0.3247	0.6670	0.3768	0.2173	0.1959	**0.0175**	0.8087	**0.0468**
Age	0.4872	0.5060	0.6615	**0.0268**	0.3344	0.1633	**0.0003**	0.2036	0.8677	0.6075	0.5993	0.4448
Gender	0.4659	0.4954	0.1957	0.6586	0.4758	0.9968	0.1102	0.6572	0.5913	0.3787	**0.0365**	0.2692
Rocket liking	0.7423	0.1993	0.0668	0.5106	**0.0001**	0.5411	0.6429	**0.0213**	0.1783	0.6086	0.1992	0.1451
Dijon's mustard liking	0.5000	0.8379	0.2144	0.1824	0.3110	0.3390	0.4133	**0.0220**	0.0974	0.3137	**0.0004**	**0.0003**
Wasabi paste liking	0.5790	0.8411	0.8535	0.6954	0.6634	0.8477	0.2027	**0.0409**	0.3716	0.0946	0.0521	**0.0140**

	Purchase intent		
Personal questions	**304**	**355**	**398**
Origin	0.3857	0.8688	0.9462
Age	0.5436	0.2138	0.7628
Gender	**0.0347**	0.2536	0.3992
Rocket liking	0.1369	**0.0236**	**0.0463**
Dijon's mustard liking	0.3483	**0.0019**	**0.0016**
Wasabi paste liking	0.6159	0.1332	0.3513

Discusión general

El ser humano ha vivido siempre en muy estrecha relación con la naturaleza. Pese al establecimiento de la agricultura, una parte de la alimentación se ha basado tradicionalmente en la recolección de frutas y hortalizas silvestres. El uso de WEPs en la alimentación ha permitido, por ejemplo, hacer frente a la carencia de alimentos en épocas de guerra, desastres naturales, situaciones de pobreza o economías de subsistencia (Tabuti, 2007; Łuczaj *et al.*, 2012; Carvalho y Barata, 2017; Geraci *et al.*, 2018). Las plantas silvestres han sido, además, utilizadas durante milenios en medicina (Abbasi *et al.*, 2013).

Si bien el consumo de WEPs se ha ido abandonando durante las últimas generaciones, persiste todavía un conocimiento etnobotánico tradicional respecto al aprovechamiento de algunas de ellas. Éste se conserva generalmente en las personas de edades más avanzadas (Schunko *et al.*, 2015); se están haciendo además esfuerzos por recuperarlo en publicaciones etnobotánicas y científicas, evitando así que se pierda (e.g., Lentini y Venza, 2007; Couplan, 2015; Geraci *et al.*, 2018). El conocimiento etnobotánico incluye aspectos como la identificación de la especie y órganos comestibles, momento óptimo de recolección e incluso tratamientos que debieran aplicarse de ser necesarios previo al consumo (e.g., tratamiento térmico). Incluye además distintos usos y propiedades medicinales que se atribuyen a las WEPs, como recogen por ejemplo Guarrera y Savo (2013) en su revisión.

Las sociedades modernas son cada vez más conscientes de la importancia de una dieta de calidad en el mantenimiento de la salud (Olayanju, 2018). Así, en las últimas décadas ha aumentado la demanda de alimentos funcionales que ejerzan un beneficio complementario sobre la salud, además del estrictamente nutricional (Nutrition Society, 1999). Por otro lado, hay corrientes de consumidores que buscan "volver a los sabores de antes", o simplemente enriquecer la alimentación con una mayor experiencia gastronómica. La interacción de estos dos factores supone una oportunidad de mercado para el desarrollo de nuevos cultivos a partir de WEPs, apoyándose con este fin en el conocimiento etnobotánico tradicional.

En este contexto, se desarrolla la presente Tesis como trabajo de evaluación y pre-domesticación de especies que pudieran resultar de interés

por su calidad funcional, pero además también por su calidad organoléptica. En concreto, el trabajo se ha centrado en la evaluación de la berraza (*A. nodiflorum*) y la rabaniza (*D. erucoides* subsp. *erucoides*). Ambas son hortalizas silvestres de consumo tradicional popular entre las culturas mediterráneas (Parada *et al.*, 2011; Couplan, 2015; Guarrera y Savo, 2016; Licata *et al.*, 2016; Pinela *et al.*, 2017; Geraci *et al.*, 2018), y que presentan además un alto potencial funcional (Guarrera y Savo, 2013).

Entre los diferentes trabajos, se incluye en la presente Tesis el primero que describe el perfil en agliconas fenólicas en la berraza. Si bien los trabajos de adaptación a cultivo en suelo no han sido prometedores, los resultados obtenidos aumentan el conocimiento en esta especie. Ofrecen además un punto de partida para futuros programas de domesticación y selección de la berraza. Por otra parte, en esta Tesis se han concentrado grandes esfuerzos encaminados a la adaptación de la rabaniza como cultivo en condiciones mediterráneas. Es además uno de los pocos trabajos en los que se ha valorado el potencial comercial de esta última hortaliza, en concreto mediante un test afectivo con aproximadamente cien consumidores potenciales. También en esta Tesis se ha llevado a cabo el primer trabajo, hasta donde sabemos, que analiza el perfil volátil de esta hortaliza, y que ha permitido conocer la co-ocurrencia de diversos glucosinolatos no estudiados hasta la fecha en la rabaniza.

1. La berraza como hortaliza silvestre y cultivo potencial

1.1. Importancia de los compuestos fenólicos en el valor funcional de la berraza

La berraza no es una especie extensamente estudiada desde un punto de vista alimentario, a pesar de haberse descrito su consumo en diversas regiones mediterráneas (Lentini y Venza, 2007; Parada *et al.*, 2011; Pinela *et al.*, 2017; Geraci *et al.*, 2018). De los distintos compuestos nutricionales y bioactivos estudiados previamente, destaca su contenido en compuestos fenólicos y flavonoides, así como su actividad reductora de radicales libres

(Morales *et al.*, 2012). El primer trabajo presentado en esta Tesis se centra pues en el estudio de este potencial funcional.

Se ha determinado en la berraza una capacidad antioxidante superior a los materiales de referencia utilizados para su comparación, apio y perejil. Esta superioridad ha resultado especialmente significativa en el caso de la actividad reductora de radicales libres (método de reducción del reactivo DPPH·). Los resultados obtenidos confirman así la clasificación de Morales *et al.* (2012) como hortaliza de alta capacidad funcional en estos términos. Además, la alta correlación establecida entre actividad reductora y contenido en fenoles totales (método de reducción del reactivo Folin-Ciocalteu) indica un gran peso de dichos compuestos sobre la capacidad reductora de la especie.

Hasta la fecha no se había descrito, sin embargo, el perfil fenólico de la berraza. Sus hojas acumulan principalmente flavonoides, y en menor cantidad relativa, ácidos hidroxicinámicos. Los flavonoides se acumulan, de hecho, en altas concentraciones las hojas, en concreto en su epidermis (Cartea *et al.*, 2011), tal y como sugieren los resultados obtenidos. La berraza presenta quercetina como principal flavonoide, a diferencia del perejil y el apio. Por su estructura, este compuesto tiene una capacidad antioxidante mayor que otros flavonoides (Yildiz *et al.*, 2008), hecho que explicaría la alta actividad reductora de la berraza. Los resultados sugieren pues que el consumo de brotes tiernos de berraza como sustituto parcial del apio y/o perejil puede aumentar la calidad funcional de la dieta. No obstante, es importante indicar que un consumo excesivo de esta hortaliza es desaconsejable dado su alto contenido en ácido oxálico (Morales, 2011).

1.2. Perfil volátil de la berraza como hortaliza

Más allá de la importancia funcional de la berraza, esta especie silvestre se aprecia sobre todo por su aroma y sabor. El factor socio-cultural resulta determinante en las sociedades modernas para entender el consumo de frutas y hortalizas silvestres, altamente influenciado precisamente por el aroma y sabor que aportan (Serrasolses *et al.*, 2016; Thakur *et al.*, 2017). En la presente Tesis se ha analizado por primera vez el perfil volátil de la berraza en fresco. Los trabajos realizados hasta la fecha, por el contrario, se

centraban en el estudio de su aceite esencial con fines medicinales y/o ecológicos (Menghini *et al.*, 2010; Maxia *et al.*, 2012; Benelli *et al.*, 2017; Heshmati Afshar *et al.*, 2017; Koutsaviti *et al.*, 2017).

En el perfil volátil de esta hortaliza silvestre destaca la abundancia (en número de compuestos y porcentaje de área total) de monoterpenos y sesquiterpenos hidrocarburos. La mezcla de estos compuestos daría lugar a la apreciación de notas herbáceas, dulces, e incluso cítricas. También es relevante en su perfil la fracción fenilpropanoide, principalmente determinada por dilapiol y/o su precursor miristicina. Distintos terpenoides, así como el dilapiol, aportan notas ligeramente picantes, y por lo tanto su presencia justificaría el sabor picante descrito por Guarrera y Savo (2016). Por otro lado, su perfil volátil tiene ciertas similitudes con los también determinados en la presente Tesis para apio, zanahoria y perejil. Estos resultados apoyan la apreciación previamente descrita para la berraza como "aroma que recuerda a apio, zanahoria o una mezcla de ambos" (Nebel *et al.*, 2006; Heshmati Afshar *et al.*, 2017; Wild Food UK, 2018), así como la semejanza a perejil detectada por los autores de este trabajo (Guijarro-Real *et al.*, 2019).

Sin embargo, la presencia, cantidades absolutas y relativas de compuestos volátiles específicos permite diferenciar en su conjunto el perfil aromático de las distintas especies, como se ha observado en el análisis de componentes principales. Estas diferencias cualitativas y cuantitativas serían aparentemente responsables del flavor específico de la berraza. Más aún, esta especie ha mostrado un perfil cuantitativamente superior al resto de materiales. Dicha superioridad en su conjunto daría lugar a un aroma más intenso, que resulta al parecer apreciado por las personas que consumen esta WEP.

1.3. Perspectivas de mejora y manejo como nuevo cultivo

En la presente Tesis se ha determinado la existencia de diversidad, aunque moderada, para la calidad antioxidante de la berraza. La existencia de variabilidad puede ser explotada en programas de mejora (Herraiz *et al.*, 2016), por ejemplo para seleccionar materiales de mayor riqueza funcional o

con un aroma y sabor destacado. En el caso de la actividad antioxidante, la variabilidad detectada en el trabajo tiene una aparente relación con el origen geográfico de los materiales. Este hecho podría deberse a una relación genética entre materiales próximos, pero también a la influencia de factores ambientales concretos. Sin embargo, los materiales no han podido ser probados en condiciones controladas de cultivo, de modo que no se han podido determinar las razones detrás de esta relación. Así pues, queda una incógnita que podría ser punto de partida para nuevas investigaciones. En este sentido, resultaría de gran interés incluir materiales de orígenes geográficos más alejados, aumentando así la posible variabilidad prospectada.

Se ha determinado asimismo cierta variabilidad en el perfil aromático de la especie. En concreto, una de las poblaciones evaluadas destaca por unos niveles relativos de limoneno superiores al resto. De confirmarse estos valores relativos en condiciones de cultivo, se podrían establecer programas dirigidos al desarrollo de diversos cultivares de aroma y sabor con matices diversos. En ellos se debería considerar, además, el mantenimiento del alto valor cuantitativo del perfil volátil, que como se ha dicho resulta superior que en las especies cultivadas.

En resumen, nuestros resultados apuntan a una posibilidad de mejora para explotar el potencial funcional y aromático de la berraza. Sin embargo, tal y como se ha mencionado anteriormente, no ha sido posible en la presente Tesis cultivar materiales con éxito. El cultivo común sobre sustrato comercial resultó en un producto de calidad no aceptable –desarrollo de hojas coriáceas y poco turgentes–. Como alternativa, un cultivo en sistema hidropónico podría resultar en una mayor calidad, dada su similitud con las condiciones naturales de crecimiento de la berraza. Comparado con sistemas convencionales en sustrato, el cultivo hidropónico aumenta el contenido en agua de hojas (Fontana y Nicola, 2009; Kovacic *et al.*, 2015), reduciendo así el aspecto coriáceo de las mismas. Finalmente, su aplicación podría utilizarse para tratar de reducir los niveles de ácido oxálico. Sin embargo, el sistema no pudo ser implantado en las instalaciones donde se ha desarrollado la Tesis y por lo tanto no se continuaron los trabajos de adaptación a cultivo de la berraza. Por lo tanto, la Tesis deja una vía abierta para continuar con el

estudio de esta especie y su conversión a cultivo con explotación de su alto valor añadido.

2. La rabaniza como potencial cultivo de alto valor añadido

La rabaniza es una especie arvense de bajas condiciones ecológicas. Tiene así una alta capacidad para desarrollarse en multitud de sustratos y un amplio rango de regímenes hídricos, pudiendo crecer en condiciones tanto de secano como de regadío (Martínez-Laborde, 1990). Por lo tanto, y a diferencia de como ocurría en la berraza, las tareas de prospección llevadas a cabo durante esta Tesis se dirigieron a la recolección de semilla como material reproductivo, para su posterior crecimiento en nuestras instalaciones experimentales.

Así pues, cada trabajo experimental realizado en esta Tesis para la rabaniza parte de material desarrollado bajo condiciones controladas. Ello permite una mejor comparación entre las distintas poblaciones, al reducirse el efecto ambiental. Ha permitido, además, analizar diversos aspectos morfológicos y agronómicos que no podrían haberse evaluado sobre poblaciones silvestres.

2.1. Caracterización morfológica de la rabaniza

La rúcula (*E. sativa*) incluye materiales de alta diversidad morfológica, especialmente para caracteres de hoja (Egea-Gilabert *et al.*, 2009; Bell *et al.*, 2017a; Bell *et al.*, 2017b). Diferencias en la morfología de hoja pueden resultar de gran interés desde un punto de vista comercial. Es más, Bell *et al.* (2017b) concluyen que la morfología puede resultar determinante para su aceptación por los consumidores. Por el contrario, la diversidad que presenta la rabaniza como especie no ha sido estudiada. En este sentido, caracterizar y conocer la variabilidad morfológica de la rabaniza se convierte en un punto clave para el desarrollo de variedades comerciales.

Esta Tesis se ha centrado principalmente en evaluar caracteres morfológicos de hoja como parte comestible de la rabaniza. No obstante, se han incluido además una selección de caracteres descriptivos de planta que consideramos pueden tener un efecto importante para el productor. Al no existir descriptores normalizados para *D. erucoides*, se utilizó el trabajo publicado para la rúcula (IPGRI, 1999). Curiosamente, estos descriptores están adaptados a *Eruca* spp. y no a *Diplotaxis* spp., si bien el cultivo más popularizado en España y otros países europeos corresponde con *D. tenuifolia*. La caracterización de las entradas de rabaniza siguiendo los descriptores IPGRI para rúcula nos ha llevado a la necesidad de adaptar ciertos caracteres, dadas las diferencias entre especies (por ejemplo, en cuanto al patrón y desarrollo del lobulado).

Se ha determinado entre los materiales cierta variabilidad morfológica, especialmente entre caracteres de tamaño de hoja, pero también en algunos casos para el hábito de crecimiento. Estas diferencias suponen inicialmente una oportunidad para la selección dentro de programas de mejora. Sin embargo, tanto las posibilidades de mejora como el número potencial máximo de variedades comerciales van a verse limitados por la limitada variabilidad registrada. Una situación diferente se da por ejemplo en *E. sativa*, con un grado mayor de diversidad (Taranto *et al.*, 2016; Bell *et al.*, 2017b). Asimismo, la baja a moderada heredabilidad determinada es un factor más a tener en cuenta para asegurar el éxito en la selección de materiales.

2.2. Valor funcional de la rabaniza

Al igual que ocurría con la berraza, existe poca información en referencia al valor nutricional de la rabaniza. De acuerdo a los trabajos realizados previamente y considerando además su relación taxonómica y agronómica con la rúcula (Bianco *et al.*, 1998; Salvatore *et al.*, 2005; Bennett *et al.*, 2006; D'Antuono *et al.*, 2008, 2009; Disciglio *et al.*, 2017; Di Gioia *et al.*, 2018), se puede definir esta hortaliza como rica en vitamina C, compuestos fenólicos y glucosinolatos. Por otro lado, estos estudios subrayan que es un potencial bioacumulador de nitratos.

En esta Tesis se confirma que la rabaniza acumula cantidades destacadas de vitamina C y compuestos fenólicos. La vitamina C es uno de los compuestos más destacados de la rúcula (Spadafora *et al.*, 2016; Tripodi *et al.*, 2017); tanto es así que su valor va explícitamente reflejado en el etiquetado de diversos productos comerciales. En este sentido, y considerando la comparación entre especies realizada a lo largo de esta Tesis, la rabaniza se puede explotar comercialmente por su valor añadido en vitamina C. Más aún, la mayor fracción de este elemento se encuentra en forma de ácido ascórbico. Así, el consumo de hojas de rabaniza no sólo va a favorecer la ingesta mínima necesaria por su actividad como elemento esencial (Carr y Frei, 1999), sino que además permite aumentar el estatus antioxidante del organismo.

Se ha evaluado, además, el contenido en sinigrina acumulado en hojas de rabaniza. La sinigrina es el glucosinolato mayoritario de esta hortaliza, y es uno de los caracteres de la calidad funcional (pero también aromática) que diferencia la rabaniza de los cultivos de rúcula (*E. sativa* y *D. tenuifolia*) emparentados. Su degradación enzimática libera un compuesto mayoritario de alto valor funcional (Rajakumar *et al.*, 2015; Sávio *et al.*, 2015). Los valores determinados en esta Tesis son inferiores a los previamente obtenidos por Di Gioia *et al.* (2018). Así pues, hay una oportunidad de mejora aparente para este compuesto, la cual podría corresponder a una mejora genética y/o de las prácticas agrícolas de producción. Se debe considerar, sin embargo, que un incremento en glucosinolatos provocaría un aumento en la intensidad de aroma y sabor, hecho que puede aumentar el rechazo por el consumidor.

Por otro lado, diferentes ensayos realizados en la Tesis han confirmado el carácter bioacumulador de la rabaniza para iones nitrato. Dada la potencial toxicidad asociada al consumo de nitratos se han fijado unos límites máximos a nivel europeo para la rúcula (European Comission, 2011). Tal restricción afecta específicamente a *Diplotaxis* sp. entre otras especies, y por lo tanto el cultivo futuro de rabaniza quedaría igualmente sujeto al límite establecido para rúcula. Así pues, el contenido en nitratos es un carácter necesariamente a considerar en todo programa de mejora que se establezca para la rabaniza. Más aun, el contenido en nitratos va a resultar clave

asimismo en la selección de las prácticas agrícolas de cultivo, dada la influencia de factores ambientales y agronómicos (e.g., intensidad de luz, fertilización) sobre su acumulación (Weightman *et al.*, 2012; Stagnari *et al.*, 2015; Colonna *et al.*, 2016; Colla *et al.*, 2018).

No obstante, los altos niveles en ácido ascórbico y compuestos fenólicos permiten reducir el efecto negativo del consumo de rabaniza por acumulación de nitratos. Estos compuestos antioxidantes tienen una correlación negativa con la formación de compuestos *N*-nitroso y favorecen, por el contrario, la formación de óxido nítrico a partir de nitratos (Helser y Hotchkiss, 1994; Lundberg *et al.*, 2018), con el consecuente beneficio sobre la salud (Bondonno *et al.*, 2018; Lundberg *et al.*, 2018).

2.2.1. Efecto de la digestión sobre la bioaccesibilidad de compuestos bioactivos

Como parte del estudio funcional de la rabaniza, se planteó un ensayo para determinar cómo puede afectar el proceso de digestión sobre diversos componentes. El análisis se centró en el estudio de la sinigrina como principal glucosinolato, y de la capacidad reductora por el método Folin-Ciocalteu. Se incluyó además la determinación de clorofilas y carotenoides, compuestos no evaluados hasta el momento en la Tesis pero comunes en hortalizas de hoja (Farnham *et al.*, 2012; Guzman *et al.*, 2012; Neugart *et al.*, 2018). Por el contrario, no se analizó el contenido en ácido ascórbico dada la rápida degradación que puede sufrir este compuesto durante la manipulación del material.

La mayoría de trabajos con alimentos vegetales determinan el contenido en biomoléculas de capacidad nutracéutica sobre materiales crudos o cocinados. Otros trabajos han dado un paso más con el estudio de su efecto sobre, por ejemplo, cultivos celulares *in vitro* (e.g., Plazas *et al.*, 2014; Savio *et al.*, 2014; Sávio *et al.*, 2015; Tang *et al.*, 2015). Sin embargo, resulta igualmente importante entender cómo pueden verse afectados los distintos biocompuestos por el proceso de digestión de los alimentos funcionales. De hecho, sólo la fracción que resulta bioaccesible tras la

digestión es capaz de ejercer una acción protectora sobre el cuerpo (Sengul *et al.*, 2014).

Por un lado, la digestión de material de rabaniza ha producido una degradación de glucosinolatos (sinigrina), clorofilas y carotenoides, conjuntamente con una baja bioaccesibilidad. Sin embargo, la degradación de sinigrina no coincide necesariamente con una pérdida de funcionalidad. De hecho, son los productos liberados tras la hidrólisis enzimática (principalmente isotiocianatos), y no los precursores glucosinolatos, los compuestos de potencial bioactivo (Vig *et al.*, 2009; Romeo *et al.*, 2018). Por otra parte, la capacidad reductora aumenta durante la digestión, presumiblemente a consecuencia de la liberación de agliconas fenólicas por hidrólisis ácida en el estómago.

2.3. Perfil volátil de la rabaniza

Tradicionalmente se ha relacionado el aroma y sabor de distintas brasicáceas con el contenido en glucosinolatos. Sin embargo, hasta donde sabemos existía un desconocimiento completo del perfil volátil en la rabaniza.

Para evaluar el perfil de esta hortaliza, se ha utilizado la misma técnica empleada en los materiales de berraza. A diferencia de aquéllos, la rabaniza es pobre en compuestos terpenoides y carece de fenilpropanoides. Por el contrario, su perfil es rico principalmente en compuestos isotiocianatos. Tal y como cabría esperar, el compuesto alil isotiocianato es el principal volátil identificado en el perfil de esta especie. El alil isotiocianato es el principal compuesto derivado de la sinigrina (Bell *et al.*, 2018), y como otros isotiocianatos se produce en condiciones de pH neutro y sin la actuación de proteínas e iones metálicos específicos (Hanschen y Schreiner, 2017). Pero en el perfil de la rabaniza se han detectado además otros isotiocianatos. La hidrólisis enzimática de un glucosinolato puede derivar en diversos compuestos según las condiciones en que tenga lugar, tales como isotiocianatos, tiocianatos, nitrilos o epitionitrilos (Bell y Wagstaff, 2014). Por el contrario, cada isotiocianato tiene un precursor glucosinolato concreto (Bell *et al.*, 2018). Significa pues que la rabaniza biosintetiza otros

glucosinolatos más allá de la sinigrina, que de acuerdo a nuestros resultados podrían incluir gluconapina, gluconasturtina y/o glucotrapeolina. Este descubrimiento aumenta el conocimiento del perfil en glucosinolatos de la rabaniza, puesto que hasta el momento no se ha descrito la co-ocurrencia de sinigrina y otros glucosinolatos en esta hortaliza (Bennett *et al.*, 2006; D'Antuono *et al.*, 2008; Di Gioia *et al.*, 2018).

Por otro lado, la rabaniza acumula también esteres derivados principalmente del alcohol *cis*-3-hexan-1-ol. Aunque se encuentren en menor cantidad relativa, su presencia puede definir las "notas verdes" en el aroma y sabor de la rabaniza (López-Gresa *et al.*, 2017; Raffo *et al.*, 2018). Esta presencia puede, además, reducir la sensación picante que aportan los isotiocianatos (Bell *et al.*, 2017a).

2.3.1. Aceptación por el consumidor

El aroma y sabor de muchas especies sintetizadoras de glucosinolatos define precisamente la aceptación por parte de los consumidores. D'Antuono *et al.* (2009) reconocen el contenido en sinigrina de la rabaniza como un factor negativo que provoca un rechazo de esta hortaliza. Estos resultados contradicen sin embargo la apreciación de la rabaniza como hortaliza silvestre consumida en la región mediterránea.

En esta Tesis hemos demostrado que la rabaniza es apreciada por los consumidores. Éste es un factor clave que debe considerarse en el desarrollo de cualquier nuevo cultivo, y en general, en la industria alimentaria. En concreto, nuestro trabajo ha puesto de manifiesto que los estados fenológicos más avanzados de entre los presentados son apreciados por individuos que disfrutan el "sabor a mostaza". Dicha correspondencia pone de manifiesto nuevamente la importancia del alil isotiocianato en el perfil volátil y aromático de la rabaniza.

Curiosamente, el trabajo que evalúa la aceptación por el consumidor revela que los niveles absolutos de isotiocianatos no estarían necesariamente correlacionados con su apreciación en la cavidad oral y retronasal. Los glucosinolatos tienden a acumularse en mayor medida en semilla y estados fenológicos iniciales, tales como brotes, brotes verdes y plántulas (Hanschen

y Schreiner, 2017). La comparación entre el perfil volátil de los distintos productos desarrollados para este trabajo confirma estas diferencias fenológicas. Sin embargo, el germinado de rabaniza resulta el más apreciado por el público general. Entra aquí en juego un aspecto no considerado hasta el momento: la cantidad de alimento ingerida. Así pues, un producto que no sería aceptable cuando se consume en grandes cantidades, puede resultar agradable como elemento decorativo en los platos. Esto significa que se puede potenciar el uso de germinados de rabaniza para, por ejemplo, su uso en restauración.

2.4. Explotación como cultivo: problemática y oportunidades

2.4.1. Superación de los mecanismos de latencia secundaria

En la presente Tesis se han estudiado dos condiciones que pueden afectar a la explotación de la rabaniza como cultivo comercial. El primer factor a considerar es la existencia de mecanismos de latencia secundaria en la semilla madura de rabaniza (Sans y Masalles, 1994; Martínez-Laborde *et al.*, 2007). Esta situación es común dentro de la familia *Brassicaceae* (González-Benito *et al.*, 2011). De entre los diversos factores y tratamientos que se pueden aplicar para reducir el estado de latencia de la semilla, un tratamiento conjunto de escarificación química con hipoclorito de sodio y aplicación posterior de ácido giberélico ha resultado efectivo para promover la germinación de la rabaniza. Se obtiene así un tratamiento de corta duración cuya aplicación facilita tanto los programas de mejora como la explotación comercial de esta hortaliza, permitiendo una germinación elevada y homogénea en el tiempo.

Una incógnita que surge como consecuencia de la aplicación de dicho tratamiento es el efecto sobre el producto final. Por ejemplo, hace más de seis décadas que se conoce el efecto del ácido giberélico como fitohormona sobre la elongación de entrenudos (Marth *et al.*, 1956). Nuestros resultados no permiten concluir que haya un efecto significativo del ácido giberélico sobre la altura final de la planta en el momento de cosecha para las condiciones de cultivo dadas. En este sentido, una valoración en campo siguiendo las prácticas de cosecha comerciales habituales en rúcula

permitiría entender mejor la relevancia de este efecto en el potencial cultivo. Por otro lado, la aplicación de un tratamiento germinativo sí puede resultar en una reducción del ciclo de cultivo. La reducción en el cultivo, siempre y cuando no esté asociada a una pérdida de rendimiento, puede suponer una ventaja productiva y económica que debe ser considerada. Finalmente, se ha confirmado que la calidad funcional no queda seriamente comprometida por la utilización de tratamientos germinativos adecuados, especialmente en el caso del contenido en ácido ascórbico.

2.4.2. Efecto del cultivo en la acumulación de nitratos y otros caracteres de la calidad

Una vez superada la germinación deficiente, el segundo factor que debe considerarse necesariamente en la explotación comercial de la rabaniza es el nivel de nitratos que puede llegar a acumular el producto final. Los niveles de fertirrigación aplicados en el primer ensayo de rabaniza han provocado una acumulación de nitratos por encima del límite máximo establecido para el cultivo de rúcula (European Comission, 2011). Esta acumulación puede deberse a una aplicación excesiva de fertilización nitrogenada (Weightman *et al.*, 2012; Colla *et al.*, 2018). Egea-Gilabert *et al.* (2009) sugieren un control de la fertirrigación en sistemas hidropónicos como método para reducir el valor final de nitratos. Otras alternativas tales como una fertirrigación sin nitrógeno los días previos a la cosecha pueden asimismo reducir este valor (Borgognone *et al.*, 2016).

Sin embargo, tras los resultados obtenidos en el primer trabajo se renunció al sistema de fertirrigación como alternativa para el cultivo de rabaniza. Por el contrario, el cultivo se desarrolló sobre sustrato en invernadero o suelo en campo, usando riego no fertilizado. Como consecuencia de este trabajo se determina que el manejo de cultivo con estos sistemas modelo lleva a una clara modificación de los niveles de nitratos en las hojas de rabaniza. Pese a suprimirse la fertirrigación, el cultivo en invernadero sigue resultando en una elevada acumulación, incluso excesiva, de nitratos. El aumento de nitratos bajo invernadero explicaría, por ejemplo, por qué los niveles máximos permitidos en lechuga bajo estas condiciones son superiores a campo (European Comission, 2011). Por el contrario, en

condiciones de campo se obtienen valores por debajo del límite establecido, con independencia del ciclo de cultivo analizado. Por lo tanto, con los resultados de esta Tesis queda patente la posibilidad de la comercialización de rabaniza por debajo de los límites permitidos de nitratos, siempre y cuando se seleccionen prácticas adecuadas para la explotación comercial de este cultivo potencial.

El cultivo en campo abierto supone además una mejora en otros caracteres que determinarán la calidad de la rabaniza. Bajo condiciones de campo, las plantas están sujetas a un mayor efecto de las condiciones ambientales. Factores como temperatura, radiación solar, corrientes de viento, humedad relativa o incidencia de precipitaciones difieren entre los sistemas de cultivo en invernadero y campo (Figàs *et al.*, 2018). Los factores determinados en los distintos sistemas pueden afectar el crecimiento y desarrollo de las plantas (Figàs *et al.*, 2018), pudiendo condicionar así la calidad visual final; pero también la acumulación de biocompuestos (Colonna *et al.*, 2016; Bell *et al.*, 2018).

En concreto, en los trabajos de comparación entre sistemas se ha evaluado el efecto sobre caracteres morfológicos de hoja y sobre la calidad funcional en términos de ácido ascórbico, fenoles totales y sinigrina. En condiciones de campo se han obtenido plantas de mayor intensidad de lobulado y contenido relativo en clorofila, lo que se traduce en una coloración más intensa. Ambos caracteres se han considerado deseables para la aceptación de la rabaniza en el mercado, tal y como determinan otros autores en hortalizas de hoja diversas (Bell *et al.*, 2017b; Egea-Gilabert *et al.*, 2013, 2014). Bajo estas condiciones se incrementa además el contenido en ácido ascórbico y fenoles totales, probablemente en respuesta al crecimiento bajo una mayor incidencia de estreses abióticos (Orsini *et al.*, 2016). La acumulación de sinigrina, por el contrario, no se ha visto claramente afectada por el sistema de cultivo.

Así pues, el cultivo de rabaniza supone un buen candidato para establecerse en condiciones de campo. Un último punto a considerar en este sentido y de acuerdo a los resultados de esta Tesis, es el efecto de unas condiciones ambientales altamente estresantes durante el ciclo en los meses más fríos (diciembre-enero). Este ciclo ha dado lugar a una producción

indiscutiblemente destacada desde un punto de vista funcional, con la co-ocurrencia de los mayores niveles de antioxidantes y menores de nitratos. Sin embargo, la baja concentración de nitratos reduce la capacidad de retención de agua (Cárdenas-Navarro *et al.*, 1999; Schiattone *et al.*, 2018), hecho que se refleja en una consistencia más coriácea que llega a resultar comercialmente no aceptable. Más aún, se ha observado durante este ciclo la acumulación de antocianinas en la lámina de las hojas. Su acumulación está relacionada con un aumento de la tolerancia estreses abióticos (D'Amelia *et al.*, 2018), y en este caso estaría inducida por las bajas temperaturas. La aparición de estas "manchas", aunque importante desde una visión fisiológica, deprecia la calidad visual. Con todo, se podría estudiar en este sentido el uso de sistemas de protección en campo para reducir el estrés abiótico de los meses más fríos. En concreto, una alternativa que podría resultar adecuada, comúnmente utilizada en diversas hortícolas, es el uso de mallas térmicas protectoras de cultivo.

2.5. Perspectivas futuras para la explotación comercial de la rabaniza

Los distintos trabajos realizados en esta Tesis con materiales de rabaniza están encaminados a la obtención de un cultivar comercial. Hasta el momento se han seleccionado dos fenotipos considerados de gran interés potencial. Dichos fenotipos se caracterizan por un aroma y sabor intensos, con una morfología de hoja diferenciada –hoja obovada o con tendencia más lanceolada y de lóbulo terminal agudo–. No obstante, el grado de variación morfológica intrapoblacional hace necesario establecer unos ciclos adicionales de homogenización para asegurar una mayor uniformidad en la variedad población final. Por ello los esfuerzos actuales se están centrando precisamente en la homogenización de estas dos pre-variedades seleccionadas.

Además, la caracterización realizada en otros materiales deja abierta una línea para el desarrollo futuro de nuevas variedades. Una idea interesante en este sentido sería explotar las correlaciones negativas entre ésteres e isotiocianatos para obtener variedades cuyo aroma y sabor "a mostaza" sea menos intenso. Bell *et al.* (2017b) sugieren una relación entre las abundancias relativas de compuestos "con notas verdes" e isotiocianatos

con la percepción de sabores picantes. La cata realizada con consumidores potenciales ha puesto de manifiesto una diferencia de opiniones en cuanto a la aceptabilidad de un sabor tan intenso. Así pues, desarrollar otras variedades donde la percepción de las notas picantes sea menor puede aumentar el alcance a consumidores potenciales.

Por otro lado, esta Tesis deja abierta dos vías de trabajo para nuevos estudios. Por un lado, ha quedado patente la necesidad de diseñar una guía de descriptores específicos que sean de utilidad para la evaluación morfoagronómica de *D. erucoides*. Para ello se necesita una mayor exploración de la variabilidad existente en la especie, traduciéndose esto en la necesidad de aumentar las labores de colección en otras regiones no prospectadas para la presente Tesis. Esta guía resultará de utilidad no sólo para clasificar el germoplasma recolectado, sino también para una correcta caracterización de las variedades comerciales que puedan desarrollarse en el futuro.

La segunda vía de trabajo consiste en trasladar los resultados obtenidos en la Tesis a una escala productiva mayor, en condiciones de producción comercial. El siguiente paso en este sentido será presumiblemente adaptar el protocolo de germinación desarrollado en el laboratorio a un manejo comercial. Con esta adaptación será posible re-evaluar el comportamiento de los materiales finalmente seleccionados más allá de los sistemas modelo (invernadero y campo) utilizados en nuestra estación experimental.

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Conclusiones

- This Doctoral Thesis has studied the potential of two wild vegetables to be domesticated and introduced into cultivation. Both species have shown strong functional potential. This is the first work describing the phenolic profile of water celery. It is also pioneering research addressing the domestication of wall rocket varieties adapted to the Mediterranean conditions, including morphological, nutraceutical and aromatic characterization tasks; studies of the available variability for these traits and the effect of the cultivation conditions; the bioaccesibility of target compounds; and the consumers acceptance of the final product.

- This work has confirmed the classification of water celery as a wild vegetable with high antioxidant activity. The correlation established between the DPPH free radical-scavenging activity and the estimated content in total phenolics suggests that this activity in water celery is mainly related to the accumulation of phenolic compounds.

- Quercetin has been identified as the main flavonoid aglycon in water celery. The high reducing capacity of this molecule would explain the greater antioxidant activity of water celery compared to the related crops. This superiority may promote its introduction as a crop with high functional value.

- The volatile profile of fresh water celery has been determined as rich in terpenoids and phenylpropanoids. Despite some similarities with related crops, the unique combination of volatile compounds and their relative quantities would be responsible of the characteristic flavour of water celery. This distinctive flavour may promote its commercial production as a different crop.

- Differences in the volatile profile among individual populations, as well as differences in the antioxidant activity among populations grouped by geographical origin, have been determined. The hydroponic cultivation under controlled conditions may allow identifying the genetic component responsible of such differences, thus establishing a basis for the future domestication and breeding programmes.

- Wall rocket can be considered as a vegetable with high functional quality, but it also accumulates high levels of nitrates. The materials evaluated have displayed moderate variability for wall rocket quality, a trait that must be considered for the domestication and breeding programmes.

- The leaf morphology of wall rocket is significantly different to the morphology of rocket crops (*E. sativa* y *D. tenuifolia*). This distinctiveness can promote its introduction into markets as a new crop. Moderate variability among materials has been determined for morphological and growth traits. Thus, the quantity of commercial varieties that can be obtained is limited. In fact, only two varieties with differential morphologies are being obtained at this moment.

- The volatile profile of wall rocket is rich in isothiocyanates and, to a lower extent, esters. Allyl isothiocyanate has been identified as the main volatile compound. Allyl isothiocyanate is the main compound derived from the hydrolysis of sinigrin, and is great responsible of the aroma and pungent flavour of wall rocket. In addition, the identification of other isothiocyanates indicates the accumulation of several glucosinolates together with sinigrin. This information increases the knowledge in the species.

- Results of the hedonic test indicate a market opportunity for wall rocket microgreens and baby-leaves. Baby-leaves have a specific cohort defined by consumers that appreciate pungent flavours found in some *Brassicaceae.* On the contrary, microgreens are appreciated by the general public in spite of their higher isothiocyanates accumulation as they are designed as a decorative element of dishes present in small amounts.

- The growing system and crop cycle have influenced the visual and functional quality of wall rocket. Plants growing in the field increase the intensity of lobation and colour of leaves, have an enhanced antioxidant quality and decrease the content in nitrates. However, highly stressful conditions during the coldest months can compromise the commercial quality. In this sense, it would be interesting to evaluate the use of thermal blankets for protecting the crop under these conditions.

- A germination protocol combining the scarification of seeds with sodium hypochlorite and the use of gibberellic acid has been suggested as a quick treatment aimed at breaking the secondary dormancy of wall rocket seeds. These germination factors allow a high and uniform germination, without compromising the quality of the final product. Thus, we suggest the use of this protocol in breeding programmes but also its adaptation to large-scale commercial production.

- The results obtained in this Doctoral Thesis provide relevant information for the domestication and revalorization of these two species, and are a basis for new studies on this topic. In the case of water celery, efforts should be firstly focused on the adaptation into hydroponic conditions, and then selection programmes could be developed. Regarding wall rocket, these results are a basis for new works in the context of commercial production, aimed at testing the varieties that are being developed.