



Universidad
Rey Juan Carlos

TESIS DOCTORAL

*Análisis ecogeográfico como herramienta para la
conservación de parientes silvestres de cultivos*

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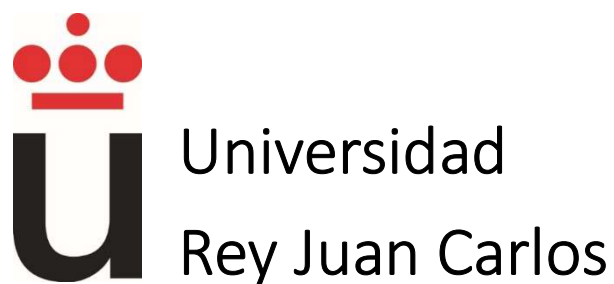
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Programa de Doctorado en Conservación de Recursos Naturales
Escuela Internacional de Doctorado

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A mis abuelos.

A mis padres.

A Vega, a Inés, a Ana.

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RESUMEN

Antecedentes

La domesticación de los cultivos comenzó hace cerca de 11000 años, durante el periodo Neolítico. El proceso de domesticación conllevó la selección de plantas silvestres con potencial para alimentar a los asentamientos humanos. La selección artificial homogeneizó y redujo la diversidad genética inicialmente contenida en el acervo de estas. La evolución de estas plantas seleccionadas desde los ancestros de los cultivos resultó en la aparición de las variedades tradicionales de los cultivos, la inmensa mayoría de ellas adaptadas a los ambientes en los que fueron seleccionadas y desarrolladas. Sin embargo, a mediados del siglo XX, el perfeccionamiento de las técnicas de mejora genética condujo al desarrollo de las actuales variedades comerciales, con una altísima productividad y una gran homogeneidad genética. Las nuevas variedades comerciales desplazaron definitivamente a las variedades tradicionales de los cultivos, en detrimento de la diversidad genética, limitando seriamente de este modo, la capacidad de adaptación de la agricultura.

Los parientes silvestres de los cultivos (PSC) son plantas estrechamente vinculadas a las cultivadas, que pueden utilizarse como fuente de diversidad genética para la mejora genética de los cultivos. A lo largo de la historia evolutiva, los PSC han adquirido adaptaciones evolutivas tanto a los ambientes físicos como a los estreses bióticos a los que se han enfrentado. De acuerdo con los informes del Grupo Intergubernamental de Expertos sobre el Cambio Climático (IPCC) el impacto del cambio climático sobre la agricultura está ya amenazando la seguridad alimentaria. En este sentido, la Agenda del Desarrollo Sostenible de las Naciones Unidas contempla la necesidad de conservar los recursos genéticos, incluyendo los Parientes Silvestres de los Cultivos, garantizando la conservación de su diversidad genética, así como el acceso y uso de los mismos.

La evaluación de la diversidad genética de valor adaptativo es un paso indispensable en el uso de parientes silvestres como fuente de genes para las plantas cultivadas. Sin embargo, resulta inviable acometer una evaluación fenotípica y una caracterización genética de cada una de las poblaciones existentes de parientes silvestres de los cultivos. Afortunadamente, se pueden realizar aproximaciones a la diversidad genética de valor adaptativo de los PSC a través del uso de mapas ecogeográficos, capaces de diferenciar escenarios adaptativos mediante la caracterización del territorio de estudio en función de variables abióticas. Asimismo, es necesario identificar de entre las poblaciones de PSC aquellas con mayores probabilidades de contener los rasgos adaptativos deseados para la mejora vegetal. En este sentido, las técnicas de Caracterización Predictiva, ya sea utilizando el método de filtrado ambiental o el método de calibración, se utilizan con éxito en la selección de genoplasma silvestre potencialmente útil para la mejora genética de los cultivos.

Objetivo

El objetivo de esta tesis doctoral es establecer un marco que contribuya al continuo de conservación, acceso y uso de los parientes silvestres de los cultivos en España. Para ello se delimitaron cinco objetivos específicos: **i)** identificar y priorizar parientes silvestres de los cultivos de importancia para España, **ii)** evaluar su estado de conservación, **iii)** aplicar y evaluar aproximaciones experimentales que permitan la conservación multi-especie, **iv)** tener en cuenta el componente de diversidad genética infraespecífica en el diseño de planes de conservación y recolección de parientes silvestres de los cultivos y **v)** aplicar y evaluar técnicas de caracterización predictiva que ayuden a identificar las poblaciones de parientes silvestres de los cultivos con mayores probabilidades de poseer características deseables para la mejora genética de los cultivos, facilitando de este modo el acceso y uso de las mismas.

Metodología

Para abordar estos objetivos específicos se planteó un trabajo multidisciplinar, que facilitara el flujo desde la identificación de especies, hasta la propuesta de lugares para su recolección y conservación y el acceso a las mismas.

En primera instancia se utilizó una aproximación monográfica para la identificación y priorización de especies parientes de los cultivos de importancia para España (Capítulo 1). Se caracterizaron las especies en base a criterios que posteriormente permitirían priorizar los parientes silvestres de cultivos más importantes para España. Para conocer la distribución de las especies priorizadas en España se llevó a cabo una descarga y un filtrado de calidad de datos corológicos. Los datos de distribución fueron descargados desde la Infraestructura Mundial de Información en Biodiversidad (GBIF, www.gbif.org). Además, se evaluó el estado de conservación *ex situ* de los parientes silvestres priorizados, al objeto de establecer prioridades de recolección.

En la siguiente etapa, con la intención de identificar los parientes silvestres de los cultivos prioritarios para España que necesitan medidas urgentes de conservación (Capítulo 2), se llevó a cabo una priorización adicional aplicando criterios de facilidad de cruzamiento con las plantas cultivadas, grado de amenaza y endemidad, seleccionando aquellas especies con datos de distribución poblacional georreferenciados de alta calidad. Se comprobó la presencia de estas especies en el Catálogo Nacional de Especies Amenazadas y en los catálogos autonómicos de especies amenazadas. Además, se utilizaron los datos corológicos de las especies para evaluar su estado de conservación *in situ*, en la red de espacios protegidos de Natura 2000, aplicando técnicas de *gap analysis*. Asimismo, se evaluó la representación de las especies seleccionadas en bancos de germoplasma nacionales (bancos de germoplasma pertenecientes a REDBAG, de la Asociación de Ibero-Macaronésica de Jardines Botánicos) e internacionales (el Catálogo Europeo de Búsqueda de Recursos Fitogenéticos – EURISCO y la base de datos de la Red de Información de Recursos de Germoplasma, del Programa Nacional de Recursos Genéticos del Departamento de Agricultura de los Estados Unidos). Dada la falta de información en algunas de las fuentes consultadas, la evaluación *ex situ* se llevó a cabo en términos de presencia-ausencia de semillas conservadas de las especies en los bancos de germoplasma.

En tercer lugar, se usaron aproximaciones multi-especie que permitieran trabajar con varias especies de manera simultánea, utilizando para ello asociaciones fitosociológicas que contuvieran parientes silvestres de los cultivos forrajeros entre sus especies (Capítulo 3). Además, se incorporó el componente genético de valor adaptivo de las poblaciones a través de la combinación de los datos de distribución de las asociaciones seleccionadas y las categorías ecogeográficas en las que se encuentran. La selección de variables para la construcción del mapa ecogeográfico se llevó a cabo con un proceso de consulta con un panel de expertos. Las variables climáticas, edáficas y geofísicas seleccionadas fueron utilizadas a continuación como parámetros en la generación del mapa ecogeográfico para la Península Ibérica y Baleares, determinando el número óptimo de agrupamientos mediante un algoritmo *k-means*. Con el propósito de evaluar el estado de conservación *in situ* de las combinaciones Asociación-Categoría ecogeográfica (Aso-Eco), de nuevo se aplicaron técnicas de *gap analysis* para identificar qué combinaciones Aso-Eco se encuentran protegidas de manera pasiva en la Red Natura 2000. Con el objeto de identificar áreas para el establecimiento de reservas genéticas de parientes silvestres de los cultivos forrajeros, se llevó a cabo un análisis de complementariedad, que permitió identificar las áreas protegidas de Natura 2000 que maximizaban el número de combinaciones Aso-Eco. Además, se hizo un análisis de cobertura para evaluar la idoneidad de la Red Natura 2000 para la conservación de parientes silvestres de los cultivos forrajeros. Todos los análisis también se realizaron con las asociaciones como unidades de conservación, para poder evaluar los beneficios y/o limitaciones de la incorporación del componente ecogeográfico en el diseño de planes de conservación.

Para finalizar, y completar el flujo a seguir en el continuo identificación-conservación-utilización, se identificaron poblaciones silvestres de lenteja potencialmente tolerantes a sequía, salinidad y encharcamiento y potencialmente resistentes a roya y jopo. En este caso, con objeto de tener una base de datos mayor que permitiera a su vez una mayor robustez de los análisis, se amplió el ámbito geográfico del trabajo a Europa y Turquía, y las especies seleccionadas de lenteja silvestre a todas las especies nativas de Europa. Se generó un nuevo mapa ecogeográfico para el nuevo rango geográfico, identificando variables ecogeográficas (climáticas, edáficas y geofísicas) que pudieran estar afectando a la distribución de las especies de lentejas silvestres. La selección de variables ecogeográficas se llevó a cabo a través de procesos objetivos, aplicando algoritmos matemáticos (*Random Forest*) y correlaciones bivariadas. Se aplicaron técnicas de caracterización predictiva para la generación de subconjuntos de poblaciones con mayor probabilidad de contener los rasgos deseados, siempre teniendo en cuenta y maximizando la representatividad de las poblaciones en las diferentes categorías ecogeográficas en las que se distribuyen. Mediante el método de filtrado ambiental se identificaron poblaciones

de lentejas silvestres potencialmente tolerantes a sequía, salinidad del suelo y encharcamiento. Mediante el método de calibración se identificaron poblaciones potencialmente resistentes a la roya y se modeló asimismo la potencial resistencia al jopo.

Resultados

Capítulo 1

La aproximación monográfica para la selección de especies, comenzando por la selección de cultivos, dio lugar a la selección de 203 cultivos de importancia para España y el mundo. En España, hay un total de 929 especies silvestres y nativas del país relacionadas con estos cultivos. Atendiendo a los grupos de uso, 223 especies están categorizadas dentro del grupo de Alimentación Humana, 260 especies en el de Pastos y Forraje, 240 especies en el grupo de Ornamentales y finalmente, 206 especies pertenecen al grupo de Uso Industrial y Otros Usos. La aplicación de los criterios de priorización redujo la lista a 578 especies, de las que 137 pertenecen al grupo de Alimentación Humana, 185 al de Pastos y Forraje, 161 al Ornamental y 95 al de Uso Industrial y Otros Usos. De estas especies priorizadas, 107 están clasificadas en el *gene pool* primario (*i.e.*, la misma especie en su forma silvestre) y 95 en el secundario (especies silvestres próximas con las que se puede cruzar el cultivo mediante métodos convencionales). Cerca del 70% de las especies priorizadas carecían de información de cruzamiento con los cultivos, por lo que fueron clasificadas siguiendo el criterio de Grupo Taxonómico. Así, 203 especies fueron clasificadas en el Grupo Taxonómico Secundario (misma sección o subsección que el cultivo), y por lo tanto se espera que también se puedan cruzar con éste utilizando métodos convencionales. El 40% de las especies priorizadas son endémicas de España y aproximadamente el 25% se encuentran bajo alguna categoría de amenaza de acuerdo con los criterios de la UICN. Además, 15 de estas especies están también amenazadas a nivel europeo y 43 de las especies priorizadas se encuentran en el Catálogo Nacional de Especies Amenazadas. Se constata que la distribución de Parientes Silvestres en España es más o menos homogénea en todo el territorio, aunque en término de riqueza de especies destacan tres comunidades autónomas, Andalucía en primer lugar, Castilla y León en segundo lugar y Castilla La Mancha en tercer lugar (303, 234 y 224 especies, respectivamente).

Los resultados globales de la conservación de accesiones de semillas de parientes silvestres de los cultivos en bancos de germoplasma muestran que el 70% de las especies cuentan con al menos una accesión conservada, siendo por lo tanto 176 especies las que no están representadas en bancos. Menos del 30% de las especies cuenta con más de 20 accesiones conservadas y hasta el 34% de las especies priorizadas solo tenían entre una y cuatro accesiones en bancos de germoplasma. Cabe destacar que se identificaron 51 especies prioritarias que tienen menos de cinco accesiones conservadas en bancos de semillas y que son de gran importancia por pertenecer al *gene pool* primario o secundario, o Grupo Taxonómico Secundario, de especies cultivadas, ser endémicas y tener algún grado de amenaza según las categorías de amenaza de la UICN. De estas 51 especies, 23 no están representadas en bancos de germoplasma y se propusieron como prioridad muy urgente de recolección. Asimismo, se establecieron otras categorías de prioridad de recolección teniendo en cuenta la representación en bancos de germoplasma. En la siguiente categoría – prioridad urgente de recolección – se incorporan 153 especies (no endémicas y amenazadas) que tampoco están representadas en bancos de semillas. De la misma manera, en la categoría “prioridad para la recolección” se identifican 195 especies que cuentan con menos de 5 accesiones conservadas y por último 205 especies que tienen más de cinco accesiones de semillas fueron consideradas como no prioritarias para la recolección.

Capítulo 2:

Se identificaron 47 especies prioritarias de gran importancia para España de acuerdo con los criterios de facilidad de cruzamiento con los cultivos (*gene pool* primario y secundario o Grupo Taxonómico Secundario), amenaza y endemidad. Estos criterios se aplicaron sobre las especies prioritarias que tenían datos de

distribución de alta calidad. La aplicación de estos mismos criterios sobre el cómputo total de especies prioritarias, sin tener en cuenta la calidad de los datos corológicos, habría seleccionado 26 especies adicionales.

La categoría de uso predominante entre las especies seleccionadas fue la categoría Ornamental (32 especies), en la que las especies se distribuyen fundamentalmente en tres familias: Plumbagináceas (40%), Lamiáceas (19%) y Amarilidáceas (15%). En el grupo de Alimentación Humana se seleccionaron tres especies, en el de Pastos y Forraje tres especies y finalmente en el grupo de uso Industrial y Otros Usos se seleccionaron nueve especies. En cuanto al grado de amenaza, las especies no están distribuidas de manera uniforme según las categorías establecidas por la UICN, encontrándose cinco especies en la categoría Casi Amenazada, 22 en la categoría Vulnerable, ocho en Peligro y 12 en Peligro Crítico. Aproximadamente el 21% de las especies (10 especies) cuentan con protección legal en España al estar incluidas en el Catálogo Nacional de Especies Amenazadas. Estas especies están clasificadas como En Peligro de Extinción (4 especies), Protegidas (4 especies) y Vulnerables (2 especies). Cabe destacar que 11 especies clasificadas como En Peligro Crítico de acuerdo con los criterios de la UICN no están incluidas en el Catálogo Nacional de Especies Amenazadas. Sin embargo, al consultar los Catálogos Autonómicos se constató que aproximadamente el 74% de las especies estaba en al menos un catálogo autonómico y que seis de ellas están protegidas bajo el marco de dos catálogos autonómicos. Además, 10 de las 11 especies que no estaban listadas en el Catálogo Nacional de Especies Amenazadas se encuentra en algún catálogo regional.

La evaluación del estado de conservación *in situ*, bajo la protección pasiva de la Red Natura 2000, mostró que el 39% de las poblaciones conocidas de las especies focales se encuentran en áreas protegidas. El 74% de las especies cuenta con al menos una población en áreas protegidas y el 38% tiene más de cinco poblaciones en áreas protegidas. En caso de que se fijara como objetivo la presencia de más del 35% de las poblaciones de una especie en áreas protegidas, serían 27 especies (cerca del 58%) las que cumplirían con ese criterio. La evaluación de estado de conservación *ex situ* mostró que 40 especies tienen al menos una accesión conservada en bancos de germoplasma nacionales o internacionales. Finalmente, se identificaron dos especies que no tienen ninguna población en áreas protegidas de la Red Natura 2000 y que de manera simultánea tampoco tienen accesiones en bancos de germoplasma: *Astragalus cavanillesii* Podlech y *Sideritis reverchonii* Willk. Estas especies, clasificadas como En Peligro Crítico y En Peligro, respectivamente, no se encuentran listadas en el Catálogo Nacional de Especies Amenazadas, aunque la primera sí aparece en dos catálogos autonómicos.

Capítulo 3:

La selección de especies pertenecientes al *gene pool* primario de los cultivos forrajeros conformó un grupo de 45 especies. El requisito de pertenecer a, al menos, dos asociaciones fitosociológicas, generó una lista de 21 asociaciones. Dos especies, *Lupinus consentinii* Guss. and *Trifolium vesiculosum* Sav., no fueron consideradas en los análisis por no pertenecer a ninguna asociación fitosociológica de entre las citadas en el Sistema de Información de la Vegetación Iberomacaronésica (SIVIM), que fue la fuente utilizada para la descarga de datos de inventarios fitosociológicos. El posterior filtrado de calidad de datos geográficos de los inventarios que indican los lugares de presencia de dichas asociaciones conllevó la eliminación de 6 asociaciones. De este modo, los análisis se realizaron con un total de 1283 inventarios fitosociológicos, que representan 15 asociaciones fitosociológicas en las que se encuentran 39 especies silvestres emparentadas con cultivos forrajeros.

El mapa ecogeográfico generado contiene 49 categorías o unidades ecogeográficas. Las asociaciones fitosociológicas se distribuyen en 39 de estas categorías y se observa que las asociaciones representadas por un mayor número de inventarios fitosociológicos se encuentran representadas en un mayor número de categorías ecogeográficas, excepto dos asociaciones (*Lino biennis*-*Cynosuretum cristati* and *Festuco amplae*-*Agrostietum castellanae*) que con una misma intensidad de muestreo se verían representadas en menos categorías ecogeográficas. La combinación de los inventarios de las asociaciones con las categorías ecogeográficas en las que se encuentran distribuidas generó 165 combinaciones Asociación-Categoría ecogeográfica (Aso-Eco).

El *gap analysis* mostró que aproximadamente el 36% de los inventarios (447) se encuentran dentro de las áreas protegidas de la Red Natura 2000. Todas las combinaciones Aso-Eco y las 15 asociaciones fitosociológicas bajo estudio están representadas, al menos una vez, dentro de la Red. El **análisis de cobertura** reveló que estas

combinaciones Aso-Eco y asociaciones se encuentran en el 8.8% de los espacios de Natura 2000 (127 áreas). Cabe resaltar que 37 áreas protegidas de Natura 2000 estarían dando cobertura a un mayor número de combinaciones Aso-Eco en comparación con las asociaciones que en ellas se encuentran. El **análisis de complementariedad** identifica 52 áreas de la Red Natura 2000 como el mínimo número de áreas necesarias para proteger de manera pasiva a las 114 combinaciones Aso-Eco que se encuentran dentro de la Red. . En el caso de las asociaciones, son siete áreas de Natura 2000 el número mínimo áreas seleccionadas para proteger de manera pasiva la totalidad de asociaciones estudiadas. Bajo este marco se seleccionaron 15 lugares como propuesta para el establecimiento de Reservas Genéticas. Catorce de estos lugares contienen al menos 3 combinaciones Aso-Eco no representadas en otras áreas protegidas y se estaría protegiendo de este modo el 41% de Aso-Eco que están dentro de área protegidas y 14 de las asociaciones. El decimoquinto lugar propuesto añadiría la asociación faltante.

Capítulo 4:

La apertura del ámbito geográfico del estudio a Europa y Turquía permitió trabajar con 624 poblaciones pertenecientes a tres especies y una subespecie de parientes silvestres de la lenteja. Esto supone la adición de una especie y una subespecie con respecto a las especies prioritarias seleccionadas en el Capítulo 1. Los países con mayor representación de poblaciones silvestres de lentejas fueron Grecia (173 poblaciones), Francia (165 poblaciones) y España (128 poblaciones).

El mapa ecogeográfico generado contiene 28 categorías y en 13 de ellas se encuentran poblaciones silvestres de lentejas. El método de **filtrado ambiental** de la caracterización predictiva seleccionó 13 poblaciones potencialmente tolerantes a la sequía: tres en Grecia, nueve en España y una en Turquía, que representan tres taxones y se encuentran en una sola categoría ecogeográfica. En cuanto a las poblaciones potencialmente tolerantes a la salinidad del suelo, solo una población en Francia fue seleccionada. Finalmente, el método de filtrado ambiental seleccionó 21 poblaciones, en las que están representados los cuatro taxones, como potencialmente tolerantes al encharcamiento. Estas poblaciones se encuentran en cuatro categorías ecogeográficas diferentes y cinco países: Ucrania (8 poblaciones), España (6 poblaciones), Turquía (cuatro poblaciones), Grecia (dos poblaciones) y Francia (1 población).

Finalmente, la proyección del mejor modelo del algoritmo GBM, seleccionado por **el método de calibración** de la caracterización predictiva, clasifica 529 poblaciones como potencialmente resistentes a la roya. De estas poblaciones, se seleccionaron las 30 primeras con mayores valores de idoneidad como propuesta para su evaluación en el rasgo. Estas poblaciones pertenecen a dos especies distintas (*L. ervoides* (Brign.) Grande – 21 poblaciones – y *L. nigricans* (M. Bieb.) Godr. – nueve poblaciones), se encuentran distribuidas en cinco categorías ecogeográficas y en seis países, todos en el área de los Balcanes y Turquía. Respecto a la resistencia al jopo, los algoritmos y modelos aplicados fueron fallidos y no pudieron proyectarse. Por último, cabe destacar que ninguna de las poblaciones de los subconjuntos seleccionados se encuentra de manera simultánea en los cuatro subconjuntos. Sin embargo, sí hay solapamientos parciales en los que algunas de las poblaciones identificadas como potencialmente tolerantes a la sequía, salinidad del suelo y encharcamiento, serían también potencialmente resistentes a la roya.

Conclusiones

1. La enorme diversidad taxonómica de parientes silvestres en España hace necesaria una priorización de especies para focalizar los esfuerzos de conservación. Aplicando criterios de facilidad de cruzamiento con los cultivos, grado de amenaza y endemidad, la mayoría de las 578 especies de PSC priorizadas en este estudio poseen potencialidad de cruzamiento con sus parientes cultivadas, cerca de la mitad son endémicas de España y aproximadamente un cuarto de ellas tienen algún grado de amenaza.
2. El número de especies de PSC priorizadas en cada categoría de uso es homogéneo, destacando ligeramente las categorías de Pastos y Forraje y Ornamental. Asimismo, las comunidades autónomas muestran una riqueza en especies priorizadas de PSC homogénea, si bien Andalucía, Castilla y León, y Castilla La Mancha destacan sobre las demás.
3. La conservación *ex situ* de PSC priorizados es satisfactoria en cuanto que mantiene representadas casi tres cuartas partes de las especies en bancos de germoplasma. No obstante, es necesaria una recolección adicional de muestras de semillas que complete las colecciones, tanto a nivel de representatividad de especies como en número de accesiones, que permitan una adecuada conservación de la diversidad genética de las especies seleccionadas. En esta tesis se establecen cuatro categorías de prioridad de recolección que tienen en cuenta la importancia de los PSC en términos de facilidad de cruzamiento con los cultivos, grado de amenaza y endemidad, así como la representación de dichas especies en bancos de semillas.
4. El Catálogo Español de Especies Amenazadas protege a menos de la cuarta parte de las especies PSC amenazadas y seleccionadas para la toma de medidas urgentes de conservación. Sin embargo, la mayoría de especies identificadas sí se encuentran protegidas en los catálogos autonómicos de especies amenazadas.
5. La mayoría de especies de PSC prioritarios que requieren medidas de conservación urgentes están representadas en bancos de germoplasma y en áreas protegidas de la Red Natura 2000. En este último caso, algo más de la tercera parte contarían con un número suficiente de poblaciones que, presumiblemente, permitiría conservar de manera adecuada la diversidad genética de la especie.
6. La aproximación multiespecie utilizando asociaciones fitosociológicas demuestra gran efectividad en la cobertura de PSC forrajeras, encontrándose asociaciones que las contienen concentradas en gran número. Además, la combinación de las poblaciones (inventarios georreferenciados) de asociaciones fitosociológicas con las categorías ecogeográficas (Aso-Eco) en las que se encuentran presentes recoge una gran diversidad genética de valor adaptativo entre las asociaciones.
7. Los espacios de Natura 2000 cubren de manera muy satisfactoria tanto la diversidad sintaxonómica, como la diversidad de combinaciones Aso-Eco, permitiendo proponer un reducido número de áreas protegidas (15) para el establecimiento de reservas genéticas de PSC forrajeros. El establecimiento de estas reservas genéticas protegería todas las asociaciones focales y aproximadamente el 40% de la diversidad genética de valor adaptativo estimada, siguiendo una aproximación multiespecie, que reduciría los costes de gestión, así como facilitaría el acceso a recursos genéticos para la mejora genética de cultivos forrajeros.
8. El método de filtrado ambiental de las técnicas de caracterización predictiva identificó tres subconjuntos de poblaciones de PSC de lentejas con mayor probabilidad de contener tolerancia a la sequía (21 poblaciones), la salinidad del suelo (una población) y el encharcamiento (13 poblaciones), que facilita la selección de germoplasma para ser utilizado en mejora vegetal de la lenteja, disminuyendo en gran medida el número de poblaciones candidatas para ensayos de evaluación de tolerancia a estos estreses abióticos.
9. El método de calibración de las técnicas de caracterización predictiva identificó un amplio porcentaje de poblaciones silvestres de PSC de lentejas potencialmente resistentes a la roya (cerca del 85.5%) y permite seleccionar de entre estas, un subconjunto con mayor probabilidad de resistencia (30 poblaciones), candidatas para su evaluación en este rasgo y ser utilizadas en procesos de pre-mejora y mejora genética de la lenteja cultivada. Sin embargo, con el conjunto de datos de evaluación para el modelo de entrenamiento, el método de calibración no se ha mostrado eficaz para la selección de poblaciones de PSC de lentejas potencialmente resistentes al jopo.

GENERAL INTRODUCTION

Plant Genetic Resources – Agrobiodiversity

The process of crop domestication was initiated during the Neolithic period, around 12-10,000 thousand years ago. It involved the selection of traits suiting human needs from wild plant species – ancestors – imposing selective pressures that resulted on the one hand in bottlenecks that strongly reduced the genetic diversity (Gross and Olsen, 2010; Smýkal et al., 2018), but on the other hand in a further phenotypic diversification that allowed the adaptation of crops and emergence of new varieties in a complex and multi-stage process (Meyer and Purugganan, 2013). According to FAO (2009) the concept *Plant Genetic Resources* (PGR) includes any plant material of actual or potential value for food and agriculture. Thus, PGR encompass the cultivated plants – commercial varieties, modern cultivars, landraces, etc. – and also their ancestors and wild relatives. This diversity of resources for food and agriculture is part of the *agrobiodiversity*, that includes also other biological fundamental resources for the agriculture (Thrupp, 2000). The selection of traits and the search for highest yields reached a milestone in the so-called Green Revolution. The Green Revolution started in the decade of the 1950's, when modern varieties started to be developed, mainly in wheat and rice but also in other crops, which allowed to increase production worldwide resulting in a gain in health and life expectancy (Evenson et al., 2003). However, it also brought homogeneity in crop genetic diversity, leading to *genetic erosion*, that is, the loss of genetic diversity of unique value (FAO, 1996). Genetic erosion has been identified as a major threat for food security (Esquinas-Alcázar, 2005; Khoury et al., 2014) mainly due to the adoption of monocropping and the genetic uniformity of cultivated varieties, which aggravates the threat (Conway and Barbie, 1988; Ehrlich et al., 1993).

Climate change and crops

Trends in global climate change are confirmed by the provisional report of the World Meteorological Organization (WMO, 2021) in which tendencies of strong increments in global mean temperatures are ratified by all analyzed datasets and extreme climate events – droughts, heat and cold waves, floods and disruption in precipitation patterns – are shown to be more frequent. Additionally, it also reports a peak in world risk of famine in 2020 which will probably be higher in 2021 due to humanitarian crises and the impact of climate change on cultivated plants and agricultural areas. The vulnerability of agriculture to climate change is known since decades ago. Whereas some countries at higher latitudes could be benefited in some aspects by climate change, some others, especially those at developing countries would be strongly affected by it (Rosenzweig and Parry, 1994; Kotschi, 2006). Nevertheless, most of the predictions on climate change impact on crops project world scenarios in which crop yields would be reduced in many world areas (Rosenzweig et al., 2014). Particularly in the Mediterranean area, climate change is hitting with force and all climatic indicators are worrying: just to cite some, mean temperatures have increased at a higher rate than global mean temperatures, precipitation has reduced over the years and droughts are more frequent (MedECC, 2019). These impacts are expected to increase food insecurity in the area, reducing yields in many crops and specially in the Southern areas (MedECC, 2019). Spain – the main studied area in this Thesis – is especially vulnerable to climate change (MTERD, 2021), and the impact in agricultural practices and crops cycles has already been assessed (García-Mozo et al., 2010; Vargas-Amelin and Pindado, 2014) mainly with negative effects, although it depends on the crops (Iglesias et al., 2000). To overcome such impacts and protect future food security, the development of new varieties, resilient and adapted to new environmental and social conditions becomes critical (Massawe et al., 2016) and actions to confront climate change should be addressed at the national or local level despite the global significance of climate change (Hasegawa et al., 2018).

Crop wild relatives a source of adaptations

Under this context of climate change and food insecurity, crop wild relatives (CWR) appear as a source of novel diversity worth using to enhance genetic diversity in crop species. CWR are plants intimately related to crop plant species and to which can transfer genetic material (Heywood et al., 2007; Maxted et al., 2012), which makes them specially valuable for breeding for traits related to adaptation. CWR have evolved under natural conditions, with non-directed selective pressures, and continue doing so, providing a magnificent source of adaptations to current environmental conditions (Brozynska et al., 2016). Although its use in breeding programs is becoming more apparent, the utilization of CWR in breeding programs is not widely adopted. Some of the main constraints to use them systematically are the lack of data on genotypic and phenotypic characterization and the linkage drag (introgression of undesired characteristics into the pure lines) (Dempewolf et al., 2017). However, in this review of the past and future usage of CWR, the authors provide several arguments that lead to an optimistic forthcoming increase of their use in pre-breeding programs, such as the improved documentation of materials, advances in biotechnological tools or the development of introgressed materials, just to cite some. In this line, it is worth mentioning the efforts made to use them in crops such as beet (Thurau et al., 2010), tomato (Willits et al., 2005; Bleeker et al., 2012), pea (Aryamanesh et al., 2012) or sunflower (Seiler et al., 2017) just to cite some among many others (see Ashraf (2010); Hunter et al. (2012); Redden (2015), Dempewolf et al. (2017) and references therein). Also encouraging is the interest in the development of new tools to enhance their use such as the *introgressomics* approach (Prohens et al., 2017) that aims to the mass scale development of breeding materials from CWR, or the Predictive Characterization approach (Thormann et al., 2014a) that aims to facilitate the identification of useful germplasm or wild populations to be used with breeding purposes, applied in this PhD thesis and which will be further explained below.

Along with this growing interest and probably driven by it, CWR have been the subject of numerous research projects over the last two decades funded by the European Commission (Sixth and Seventh Framework Programmes and Horizon 2020) and has motivated the creation of international bodies aimed at their conservation (e.g., the CWR Specialist Group of the International Union for the Conservation of Nature (IUCN) or the Wild Species Conservation in Genetic Reserves Working Group of European Cooperative Programme for Plant Genetic Resources (ECPGR)). CWR have raised the attention and concern of international bodies such as the Food and Agricultural Organization of the United Nations (FAO), who included them as valuable resources for the sustainability of food security in the First and Second Plan of Action for the Conservation of PGR (FAO, 1996, 2010). Similarly, the first contract of the Convention on Biological Diversity (CBD) recognizes PGR as a component of biodiversity, advocating for their conservation and sustainable use, explicitly mentioning CWR as part of PGR (article 1 and Annex 1 of the CBD contract) (UN, 1992). Furthermore, CWR are included in the Strategic goal C, target 13 of the Strategic Plan for Biodiversity 2011-2020 and the Aichi Biodiversity Targets agreed by the CBD in 2010 (UN CBD, 2010). This increasing interest has culminated in Spain with the development and publication of the National Strategy for the Conservation of Crop Wild Relatives and Wild Food Plants (Molina et al. 2022, in press), supported by the Ministry of Agriculture, Fisheries and Food of Spain.

Conservation of crop wild relatives

The effective conservation of CWR should start with a clear delimitation of species aimed for conservation, that will depend on the scope of the action (global, regional or national) (Heywood et al., 2007). The creation of inventories or checklists delimiting CWR subject to conservation actions can follow an up-down or *floristic* approach that identifies CWR from the flora of the area of interest or a bottom-up or *monographic* approach, that firstly set priority crops and then identify their CWR in the targeted area (Maxted et al., 2011; Magos Brehm et al., 2013). Subsequently, if the generated CWR list is large in terms of number of species and might difficult management and conservation planning, CWR checklists should be further prioritized. The incorporation of conservation assessment information (*in situ* or *ex situ*) or any other relevant knowledge about their use in breeding, endemism or threat status (just to cite some), would finally turn the CWR checklist into a CWR inventory. Nowadays, we count on global, regional and national inventories and/or checklists that have been

developed over the last two decades. Vincent et al. developed in 2013 the first web-enabled global inventory of CWR containing 1667 taxa related to 173 priority crops important at the global level and identified gaps in *ex situ* collections as well as areas for collecting and fulfilling those gaps. In the same vein, Castañeda-Álvarez et al. (2016) also developed a global inventory that contained 1076 taxa related to 81 crops and likewise identified gaps in *ex situ* collections and priority areas for collecting missing diversity in genebanks. We found also efforts for inventorying CWR at the regional level, retrieving a Catalogue of CWR for Europe and the Mediterranean area (Kell et al., 2008) that recognizes 25,687 taxa as CWR with potential or actual socio-economic value (80% of the flora of the area). In the Fertile Crescent region around 4% of the flora (835 species) were identified as CWR and further prioritized, generating a checklist of 220 CWR species (Zair et al., 2018). North and South Africa regions also have their corresponding CWR inventories with 5780 CWR taxa (Lala et al., 2017) and 1900 CWR taxa (Allen et al., 2019) respectively, the latter further prioritized into 745 taxa. At the national level, we can see notable advances in the generation of CWR inventories and checklists (please see Iriando et al., 2016 and Labokas et al., 2018). Apart from Portugal which developed its inventory for CWR and wild harvested plants in 2008, the major advances in inventorying CWR have been achieved in the last decade. Since then, many countries have boosted the development of inventories or checklists of CWR. Europe has been very prolific in this sense and we find published inventories for Finland (Fitzgerald, 2013), Cyprus (Phillips et al., 2014), , England (Fielder et al., 2015a), Scotland (Fielder et al., 2015b), Norway (Phillips et al., 2016), the Czech Republic (Taylor et al., 2017), The Netherlands (van Treuren et al., 2017) and Italy (Ciancaleoni et al., 2021) who updated the work done by Landucci et al. (2014). African countries such as Benin (Idohou et al., 2013) and Zambia (Mwila et al., 2019) also have inventories, as well as the Asiatic Israel (Barazani et al., 2008), China (Kell et al., 2014) and Turkey (Tas et al., 2019). In America there are published CWR lists for Venezuela (Berlingeri and Crespo, 2012), the United States (Khoury et al., 2013) and Mexico (Contreras-Toledo et al., 2019).

However, the concern for the conservation of PGR is not new and emerged in the 1920s when the Russian botanist Nicolai Ivanovitch Vavilov was asked to gather germplasm for the maintenance and breeding of food and industrial crops (Bacchetta et al., 2008). He started thirty-years world expeditions aimed to the collection of PGR germplasm, including crop ancestors and their wild relatives, that were subsequently *ex situ* preserved, in the first germplasm bank created in the world (Brush, 1989). Soon after, in 1947 the United States also established seed collections of crop species and, since the 1970s, the *ex situ* conservation of PGR was spread around the world (Rajasekharan, 2015). The *ex situ* conservation has significant benefits, just to cite some, it gives the possibility of storage for the mid and long term with relatively low costs, grants rapid and easy access for the characterization of the stored germplasm and the rapid access to useful germplasm and allows the conservation of wide genetic diversity (Hawkes et al., 2000). It is also a way to back up the *in situ* diversity, allowing to reintroduce or reinforce species populations if needed. However, evolutionary processes take place in natural circumstances, when species are exposed to different conditions that exert selective pressures, so we can infer that the *ex situ* preserved material is an static picture of the genetic diversity in the moment of sampling. In fact, the *ex situ* conservation must be carefully designed as the genetic diversity of samples can be compromised (Hamilton, 1994). Regarding CWR, *ex situ* conservation has traditionally been the main pathway to conserve their diversity (Meilleur and Hodgkin, 2004).

Even so, the *in situ* conservation of CWR is pointed out as the first choice for preserving their diversity along with the habitats where they occur, in an efficient manner to conserve also the genetic diversity in continuous evolution, as reviewed by Meilleur and Hodgkin (2004). Diverse approaches and concepts for the implementation of such type of conservation targeting CWR have been developed especially in the last two decades (Heywood and Dulloo, 2005; Maxted and Kell, 2009; Maxted et al., 2015). Nowadays, the best strategy for the implementation of *in situ* conservation actions for CWR protection is through the establishment of *Genetic Reserves*. The genetic reserve concept (Maxted et al., 1997b) involves the active managing of the sites together with the design of strategies that would guarantee their long-term endurance (Kell et al., 2012). The rationale for the establishment of a genetic reserve is the dynamic conservation of the genetic diversity of the targeted CWR (Iriando et al., 2012). In this sense, a genetic reserve must comply with a minimum of quality standards that involve the identification of suitable localities, with a sufficient extent to take the action, a concrete list of targeted taxa and populations and a management plan (Iriando et al., 2012). Furthermore, the

genetic reserve approach enables the conservation of multiple species in the same place, following a multi-species approach. A multi-species approach would – under the same costs – target multiple taxa which will increment the efficiency of the action. Ideally, the establishment of a genetic reserve must be accompanied by *ex situ* planning measures that ensure a safe back up of the genetic diversity of species protected in the reserve. That is the case, for example of the three recent created genetic reserves for CWR in the Biosphere Reserve Sierra del Rincón, in Madrid (Spain) (OAPN, 2020). For the establishment of these genetic reserves under the protection of the biosphere reserve, 15 CWR species were identified, populations inventoried and biotic and abiotically characterized and main risks for the populations survival identified (OAPN, 2021). Furthermore, the seeds from the 15 CWR species were sampled and sent to the germplasm bank ‘César Gómez Campo’, belonging to the Polytechnique University of Madrid for their long-term storage. This procedure of exploiting the synergies between *in situ* and *ex situ* techniques, is known as *complementary conservation*. The complementary conservation is endorsed by the CBD in its ninth article, that mandate the contracting countries to, “*as far as possible and as appropriate*”, complement *in situ* measures with *ex situ* actions (UN, 1992). Therefore, it is the recommended pathway to fully conserve CWR diversity and make it accessible to users (Maxted et al., 1997a; Fielder, 2015 and references therein). However, and despite the extensive literature and growing interest in CWR protection, their conservation has been neglected during decades, both *ex situ* (Hunter et al., 2012) and *in situ* (Maxted, 2003): still very few coordinated actions regarding their *in situ* conservation have been released (Álvarez-Muñiz et al., 2021) and their *ex situ* conservation is poorly addressed (Castañeda-Álvarez et al., 2016).

Genetic diversity and its conservation

An effective network design for *in situ* conservation is dependent on the location of the components of biodiversity, including genetic diversity (Humphries et al., 1995). The genetic component should be considered when planning conservation actions (Thomassen et al., 2011) and the genetic diversity of the species systematically monitored (Schwartz et al., 2006) to develop conservation strategies that preserve the genetic diversity of the species and its distribution within and among populations. Accordingly, populations selected for *in situ* and *ex situ* collection should be representative of the overall genetic diversity of the species (Parra-Quijano et al., 2012a) and various populations of the same species might be needed to achieve such representation. The development of conservation genomics techniques, *i.e.*, “use of new genomic techniques to solve problems in conservation biology”, is expected to generate great advances in conservation biology (Allendorf et al., 2010). The investment in developing new and affordable techniques to characterize species and populations genetics will allow to target species that otherwise would be relegated to lower priorities (*i.e.* non-threatened or endemic species). Still, nowadays, the molecular characterization of all populations of every target species to evaluate their genetic diversity and link it to conservation actions is currently not feasible. Consequently, only a select group of emblematic threatened species is genetically characterized for most or all of their populations, which leaves apart many important CWR.

The minimum standards for the conservation of the genetic diversity have been object of debate in the last decades, both for its *in situ* or *ex situ* preservation. For instance, Brown and Briggs (1991) suggested that the seeds of a minimum of five populations should be collected and *ex situ* preserved to ensure a good representation of the genetic diversity contained by endangered plant species. In fact, this minimum five populations has been used as reference targeting CWR conservation, and applied to *in situ* and *ex situ* conservation planning and assessment (Dulloo et al., 2008; Fielder et al., 2015a; Phillips et al., 2016). On the other hand, Whitlock et al. (2016) suggested that at least 35% of the populations of a given species should be *in situ* conserved to meet the CBD recommendation of preserving 70% of plant species genetic diversity (Objective II, target 9 of the Global Strategy for plant conservation targets 2011-2020) (UN CBD, 2010).

Genetic diversity and environment: Ecogeographic tools

Some of the pioneer studies on the role of the environment in the genetic polymorphisms of species date back more than 40 years (Hedrick et al., 1976). Throughout this period, many studies have found that environmental pressures may result in the genetic differentiation of populations (Wang and Bradburd, 2014) and thus, in genetic diversity of adaptive value. Because different environments may induce diverse adaptation patterns in

the genomes, one could infer that the diversity of environmental conditions could be a proxy for the estimation of genetic diversity of adaptive value. Different climatic, geographic, soil and other environmental variables have been used to construct ecoregional maps at different levels (global, regional, national) (Omernik, 1987; Olson et al., 2001; Abell et al., 2008; Sayre et al., 2020) that have been used for management or conservation purposes. However, they have been designed with little consideration for their possible link with species genetic diversity. Even so, Egan et al. (2018) found a good association between genetic variation and climate and landscape proxies as predictors for evolutionary processes in wild woodland strawberry and ecogeography was found to be extremely useful for the discrimination of wild *Helianthus* species (sunflower relatives) (Kantar et al., 2015) and wild *Ipomoea* species (sweetpotato relatives) (Khoury et al., 2015), finding patterns of adaptations, in the search for germplasm to be used with breeding purposes. On the contrary, Thormann et al. (2016) did not find such correlations when assessing wild barley germplasm in Jordan. Notwithstanding with this, if the aim is to protect adaptive variability, it is essential to conserve populations from all environments where the target species is found (Maxted et al., 2012).

Ecogeographical Land Characterization maps (ELC maps) classify a given territory according to its climatic, geophysical and geographical variables but, on the contrary to the above-mentioned maps, attempt to link possible adaptation patterns in plant species to differences in environments (Parra-Quijano et al., 2012a, 2012c). To do so, the variables that may be shaping a given species genome are taken into account. Is the researcher who, based on expert advice/knowledge or on objective processes (*i.e.*, multivariate analyses), decides which variables are most likely to be affecting the plant species ecology and distribution. Main differences with former maps rely on the inclusion of a larger number of abiotic variables to define the territory and the obtention of more reticulated maps, avoiding large, continuous and homogenous regions or units (Parra-Quijano et al., 2020), which are less likely to be operating selective pressures. The generation of an ELC map allows the researcher to decide the resolution according to the territory to be classified and its extension, which help to better adjust environmental variables and targeted plant species searching for adaptive traits. The utilization of this approach for conservation in CWR started to be widely used in the late 2000 and 2010 decades using them as a proxy for genetic diversity as shown by many recent works (Table 1) dealing with CWR conservation and access, for example: *i)* the development of national strategies for CWR conservation (see Labokas et al. (2018) and references therein), *ii)* identification of areas for *in situ* conservation and *iii)* *ex situ* assessment and collection design.

Table 1: Main applications of Ecogeographical Land Characterization maps in crop wild relative conservation and use, as a proxy to estimate and properly conserve CWR genetic diversity of adaptive value.

Development of National Strategies	Main results	Reference
Norway	Close to 48% (average) ELC diversity found in protected areas. Identification of priority of areas for <i>ex situ</i> collection.	(Phillips et al., 2016)
Czech Republic	Identification of 22 ELC areas for priority CWR <i>in situ</i> conservation Representativeness in genebanks assessed, low scores found.	(Taylor et al., 2017)
Mexico	Low representativeness of ELC diversity in protected areas. Most of ELC diversity underrepresented in genebanks, only 22% mid-high or highly represented.	(Contreras-Toledo et al., 2019)
Sweden	54% of ELC diversity represented in Protected areas selected for maximum representation of species.	(Weibull and Phillips, 2020)

Table 1: Main applications of Ecogeographical Land Characterization maps in crop wild relative conservation and use, as a proxy to estimate and properly conserve CWR genetic diversity of adaptive value.

Direct application for <i>in situ</i> conservation	Main results	Reference
Global level	Identification of priority areas for CWR conservation. Identify current and projected loss of potential genetic diversity in protected areas.	(Vincent et al., 2019)
<i>Ex situ</i> conservation	Main results	Reference
<i>Lupinus</i>	Designed and executed collection missions targeting underrepresented ELC categories in the Spanish <i>Lupinus</i> collections. Found phenotypic differences between populations from different ELC categories.	(Parra-Quijano et al., 2012b)
<i>Aegilops</i>	Identify areas for priority <i>ex situ</i> collection of <i>Aegilops</i> species inhabiting underrepresented ELC categories.	(Garcia et al., 2017)
Wild relatives of legumes and cereals	Identified ecogeographical gaps in Spanish seed banks collections for 98 wild relatives of legumes and cereals. Identified and ranked optimal areas for <i>ex situ</i> collection of gaps.	(García et al., 2017)
Priority CWR for the Fertile Crescent	Working with 441 priority CWR, reported poor or null representation of ecogeographical diversity in genebanks.	(Zair et al., 2021)

One of the most important advantages of the ecogeographic approaches are the association of traits of adaptive value to populations of CWR in particular environments, ready to be used in breeding (Kantar et al., 2015; Khoury et al., 2015). Such relationships were lucidly suspected to be worth explorable in the search for germplasm for crop breeding by Mackay and Street (2004), who conceived the Focused Identification of Germplasm Strategy (FIGS). The FIGS methodology aimed to identify candidate landrace accessions for breeding abiotic tolerances and biotic resistances, with higher success probabilities than if randomly selected, based on the environmental characteristics of the collecting sites. The FIGS technique was further developed and conceptually supported by the successful correlations found in nordic barley landraces between ecogeographic data and morphological traits (Endresen, 2010) and biotic stresses (Endresen et al., 2011) and the agreement between drought tolerance scores given by tested models and classification given by genebank curators (Bari et al., 2016). FIGS was also experimentally tested in many works, identifying subsets of wheat accessions resistant to powdery mildew (Bhullar et al., 2010), association of resistance to wheat stem rust to certain environments (Bari et al., 2012) and successfully predicting drought tolerance traits in *Vicia faba* L. accessions (Khazaei et al., 2013). Based on the FIGS methodology, Thormann et al. (2014b, 2014a) proposed the Predictive Characterization technique for its application in CWR. Mirroring back the FIGS methodology, Predictive Characterization aims to the identification of populations and accessions of CWR with higher probabilities of containing target adaptive traits that if randomly selected. They argued that associations between environment and genetic diversity of adaptive value in CWR would be stronger than for cultivated varieties and landraces, due to their evolution in natural conditions. The Predictive Characterization technique can be applied by means of two methods: the ecogeographic filtering method or the calibration method (Figure 1). The ecogeographic filtering method is established under the basis of associations between environmental conditions and the adaptive characteristics that are likely to occur in plants inhabiting those particular environments. That is, if a population of a plant species is inhabiting an arid environment, this population will be more likely to possess adaptation to drought than other populations that do not occur in arid where such conditions do not concur. On the other hand, the calibration method, also based on that hypothesis, uses mathematical models to infer the probabilities of containing the desired trait, using as training data accessions of populations already characterized for the targeted trait, for example resistance to a plant disease.

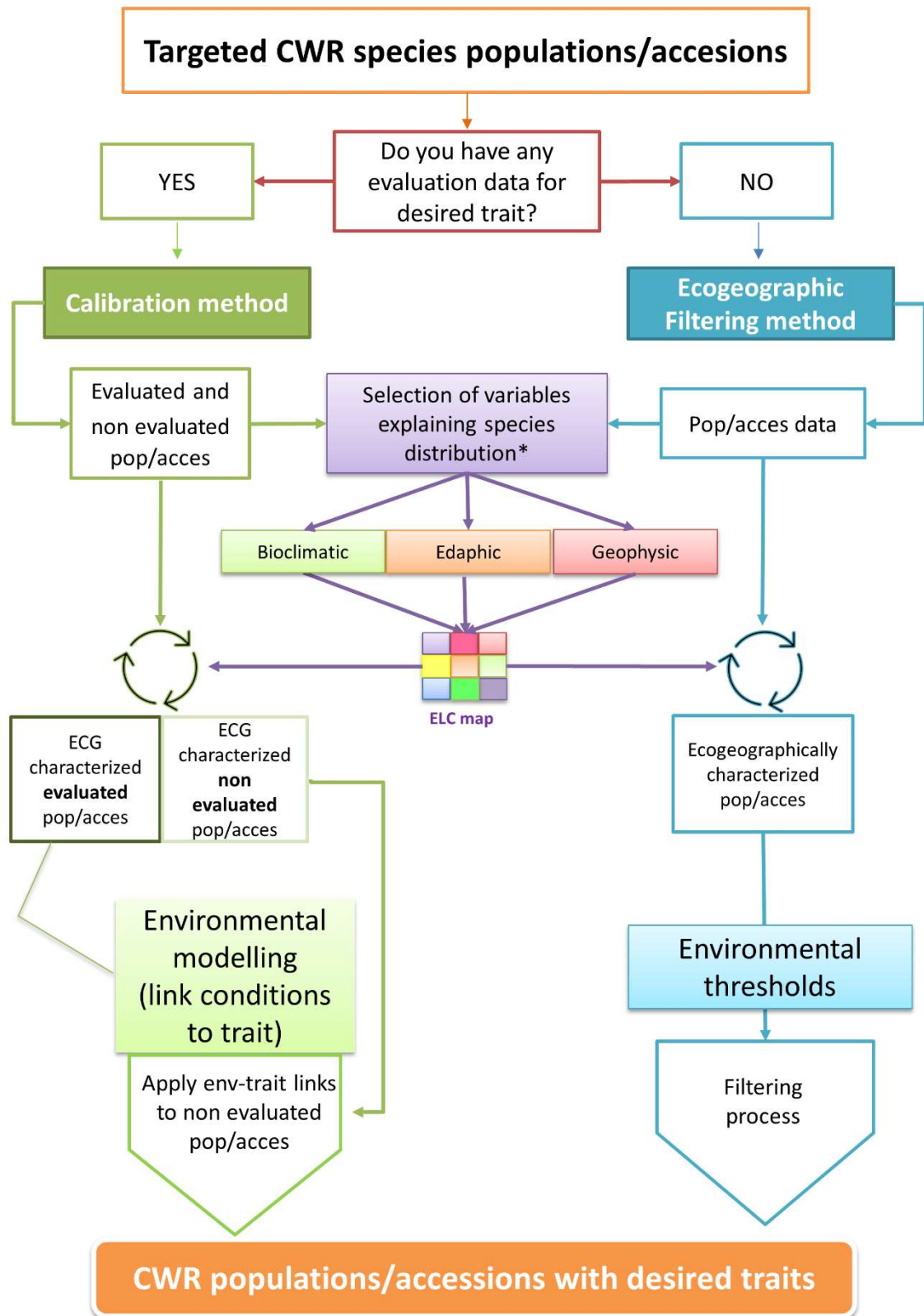


Figure 1: Steps for the application of the Predictive Characterization techniques.

Rationale for the thesis and objectives

This doctoral thesis was conceived at a time where no previous studies were available on the identification of priority crop wild relatives for Spain or their conservation status. Taking into account the relevance of the problem of the genetic erosion in crops in the context of global change, the main objective of this thesis was to contribute to setting the conceptual and methodological basis for an effective and sustainable conservation, access and use of crop wild relatives in Spain.

To achieve this goal, we posed five specific objectives: **I)** Delimitation and prioritization of CWR in Spain, **II)** Assessment of conservation status of priority CWR of Spain, **III)** Application and evaluation of experimental approaches that focus on multiple CWR species conservation, **IV)** Addressing the infraspecific genetic diversity component when designing CWR conservation and collecting plans, and **V)** Application and evaluation of predictive characterization techniques that identify CWR populations more likely to contain desired traits for plant breeding, facilitating their access and use. These specific goals contribute to addressing challenges and gaps identified at the different stages of the conservation-access-utilization continuum (Figure 2).

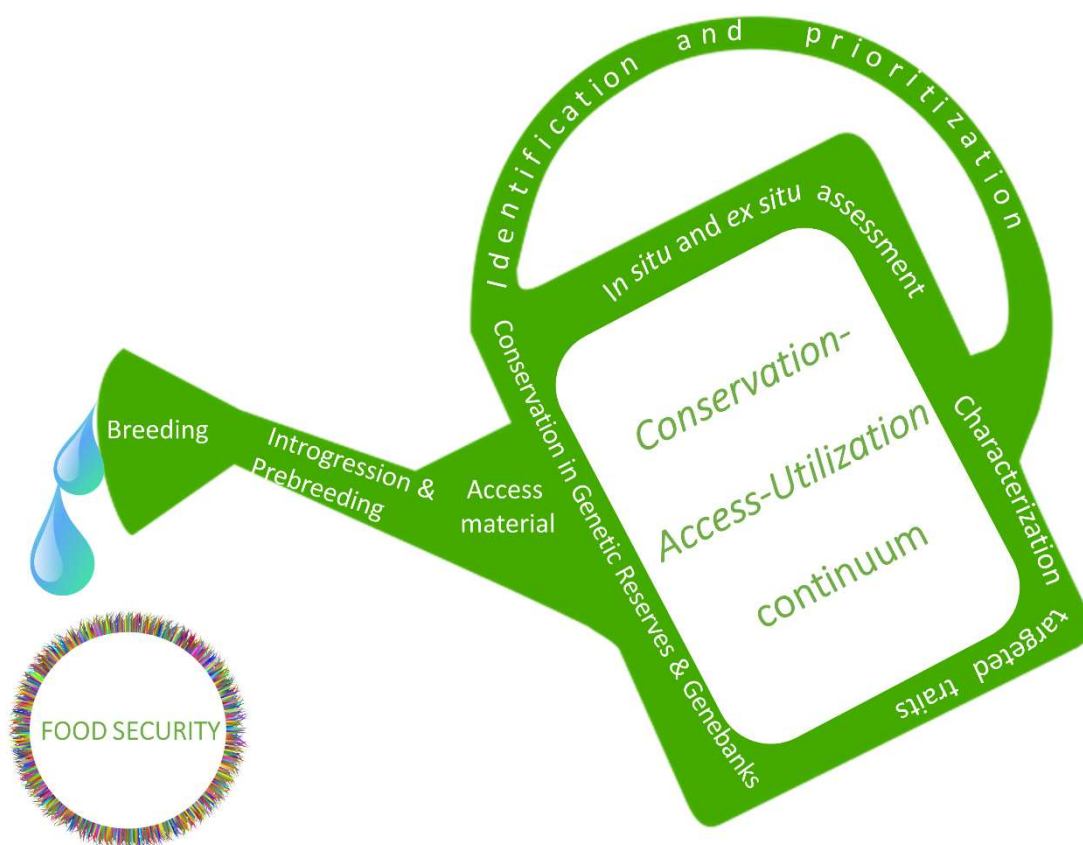


Figure 2. Main steps of the conservation, access and utilization continuum of plant genetic resources in general, and CWR in particular, and placement of the specific objectives along this continuum.

Workplan

This thesis is structured in four interconnected chapters (Figure 3). The chapters have been designed to achieve the main objective of the thesis and structured following the typical configuration of research articles, to be published in international peer-reviewed journals (i.e.: Introduction, material and methods, results, discussion and conclusions). At the end of the thesis, we present a general discussion that evaluates the achievement of the general and specific objectives mentioned above.

Objectives set to enhance the *conservation-access-utilization* continuum

Chapter 1	Objective I: Delimitation and prioritization of CWR in Spain Objective II: Assessment of conservation status of priority CWR of Spain
Chapter 2	Objective II: Assessment of conservation status of priority CWR of Spain
Chapter 3	Objective III: Application and evaluation of experimental approaches that focus on multiple CWR species conservation Objective IV: Addressing the infraspecific genetic diversity component when designing CWR conservation and collecting plans
Chapter 4	Objective IV: Addressing the infraspecific genetic diversity component when designing CWR conservation and collecting plans Objective V: Application and evaluation of predictive characterization techniques that identify CWR populations more likely to contain desired traits for plant breeding, facilitating their access and use

Figure 3: Schematic representation of the chapters and specific objectives addressed in each one

In the **first chapter** we face the first specific objective, aiming to define a National Inventory of CWR for Spain, setting up a dynamic baseline of CWR species important for Spain in terms of socio-economic importance of crops, crossability to crops, endemism or threat status. In this way, we asked which criteria should be applied for prioritization of CWR in Spain and what is the threat status and degree of endemism of the prioritized CWR checklist. These CWR were also assessed in their abundance in the country (number of species and populations in each Autonomous Community), in their presence in national and international genebanks, and on whether they were under any legal protection in Spain tackling objective II.

In **chapter two**, targeting specific objective II, we aimed to gain deeper insight into the *in situ* and *ex situ* conservation status of priority CWR of Spain and identify CWR that require urgent conservation measures. To that purpose, those CWR simultaneously classified as easier to cross with crops, endemic to Spain and in any threat category of the IUCN were identified and further prioritized. To understand the needs for conservation, the National Catalogue of Protected Species of Spain, as well as the Catalogues of Protected Species for each Autonomous Communities were consulted. Those CWR that were threatened but lacked legal protection were pinpointed, finding 11 species that should be proposed into such Catalogues. Finally, herein prioritized CWR were evaluated in their current conservation status, checking their representation in protected areas and in national and international genebanks.

In the **third chapter**, we tackle objectives III and IV, and work towards an integrative approach for CWR conservation. We make use of existing concepts and tools that help targeting groups of species that tend to grow together (phytosociological associations) and include the genetic diversity component of adaptive value in the conservation assessments using ELC maps, answering whether phytosociological associations can be a useful approach to design and implement *in situ* conservation measures for CWR. To illustrate the method, we work with fodder and forage priority CWR of Spain and develop an ELC map to capture potential adaptations given by bioclimatic and other environmental conditions. Through the combination of the CWR associations with the ELC environments they inhabit, we create a new target conservation unit that includes, both the associations (species diversity) and the genetic diversity component. Subsequently, we include a gap analysis to

check whether Natura 2000 network can properly conserve the target CWR conservation units and, finally, a complementarity analysis is performed to identify the priority sites for the establishment of genetic reserves for fodder and forage CWR.

Lastly, the **fourth chapter** addresses not only the inclusion of the genetic diversity component of the species into their populations, but also their potentiality to be used as gene donors in plant breeding processes. Thus, in this chapter we deal with objectives IV and V posing that it is possible to identify wild lentil populations tolerant to abiotic stresses, based on their ecological range and identify populations of wild lentils more probable to be tolerant to drought, soil salinity, waterlogging. We furthermore pose that ecogeographic variables associated with each population will allow to train mathematical models that will identify better wild lentil populations resistant to lentil rust than if randomly selected. To reach this goal, we apply Predictive Characterization methodologies that link environmental conditions to the probability of occurrence of the trait and thus, diminish the costs of field trials to confirm the existence of the desired trait against the random selection of populations. In order to increase the statistical robustness of the analyses and increment the chances of success, the geographic scope of this chapter was opened to Europe and Turkey and, consequently, the number of populations under analysis increased.

References

- Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., et al. (2008). Freshwater Ecoregions of the World: A New Map of Biogeographic Units for Freshwater Biodiversity Conservation. 58. doi:10.1641/B580507.
- Allen, E., Gaisberger, H., Magos Brehm, J., Maxted, N., Thormann, I., Lupupa, T., et al. (2019). A crop wild relative inventory for Southern Africa: a first step in linking conservation and use of valuable wild populations for enhancing food security. *Plant Genet. Resour.*, 1–12. doi:10.1017/S1479262118000515.
- Allendorf, F. W., Hohenlohe, P. A., and Luikart, G. (2010). Genomics and the future of conservation genetics. *Nat. Rev. Genet.* 11, 697–709. doi:10.1038/nrg2844.
- Álvarez-Muñiz, C., Magos-Brehm, J., Ralli, P., Palmé, A., Dulloo, M. E., Maxted, N., et al. (2021). Crop wild relative network showcases – analysis and best practices. Farmer's Pride: Networking, partnerships and tools to enhance in situ conservation of European plant genetic resources.
- Aryamanesh, N., Byrne, O., Hardie, D. C., Khan, T., Siddique, K. H. M., and Yan, G. (2012). Large-scale density-based screening for pea weevil resistance in advanced backcross lines derived from cultivated field pea (*Pisum sativum*) and *Pisum fulvum*. *Crop Pasture Sci.* 63, 612–618. doi:10.1071/CP12225.
- Ashraf, M. (2010). Inducing drought tolerance in plants: recent advances. *Biotechnol. Adv.* 28, 169–83. doi:10.1016/j.biotechadv.2009.11.005.
- Bacchetta, G., Bueno Sánchez, A., Fenu, G., Jiménez-Alfaro, B., Mattana, E., Piotto, B., et al. eds. (2008). *Conservación ex situ de plantas silvestres*. Principado de Asturias / La Caixa.
- Barazani, O., Perevolotsky, A., and Hadas, R. (2008). A problem of the rich: Prioritizing local plant genetic resources for ex situ conservation in Israel. *Biol. Conserv.* doi:10.1016/j.biocon.2007.10.014.
- Bari, A., Khazaei, H., Stoddard, F. L., Street, K., Sillanpää, M. J., Chaubey, Y. P., et al. (2016). In silico evaluation of plant genetic resources to search for traits for adaptation to climate change. *Clim. Change* 134, 667–680. doi:10.1007/s10584-015-1541-9.
- Bari, A., Street, K., Mackay, M., Endresen, D. T. F., de Pauw, E., and Amri, A. (2012). Focused identification of germplasm strategy (FIGS) detects wheat stem rust resistance linked to environmental variables. *Genet. Resour. Crop Evol.* 59, 1465–1481. doi:10.1007/s10722-011-9775-5.
- Berlingeri, C., and Crespo, M. B. (2012). Inventory of related wild species of priority crops in Venezuela. *Genet. Resour. Crop Evol.* doi:10.1007/s10722-011-9709-2.
- Bhullar, N. K., Zhang, Z., Wicker, T., and Keller, B. (2010). Wheat gene bank accessions as a source of new alleles of the powdery mildew resistance gene Pm3: A large scale allele mining project. *BMC Plant Biol.* 10, 1471–2229. doi:10.1186/1471-2229-10-88.
- Bleeker, P. M., Mirabella, R., Diergaarde, P. J., VanDoorn, A., Tissier, A., Kant, M. R., et al. (2012). Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. *Proc. Natl. Acad. Sci. U. S. A.* 109, 20124–20129. doi:10.1073/pnas.1208756109.
- Brown, A. H. D., and Briggs, J. D. (1991). “Sampling strategies for genetic variation in ex situ collectoins of endangered plant species,” in *Genetics and conservation of rare plants*, eds. D. A. I. Falk and K. E. Holsinger (New York; Oxford: Oxford University Press), 99–119.

- Brozynska, M., Furtado, A., and Henry, R. J. (2016). Genomics of crop wild relatives: Expanding the gene pool for crop improvement. *Plant Biotechnol. J.* 14, 1070–1085. doi:10.1111/pbi.12454.
- Brush, S. B. (1989). Rethinking Crop Genetic Resource Conservation: A View From the South. *Conserv. Biol.* 3, 19–29. doi:10.1111/j.1523-1739.1989.tb00227.x.
- Castañeda-Álvarez, N. P., Khoury, C. K., Achicanoy, H. A., Bernau, V., Dempewolf, H., Eastwood, R. J., et al. (2016). Global conservation priorities for crop wild relatives. *Nat. Plants* 2, 1–6. doi:10.1038/nplants.2016.22.
- Ciancaleoni, S., Raggi, L., Barone, G., Donnini, D., Gigante, D., Domina, G., et al. (2021). A new list and prioritization of wild plants of socioeconomic interest in Italy: toward a conservation strategy. *Agroecol. Sustain. Food Syst.* doi:10.1080/21683565.2021.1917469.
- Contreras-Toledo, A. R., Cortés-Cruz, M., Costich, D. E., Rico-Arce, M. D. L., Magos Brehm, J., and Maxted, N. (2019). Diversity and conservation priorities of crop wild relatives in Mexico. *Plant Genet. Resour. Characterisation Util.* 17, 140–150. doi:10.1017/S1479262118000540.
- Conway, G. R., and Barbic, E. B. (1988). After the Green Revolution. Sustainable and equitable agricultural development. *Futures* 20, 651–670. doi:10.1016/0016-3287(88)90006-7.
- Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., and Guarino, L. (2017). Past and future use of wild relatives in crop breeding. *Crop Sci.* 57, 1070–1082. doi:10.2135/cropsci2016.10.0885.
- Dulloo, M. E., Labokas, J., Iriundo, J. M., Maxted, N., Lane, A., Laguna, E., et al. (2008). “Genetic reserve location and design,” in *Conserving plant genetic diversity in protected areas*, eds. J. M. Iriundo, N. Maxted, and M. E. Dulloo (Wallingford, UK: CAB International), 23–64.
- Egan, P. A., Muola, A., and Stenberg, J. A. (2018). Capturing genetic variation in crop wild relatives: An evolutionary approach. *Evol. Appl.*, 1–12. doi:10.1111/eva.12626.
- Ehrlich, P. R., Ehrlich, A. H., and Daily, G. C. (1993). Food Security, Population and Environment. *Popul. Dev. Rev.* 19, 1–32.
- Endresen, D. T. F. (2010). Predictive association between trait data and ecogeographic data for Nordic barley landraces. *Crop Sci.* 50, 2418–2430. doi:10.2135/cropsci2010.03.0174.
- Endresen, D. T. F., Street, K., Mackay, M., Bari, A., and de Pauw, E. (2011). Predictive association between biotic stress traits and eco-geographic data for wheat and barley landraces. *Crop Sci.* 51, 2036–2055. doi:10.2135/cropsci2010.12.0717.
- Esquinas-Alcázar, J. (2005). Science and society: protecting crop genetic diversity for food security: political, ethical and technical challenges. *Nat. Rev. Genet.* 6, 946–53. doi:10.1038/nrg1729.
- Evenson, R. E., Gollin, D., and Africa, N. (2003). Assessing the Impact of the Green Revolution , 1960 to 2000. *Science* (80-.). 300, 758–763.
- FAO (1996). Report on the State of the World’s Plant Genetic Resources for Food and Agriculture. Rome, Italy.
- FAO (2009). International Treaty on Plant Genetic Resources for Food and Agriculture. Rome, Italy.
- FAO, F. and A. O. of the U. N. (2010). *The Second Report on the State of the World’s Plant Genetic Resources for Food and Agriculture*. doi:ISBN 978-92-5-106534-1.
- Fielder, H., Brotherton, P., Hosking, J., Hopkins, J. J., Ford-Lloyd, B., and Maxted, N. (2015a). Enhancing the Conservation of Crop Wild Relatives in England. *PLoS One* 10, e0130804. doi:10.1371/journal.pone.0130804.
- Fielder, H., Smith, C., Ford-Lloyd, B., and Maxted, N. (2015b). Enhancing the conservation of crop wild relatives in Scotland. *J. Nat. Conserv.* 29, 51–61. doi:10.1016/j.jnc.2015.11.002.
- Fielder, H. V. (2015). Developing methodologies for the genetic conservation of UK crop wild relatives.
- Fitzgerald, H. (2013). The National Crop Wild Relative Strategy Report for Finland.
- García-Mozo, H., Mestre, A., and Galán, C. (2010). Phenological trends in southern Spain: A response to climate change. *Agric. For. Meteorol.* 150, 575–580. doi:10.1016/j.agrformet.2010.01.023.
- García, R. M., Parra-Quijano, M., and Iriundo, J. M. (2017). Identification of ecogeographical gaps in the Spanish Aegilops collections with potential tolerance to drought and salinity. *PeerJ* 2017. doi:10.7717/peerj.3494.
- García, R. M., Parra-Quijano, M., and Iriundo, J. M. (2017). A multispecies collecting strategy for crop wild relatives based on complementary areas with a high density of ecogeographical gaps. *Crop Sci.* 57, 1059–1069. doi:10.2135/cropsci2016.10.0860.
- Gross, B. L., and Olsen, K. M. (2010). Genetic perspectives on crop domestication. *Trends Plant Sci.* 15, 529–537. doi:10.1016/j.tplants.2010.05.008.
- Hamilton, M. B. (1994). Ex situ conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks. *Conserv. Biol.* 8, 39–49. Available at: <http://www.jstor.org/stable/2386719>.

- Hasegawa, T., Fujimori, S., Havlík, P., Valin, H., Bodirsky, B. L., Doelman, J. C., et al. (2018). Risk of increased food insecurity under stringent global climate change mitigation policy. *Nat. Clim. Chang.* 8, 699–703. doi:10.1038/s41558-018-0230-x.
- Hawkes, J. G., Maxted, N., and Ford-Lloyd, B. V. (2000). “The genetic resources of plants and their value,” in *The ex situ conservation of plant genetic resources*, eds. J. G. Hawkes, N. Maxted, and B. V. Ford-Lloyd (Springer Science & Business Media), 1–18.
- Hedrick, P. W., Ginevan, M. E., and Ewing, E. P. (1976). Genetic Polymorphism in Heterogeneous Environments. *Annu. Rev. Ecol. Syst.* 7, 1–32.
- Heywood, V., Casas, A., Ford-Lloyd, B., Kell, S., and Maxted, N. (2007). Conservation and sustainable use of crop wild relatives. *Agric. Ecosyst. Environ.* 121, 245–255. doi:10.1016/j.agee.2006.12.014.
- Heywood, V. H., and Dulloo, M. E. (2005). In Situ Conservation of Wild Plant Species: A Critical Global Review of Good Practices. *IPGRI Tech. Bull.* doi:10.1663/0013-0001(2007)61[205:ISCOWP]2.0.CO;2.
- Humphries, C. J., Williams, P. H., and Vane-Wright, R. I. (1995). Measuring biodiversity value for conservation. *Annu. Rev. Ecol. Syst.* 26, 111.
- Hunter, D., Guarino, L., Khoury, C. K., and Dempewolf, H. (2012). “A community divided: Lessons from the conservation of crop wild relatives around the world,” in *Agrobiodiversity Conservation: Securing the diversity of crop wild relatives and landraces*, eds. N. Maxted, M. E. Dulloo, B. V. Ford-Lloyd, L. Frese, J. M. Iriondo, and M. A. A. Pinheiro de Carvalho (Wallingford, Oxfordshire, UK: CAB International), 298–304.
- Idohou, R., Assogbadjo, A. E., Fandohan, B., Gouwakinnou, G. N., Glele Kakai, R. L., Sinsin, B., et al. (2013). National inventory and prioritization of crop wild relatives: Case study for Benin. *Genet. Resour. Crop Evol.* 60, 1337–1352. doi:10.1007/s10722-012-9923-6.
- Iglesias, A., Rosenzweig, C., and Pereira, D. (2000). Agricultural impacts of climate change in Spain: Developing tools for a spatial analysis. *Glob. Environ. Chang.* 10, 69–80. doi:10.1016/S0959-3780(00)00010-8.
- Iriondo, J. M., Fielder, H., Fitzgerald, H., Kell, S. P., Labokas, J., Negri, V., et al. (2016). “National Strategies for the Conservation of Crop Wild Relatives,” in *Enhancing Crop Gene Pool Use. Capturing wild relative and landrace diversity for crop improvement*, eds. N. Maxted, M. E. Dulloo, and B. V. Ford-Lloyd (CAB International), 161–171.
- Iriondo, J. M., Maxted, N., Kell, S. P., Ford-Lloyd, B. V., Lara-Romero, C., Labokas, J., et al. (2012). “Quality standards for genetic reserve conservation of crop wild relatives,” in *Agrobiodiversity Conservation: Securing the diversity of crop wild relatives and landraces*, eds. N. Maxted, M. E. Dulloo, B. V. Ford-Lloyd, L. Frese, J. M. Iriondo, and M. A. A. Pinheiro de Carvalho (Oxfordshire, UK: CAB International), 72–77.
- Kantar, M. B., Sosa, C. C., Khoury, C. K., Castañeda-Álvarez, N. P., Achicanoy, H. a, Bernau, V., et al. (2015). Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (*Helianthus annuus* L.). *Front. Plant Sci.* 6, 1–11. doi:10.3389/fpls.2015.00841.
- Kell, S., Knüpfner, H., Jury, S., Ford-Lloyd, B., and Maxted, N. (2008). “Crops and Wild Relatives of the Euro-Mediterranean Region: Making and Using a Conservation Catalogue,” in, 67–109.
- Kell, S. P., Maxted, N., Frese, L., and Iriondo, J. M. (2012). “In situ conservation of crop wild relatives: A strategy for identifying priority genetic reserves,” in *Agrobiodiversity Conservation: Securing the diversity of crop wild relatives and landraces*, eds. N. Maxted, M. E. Dulloo, B. V. Ford-Lloyd, L. Frese, J. M. Iriondo, and M. A. A. Pinheiro de Carvalho (Oxfordshire, UK: CAB International), 7–19.
- Kell, S., Qin, H., Chen, B., Ford-Lloyd, B., Wei, W., Kang, D., et al. (2014). China’s crop wild relatives: Diversity for agriculture and food security. *Agric. Ecosyst. Environ.* 209, 138–154. doi:10.1016/j.agee.2015.02.012.
- Khazaei, H., Street, K., Bari, A., Mackay, M., and Stoddard, F. L. (2013). The FIGS (focused identification of germplasm strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. *PLoS One* 8, e63107. doi:10.1371/journal.pone.0063107.
- Khoury, C. K., Bjorkman, A. D., Dempewolf, H., Ramirez-Villegas, J., Guarino, L., Jarvis, A., et al. (2014). Increasing homogeneity in global food supplies and the implications for food security. *PNAS* 111, 4001–4006. doi:10.1073/pnas.1313490111.
- Khoury, C. K., Greene, S., Wiersema, J., Maxted, N., Jarvis, A., and Struik, P. C. (2013). An inventory of crop wild relatives of the United States. *Crop Sci.* 53, 1496–1508. doi:10.2135/cropsci2012.10.0585.
- Khoury, C. K., Heider, B., Castañeda-Álvarez, N. P., Achicanoy, H. A., Sosa, C. C., Miller, R. E., et al. (2015). Distributions, ex situ conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato [*Ipomoea batatas* (L.) Lam., I. series Batatas]. *Front. Plant Sci.* 6, 1–14. doi:10.3389/fpls.2015.00251.
- Kotschi, J. (2006). Coping with climate change and the role of agrobiodiversity. in *Conference on International Agricultural Research for Development* (Tropentag, University of Bonn, October 11-13), 29.
- Labokas, J., Maxted, N., Kell, S., Brehm, J. M., and Iriondo, J. M. (2018). Development of national crop wild relative conservation strategies in European countries. *Genet. Resour. Crop Evol.* 65, 1385–1403. doi:10.1007/s10722-018-0621-x.

- Lala, S., Amri, A., and Maxted, N. (2017). Towards the conservation of crop wild relative diversity in North Africa: checklist, prioritisation and inventory. *Genet. Resour. Crop Evol.*, 1–12. doi:10.1007/s10722-017-0513-5.
- Landucci, F., Panella, L., Lucarini, D., Gigante, D., Donnini, D., Kell, S., et al. (2014). A prioritized inventory of crop wild relatives and wild harvested plants of Italy. *Crop Sci.* 547, 1628–1644.
- Mackay, M. C., and Street, K. (2004). Focused identification of germplasm strategy – FIGS. in *Proceedings of the 54th Australian Cereal Chemistry Conference and the 11th Wheat Breeders' Assembly*. (Melbourne: Royal Australian Chemical Institute), 138–141.
- Magos Brehm, J., Kell, S., Thormann, I., Gaisberger, H., Dulloo, E., and Maxted, N. (2013). INTERACTIVE TOOLKIT FOR CROP WILD RELATIVE CONSERVATION PLANNING version 1.0. Resource Book for the Preparation of National Plans of Crop Wild Relatives and Landraces. Food and Agriculture Organization of the In Situ Conservation and Use of Crop Wild Rela.
- Massawe, F., Mayes, S., and Cheng, A. (2016). Crop Diversity: An Unexploited Treasure Trove for Food Security. *Trends Plant Sci.* 21, 365–368. doi:10.1016/j.tplants.2016.02.006.
- Maxted, N. (2003). Conserving the genetic resources of crop wild relatives in European protected areas. *Biol. Conserv.* 113, 411–417. doi:10.1016/S0006-3207(03)00123-X.
- Maxted, N., Avagyan, A., Frese, L., Iriondo, J., Brehm, J. M., Singer, A., et al. (2015). ECPGR Concept for in situ conservation of crop wild relatives in Europe. Wild Species Conservation in Genetic Reserves Working Group. European Cooperative Programme for Plant Genetic Resources. Rome, Italy. Available at: www.ecpgr.cgiar.org/fileadmin/temp.21. Available at: www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/WG_UPLOADS_PHASE_IX/WILD_SPECIES/%0AConcept_for_in_situ_conservation_of_CWR_in_Europe.pdf%0A.
- Maxted, N., Ford-Lloyd, B. V., and Hawkes, J. G. (1997a). “Complementary conservation strategies,” in *Plant genetic conservation: the in situ approach*, eds. N. Maxted, B. V. Ford-Lloyd, and J. G. Hawkes (Luxembourg: Springer Science & Business Media), 15–39.
- Maxted, N., Hawkes, J. G., Ford-Lloyd, B. V., and Williams, J. T. (1997b). “A Practical Model for In Situ Genetic Conservation,” in *Plant genetic conservation: the in situ approach*, eds. N. Maxted, B. V. Ford-Lloyd, and J. G. Hawkes (London, UK: Chapman and Hall), 545–592.
- Maxted, N., and Kell, S. (2009). Establishment of a Global Network for the In Situ Conservation of Crop Wild Relatives: Status and Needs. *FAO Comm. Genet. Resour. Food Agric. Rome, Italy. Backgr. study Pap. No. 39*, 266.
- Maxted, N., Kell, S., and Brehm, J. M. (2011). Options to Promote Food Security: On-Farm Management and In Situ Conservation of Plant Genetic Resources for Food and Agriculture. *Commission Genet. Resour. Food Agric.*, 25. Available at: <http://www.fao.org/docrep/meeting/022/am489e.pdf>.
- Maxted, N., Kell, S., Ford-Lloyd, B., Dulloo, E., and Toledo, Á. (2012). Toward the systematic conservation of global crop wild relative diversity. *Crop Sci.* 52, 774–785. doi:10.2135/cropsci2011.08.0415.
- MedECC (2019). Risks associated to climate and environmental changes in the Mediterranean region.
- Meilleur, B. a., and Hodgkin, T. (2004). In situ conservation of crop wild relatives: Status and trends. *Biodivers. Conserv.* 13, 663–684. doi:10.1023/B:BIOC.0000011719.03230.17.
- Meyer, R. S., and Purugganan, M. D. (2013). Evolution of crop species: Genetics of domestication and diversification. *Nat. Rev. Genet.* 14, 840–852. doi:10.1038/nrg3605.
- Molina Pertúñez, A., Torres Lamas, E., Rubio Teso, M.L., Álvarez Muñoz, C., de la Rosa Fernández, L., Rincón Martín, V., Tardío Pato, J., Guasch Pereira, L., Iriondo Alegría, J.M. (en prensa). Estrategia Nacional de Conservación y Utilización de Parientes Silvestres de los Cultivos (PSC) y Plantas Silvestres de Uso Alimentario (PSUA). Ministerio de Agricultura, Pesca y Alimentación. Madrid. 114 pp.
- MTERD (2021). Plan Nacional de Adaptacion al Cambio Climático 2021-2030. Madrid, Spain.
- Mwila, G., Gaisberger, H., International, B., Brehm, J. M., and Kell, S. (2019). Spatial analyses of occurrence data of crop wild relatives (CWR) taxa as tools for selection of sites for conservation of priority CWR in Zambia. *Plant Genet. Resour.*, 1–12. doi:10.1017/S1479262118000497.
- OAPN (2020). Conservación in situ y ex situ de parientes silvestres de los cultivos en la Reserva de la Biosfera de la Sierra del Rincón. 37–38.
- OAPN (2021). Conservación in situ y ex situ de parientes silvestres de especies cultivadas en la Reserva de la Biosfera de la Sierra del Rincón. 42–46.
- Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., et al. (2001). Terrestrial ecoregions of the world: a new map of life on Earth. *Bioscience* 51, 933–938.
- Omernik, J. M. (1987). Map Supplement: Ecoregions of the Conterminous United States. *Ann. Assoc. Am. Geogr.* 77, 118–125.
- Parra-Quijano, M., Iriondo, J. M., and Torres, E. (2012a). Ecogeographical land characterization maps as a tool for assessing

- plant adaptation and their implications in agrobiodiversity studies. *Genet. Resour. Crop Evol.* 59, 205–217. doi:10.1007/s10722-011-9676-7.
- Parra-Quijano, M., Iriondo, J. M., and Torres, E. (2012b). Improving representativeness of genebank collections through species distribution models, gap analysis and ecogeographical maps. *Biodivers. Conserv.* 21, 79–96. doi:10.1007/s10531-011-0167-0.
- Parra-Quijano, M., Iriondo, J. M., and Torres, E. (2012c). Review. Applications of ecogeography and geographic information systems in conservation and utilization of plant genetic resources. *Spanish J. Agric. Res.* 10, 419–429. doi:10.5424/sjar/2012102-303-11.
- Parra-Quijano, M., Iriondo, J. M., Torres, M. E., López, F., Maxted, N., and Kell, S. (2020). CAPFITOGEN3. A toolbox for the conservation and promotion of the use of agricultural biodiversity. 303.
- Phillips, J., Asdal, Å., Magos Brehm, J., Rasmussen, M., and Maxted, N. (2016). In situ and ex situ diversity analysis of priority crop wild relatives in Norway. *Divers. Distrib.* 22, 1112–1126. doi:10.1111/ddi.12470.
- Phillips, J., Kyrtzias, A., Christoudoulou, C., Kell, S., and Maxted, N. (2014). Development of a national crop wild relative conservation strategy for Cyprus. *Genet. Resour. Crop Evol.* 61, 817–827. doi:10.1007/s10722-013-0076-z.
- Prohens, J., Gramazio, P., Plazas, M., Dempewolf, H., Kilian, B., Díez, M. J., et al. (2017). Introgressomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. *Euphytica* 213. doi:10.1007/s10681-017-1938-9.
- Rajasekharan, P. E. (2015). “Gene Banking for Ex Situ Conservation of Plant Genetic Resources,” in *Plant Biology and Biotechnology: Volume II: Plant Genomics and Biotechnology*, eds. B. Bahadur, R. M. Venkat, L. Sahjram, and K. Krishnamurthy (New Delhi, India: Springer), 445–459. doi:10.1007/978-81-322-2283-5.
- Redden, R. (2015). “Wild relatives for the crop improvement challenges of climate change: the adaptation range of crops,” in *Crop wild relatives and climate change*, eds. R. Redden, S. S. Yadav, N. Maxted, M. E. Dulloo, L. Guarino, and P. Smith (New Jersey, USA: Wiley-Blackwell), 61–79.
- Rosenzweig, C., Elliott, J., Deryng, D., Ruane, A. C., Müller, C., Arneth, A., et al. (2014). Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. *PNAS* 111, 3268–3273. doi:10.1073/pnas.1222463110.
- Rosenzweig, C., and Parry, M. L. (1994). Potential impact of climatic change on world food supply. *Nature* 367, 133–138.
- Sayre, R., Karagulle, D., Frye, C., Boucher, T., Wolff, N. H., Breyer, S., et al. (2020). An assessment of the representation of ecosystems in global protected areas using new maps of World Climate Regions and World Ecosystems. *Glob. Ecol. Conserv.* 21, e00860. doi:10.1016/j.gecco.2019.e00860.
- Schwartz, M. K., Luikart, G., and Waples, R. S. (2006). Genetic monitoring as a promising tool for conservation and management. *Trends Ecol. Evol.* 22, 25–33. doi:10.1016/j.tree.2006.08.009.
- Seiler, G. J., Qi, L. L., and Marek, L. F. (2017). Utilization of Sunflower Crop Wild Relatives for Cultivated Sunflower Improvement. *Crop Sci.* 57, 1083–1101. doi:10.2135/cropsci2016.10.0856.
- Smýkal, P., Nelson, M. N., Berger, J. D., and Von Wettberg, E. J. B. (2018). The impact of genetic changes during crop domestication. *Agronomy* 8, 1–22. doi:10.3390/agronomy8070119.
- Tas, N., West, G., Kircalioglu, G., Topaloglu, S. B., Phillips, J., Kell, S., et al. (2019). Conservation gap analysis of crop wild relatives in Turkey. *Plant Genet. Resour. Characterisation Util.* 17, 164–173. doi:10.1017/S1479262118000564.
- Taylor, N. G., Kell, S. P., Holubec, V., Parra-Quijano, M., Chobot, K., and Maxted, N. (2017). A systematic conservation strategy for crop wild relatives in the Czech Republic. *Divers. Distrib.* 1–15. doi:10.1111/ddi.12539.
- Thomassen, H. A., Fuller, T., Buermann, W., Milá, B., Kieswetter, C. M., Jarrín-V., P., et al. (2011). Mapping evolutionary process: A multi-taxa approach to conservation prioritization. *Evol. Appl.* 4, 397–413. doi:10.1111/j.1752-4571.2010.00172.x.
- Thormann, I., Parra-Quijano, M., Endresen, D. T. F., Rubio-Teso, M. L., Iriondo, J. M., and Maxted, N. (2014a). Predictive characterization of crop wild relatives and landraces. *Biodiversity Int.*, 40.
- Thormann, I., Parra-Quijano, M., Iriondo, J. M., Rubio-Teso, M. L., Endresen, D. T. F., Dias, S., et al. (2014b). New predictive characterization methods for accessing and using CWR diversity. in *International Conference on ENHANCED GENEPOOL UTILIZATION - Capturing wild relative and landrace diversity for crop improvement, Cambridge, United Kingdom, 16-20 June 2014. Book of Abstracts.*
- Thormann, I., Reeves, P., Reilley, A., Engels, J. M. M., Lohwasser, U., B??rner, A., et al. (2016). Geography of genetic structure in barley wild relative *Hordeum vulgare* subsp. *Spontaneum* in Jordan. *PLoS One* 11, 1–19. doi:10.1371/journal.pone.0160745.
- Thrupp, L. A. (2000). Linking agricultural biodiversity and food security: The valuable role of agrobiodiversity for sustainable agriculture. *Int. Aff.* 76, 265–281. doi:10.1111/1468-2346.00133.
- Thurau, T., Ye, W., Menkhaus, J., Knecht, K., Tang, G., and Cai, D. (2010). Plant Nematode Control. *Sugar Tech* 12, 229–237. doi:10.1007/s12355-010-0056-y.

- UN (1992). Convention on Biological Diversity. doi:10.1016/B978-0-12-384719-5.00418-4.
- UN CBD (2010). United Nations Convention on Biological Diversity Conference of the Parties 10 Decision X/2. Available at: <https://www.cbd.int/decision/cop/?id=12268>.
- van Treuren, R., Hoekstra, R., and van Hintum, T. J. L. (2017). Inventory and prioritization for the conservation of crop wild relatives in The Netherlands under climate change. *Biol. Conserv.* 216, 123–139. doi:10.1016/j.biocon.2017.10.003.
- Vargas-Amelin, E., and Pindado, P. (2014). The challenge of climate change in Spain: Water resources, agriculture and land. *J. Hydrol.* 518, 243–249. doi:10.1016/j.jhydrol.2013.11.035.
- Vincent, H., Amri, A., Castañeda-Álvarez, N. P., Dempewolf, H., Dulloo, E., Guarino, L., et al. (2019). Modeling of crop wild relative species identifies areas globally for in situ conservation. *Commun. Biol.* 2, 136. doi:10.1038/s42003-019-0372-z.
- Vincent, H., Wiersema, J., Kell, S., Fielder, H., Dobbie, S., Castañeda-Álvarez, N. P., et al. (2013). A prioritized crop wild relative inventory to help underpin global food security. *Biol. Conserv.* 167, 265–275. doi:10.1016/j.biocon.2013.08.011.
- Wang, I. J., and Bradburd, G. S. (2014). Isolation by environment. *Mol. Ecol.* 23, 5649–5662. doi:10.1111/mec.12938.
- Weibull, J., and Phillips, J. (2020). Swedish Crop Wild Relatives : towards a national strategy for in situ conservation of CWR. *Genet. Resour.* 1, 17–23. doi:10.46265/genresj.2020.1.17-23.
- Whitlock, R., Hipperson, H., Thompson, D. B. A., Butlin, R. K., and Burke, T. (2016). Consequences of in-situ strategies for the conservation of plant genetic diversity. *Biol. Conserv.* 203. doi:10.1016/j.biocon.2016.08.006.
- Willits, M. G., Kramer, C. M., Prata, R. T. N., De Luca, V., Potter, B. G., Stephens, J. C., et al. (2005). Utilization of the genetic resources of wild species to create a nontransgenic high flavonoid tomato. *J. Agric. Food Chem.* 53, 1231–1236. doi:10.1021/jf049355i.
- WMO (2021). State of the Global Climate 2021: WMO Provisional report. Available at: <https://public.wmo.int/en/our-mandate/climate/wmo-statement-state-of-globalclimate>.
- Zair, W., Maxted, N., and Amri, A. (2018). Setting conservation priorities for crop wild relatives in the Fertile Crescent. *Genet. Resour. Crop Evol.* 65, 855–863. doi:10.1007/s10722-017-0576-3.
- Zair, W., Maxted, N., Brehm, J. M., and Amri, A. (2021). Ex situ and in situ conservation gap analysis of crop wild relative diversity in the Fertile Crescent of the Middle East. *Genet. Resour. Crop Evol.* 68, 693–709. doi:10.1007/s10722-020-01017-z.

CAPÍTULO 1:

National Inventory and Prioritization of Crop Wild Relatives in Spain

Manuscript published

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Abstract

Crop Wild Relatives (CWR) have recently received significant attention due to their value as plant genetic resources and their contribution to world food security. We present a prioritized checklist of CWR in Spain, where the criteria of crossability with crops of economic importance, endemism and threat status have been taken into account. First, we generated a list of the most relevant crops for Spain and at the international level. These crops were assigned to one of four use categories depending on the major crop of the genus (Food, Forage & Fodder, Ornamental and Industrial & Other uses). Subsequently, the corresponding native CWR related to these crops were listed. After evaluation by national experts in plant breeding, the resulting checklist contained 930 species which was then prioritized to obtain the prioritized Spanish checklist of CWR containing 578 species. The representation of the use categories in this list ranges from 32% (Forage & Fodder) to 16% (Industrial & Other Uses). Thirty-five percent of the prioritized species are endemic to Spain and over one-fourth of them are classified under some category of threat according to the International Union for Conservation of Nature. Endemism or threat status rates in the prioritized Spanish checklist of CWR were higher than those found in the prioritized CWR inventories of other countries. An *ex situ* conservation assessment for the Food and Forage & Fodder categories was also performed showing that around 71% of the species in these groups were present in genebanks.

Keywords: Food Security; Plant Diversity Conservation; Spanish Plant Genetic Resources; Checklist; Threatened Plants

Introduction

Crop quality and yields are known to be affected by climate change. Some models, using diverse climate change scenarios, crops and territories, predict a decrease of up to 40% in crop yields from 2010-2050 (Müller and Robertson 2014). At the same time, production risks are expected to increase, which may lead to an increase in world hunger (Tubiello and Fischer 2007). In Southern Europe, not only are temperatures expected to rise and precipitations expected to decrease under a climate change scenario, but the frequency of extreme events is also predicted to increase (Lotze-Campen 2011). In a conclusive meta-analysis, Challinor et al. (2014) report a highly significant negative impact of climate warming on crop yields that will be more pronounced in the second half of the century especially in tropical regions, as well as increases in yield variability that will further compromise food security. These hypothetical scenarios are already becoming apparent. In 2016 the greatest increase in global temperatures and variations in precipitation patterns were reported for the 137-year period of record (NOAA 2017). In Spain, the average temperature was 0.7°C higher than the mean of the reference series (1981-2010), placing 2016 among the warmest years on record (AEMET 2017).

In this context, farmers need to change their agricultural practices to effectively adapt to climate change, if they are to maintain and improve crop quality and yields. Such practices include adjusting planting times to avoid drought or heat stress and adopting new crop varieties, amongst others (Howden et al. 2007). However, these measures may not be sufficient (Turner and Meyer 2011), as modern cultivars may lack the ability to adapt to environmental change due to their narrow genetic base, resulting from selection applied in previous domestication and breeding processes (Stamp and Visser 2012).

Crop Wild Relatives (CWR) are wild plant species that are genetically related to crops (Heywood et al. 2007). As potential gene donors of desired traits for crops (Maxted et al. 2012), they have been successfully used in breeding for new traits and adaptations (Zamir 2001; Hajjar and Hodgkin 2007; Tester and Langridge 2010; Honnay et al. 2012). The wide range of adaptations found in CWR can be used as a genetic resource to mitigate the effects of climate change on crops, thereby helping to maintain and improve yields, and guarantee food security (Brozynska et al. 2016). The great value of CWR as a component of the Plant Genetic Resources for Food and Agriculture has been recognized in the United Nations Sustainable Development Goals (UN 2015), the Food and Agriculture Organization of the United Nations' Second Global Plan of Action for Plant Genetic

Resources for Food and Agriculture (FAO 2011) as well as the Convention of Biological Diversity (UN CBD 2010).

In global terms, CWR are seriously threatened by processes driven by human activities such as habitat fragmentation and loss, competition with invasive species, nitrogen depositions or changes in land uses, just like any other component of biological diversity (Ford-Lloyd et al. 2011; Heywood, 2011; Kell et al. 2012). The importance of CWR conservation and the best approaches to preserve these natural resources have been largely discussed (Heywood et al. 2007; Magos-Brehm et al. 2010; Maxted 2003; Pautasso 2012; Maxted et al. 2013) and over the past few years, several international projects have been implemented to conserve and manage CWR (see Online Resource 1). The *in situ* conservation of CWR in protected areas (Hunter and Heywood 2011), the establishment of genetic reserves (Pinheiro de Carvalho et al. 2012; Fielder et al. 2015a) and the identification of priorities and efficient sampling approaches for *ex situ* conservation (Khoury et al. 2015, García et al. 2017) are some of the procedures recently addressed for CWR conservation. In this context, the generation of CWR inventories is an essential first step in identifying the conservation needs of this group of species. Thus, listing and prioritizing existing CWR at the appropriate scale helps direct management efforts and underpins agrobiodiversity conservation. Two different approaches can be used to generate the inventories. The “crop list” approach uses a priority list of important crops to obtain their corresponding wild relatives. Alternatively, the “floristic” approach uses the flora of a territory as a starting point and matches it against a previously-existing catalogue of CWR species in the region or an exhaustive database of plants of economic use. To the best of our knowledge, seventeen CWR checklists have been published in the scientific literature: fifteen at the national level and two at the subnational level. These were generated for India (Arora and Nayar 1984), the United Kingdom (Maxted et al. 2007), Portugal (Magos-Brehm et al. 2008), Russia (Smekalova 2008), Israel (Barazani et al. 2008), Denmark (Bjørn et al. 2011), Venezuela (Berlingeri and Crespo 2012), Finland (Fitzgerald 2013), Benin (Idohou et al. 2013), the United States (Khoury et al. 2013), Italy (Panella et al. 2014), Cyprus (Phillips et al. 2014), China (Kell et al. 2014), the Czech Republic (Taylor et al. 2017), the Netherlands (van Treuren et al. 2017), England (Fielder et al. 2015a) and Scotland (Fielder et al. 2015b). Furthermore, a global inventory (Vincent et al. 2013), two European catalogues (Heywood and Zohary 1995; Kell et al. 2005) and a prioritized checklist of North Africa (Lala et al. 2017) have also been published. Similarly, some regional and country red lists of CWR have been generated (VMABCC-BIOVERSITY 2009; Bilz et al. 2011).

Governments and institutions dealing with wild and cultivated biodiversity conservation should take responsibility for the *in situ* and *ex situ* conservation of CWR. This is especially relevant for Spain, as it is one of the countries with the greatest number of plant species in Europe (7071 species, Aedo et al. 2013). In fact, it is the second country with the greatest number of CWR in Europe, hosting 26% of the Euro-Mediterranean CWR species (Kell et al. 2008). In addition, many of them are only found in this country, as the Iberian Peninsula is one of the two main centers of biodiversity in the Mediterranean Basin and presents a high number of endemics (Medail & Quezel 1999). The interest of the research community in CWR native to Spain is not new. Significant efforts have been made in the last few decades to explore, conserve and characterize wild *gramineae* (Soler et al. 1997), *Brassica* L. (Gomez-Campo et al. 2005), *Vitis* L. (De Andrés et al. 2012) and *Medicago* L. (Prosperi et al. 2006), among others. Although CWR conservation in Spain has been historically neglected by both the departments of wildlife conservation and agriculture at the national and autonomous community levels, its importance is becoming more widely recognized. Consequently, CWR have been included in the Spanish National Strategy of Plant Conservation (MAGRAMA 2014) and the Spanish Plant Genetic Resources Center has set some initiatives for the collection and preservation of CWR seed accessions. CWR germplasm is also stored in the César Gómez Campo genebank at the Polytechnic University of Madrid and the Agrifood Research and Technology Center of Aragón. REDBAG, the network of seedbanks associated with the Ibero-Macaronesian Association of Botanical Gardens, also preserves CWR seeds as part of their efforts to preserve threatened plant species. In parallel, native and exotic CWR have been actively used in breeding in Spain. For instance, Pico et al. (1999), Pérez de Castro et al. (2005), Caro et al. (2015) and Campos et al. (2017) worked on the development and evaluation of breeding tomato lines partially resistant to Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus derived from *Solanum chilense* (Dunal) Reiche and *S. peruvianum* L.. Similarly, Martín-Sánchez et al. (2003) and Fernández-Martínez et al. (2000) used different wild accessions of *Aegilops* and *Helianthus* species stored in Spanish germplasm banks to deal with Hessian fly pests in wheat and

Sunflower broomrape, respectively. So far, we have barely begun to explore the potential use of crop wild relatives from Spain. Some examples of how they are being used for breeding are found in Table 1. Taking all this into account, from a national strategy perspective, it is essential to list the main CWR taxa that occur in the country and prioritize the CWR species in need of active conservation measures.

Table 1. Some examples of the use for breeding of crop wild relatives from Spain and the traits that they provide.

Crop Wild Relative	Endemic to Spain	Crop	Target traits	Reference
<i>Aegilops triuncialis</i> L.	no	Wheat	Hessian fly resistance	Martín-Sánchez et al. (2003)
<i>Brachypodium distachyon</i> (L.) Beauv.	no	Wheat	Tolerance to water stress	Ruiz et al. (2016)
<i>Lens ervoides</i> (Brign.) Grande	no	Lentil	Drought and disease resistance	Using Crop Wild Relatives for Future Lentil Breeding Project*
<i>Solanum liliifolium</i> Sundig	yes	Eggplant	Adaptation to climate change	Eggplant Prebreeding project**
<i>Solanum vespertilio</i> Ait.	yes	Eggplant	Adaptation to climate change	Eggplant Prebreeding project**

* Using Crop Wild Relatives for Future Lentil Breeding: Evaluation of Drought and Disease Resistance of Interspecific Hybrid Lines is a project funded by the Global Crop Diversity Trust. More information at: <http://knowpulse2.usask.ca/portal/project/Using-Crop-Wild-Relatives-for-Future-Lentil-Breeding%3A-Evaluation-of-Drought-and-Disease-Resistance-of-Interspecific-Hybrid-Lines>

**Eggplant Prebreeding project (<http://eggplantprebreeding.upv.es/index.html>) is part of the initiative "Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives" which is supported by the Government of Norway. The project is managed by the Global Crop Diversity Trust with the Millennium Seed Bank of the Royal Botanic Gardens, Kew and implemented in partnership with national and international gene banks and plant breeding institutes around the world.

The aim of this paper was to develop a checklist of CWR of importance in Spain and a prioritized list for the implementation of conservation plans. In this context, we asked: a) What criteria should be applied to prioritize CWR in Spain? b) What is the threat status of the prioritized CWR checklist at the national and European levels? c) Are these species under any legal protection in Spain? d) What are the levels of endemism of the prioritized CWR checklist? e) How are their populations distributed and preserved ex situ in Spain?

Material and Methods

The process involved in the generation of the prioritized CWR list is summarized in Figure 1.

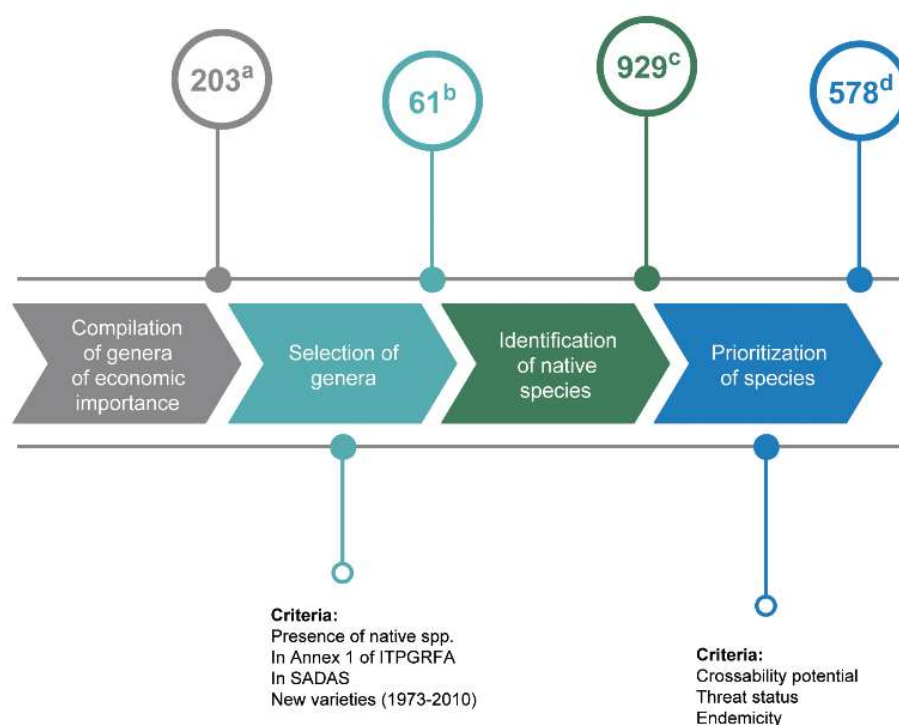


Figure 1: Complete process depicting the steps followed for the generation of the Prioritized Spanish Checklist of crop wild relatives. The process involves four steps (two of compilation of information and two of prioritization) and provides four distinct products: a) Baseline list of crop genera, b) List of selected crop genera, c) Spanish checklist of crop wild relatives and d) Prioritized Spanish Checklist of crop wild relatives.

Baseline List of Crop Genera and List of Selected Crop Genera

More than 6500 CWR species can be found in Spain according to the PGR Forum project (Kell et al. 2008). This large number of species, comprising over 80% of Spanish flora, is simply too big to manage for conservation purposes or use. Consequently, the first step in generating the national CWR checklist was to identify crops that contributed to global food security and were of economic importance in Spain and obtain a list of the corresponding genera. This was achieved in two stages: a) compilation of the Baseline List of Crop Genera and associated information and b) selection of crop genera. Crops under consideration were classified into four use categories: 1) Food, 2) Forage & Fodder, 3) Ornamental and 4) Industrial & Other uses.

a) Compilation of the Baseline List of Crop Genera and associated information: The crops considered under the Food, Forage & Fodder and Industrial & Other uses categories were obtained from those listed in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2010) and in the Spanish Annual Directory of Agricultural Statistics of 2010 (MAGRAMA 2011). From the latter, we extracted information on production and cultivated area for each crop. For the ornamental crops category, we recorded the most important crops in Europe according to the number of applications for registration of new varieties from the Annual Report of the Community Plant Variety Office in Europe (2011). The list of the taxa protected by the members of the Union by the International Union for the Protection of New Varieties of Plants (UPOV 2011) and the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA 2017) were also consulted to check for other crops of importance not yet incorporated in the list. The UPOV database was also used to collect data on the number of species, infraspecific taxa and/or hybrids associated with a particular crop. Similarly, specialized publications on trends in plant breeding were checked (Kole 2011a, b, c, d, e) as well as inventories from other countries and CWR checklists (Berlingeri and Crespo 2012; Labokas et al. 2010; Magos-Brehm et al. 2010; Markkola, 2005). The genera corresponding to these crops were listed in a database including all the information collated from the above-mentioned data sources.

This database was completed with information on whether these genera contained wild species native to Spain following *Flora Iberica* (Castroviejo 1986-2012) for Peninsular Spain and the Balearic Islands and Acebes Ginovés et al. (2010) for the Canary Islands.

b) Selection of crop genera: After careful consideration of all compiled data, we selected a list of genera based on the following criteria: 1) the genus must contain at least one species native to Spain and 2) it must comply with at least one of the following items: a) is listed in Annex 1 of the International Treaty for Plant Genetic Resources for Food and Agriculture, indicating its relevance as a contributor to global food security, b) contains a crop in the Spanish Annual Directory of Agricultural Statistics (MAGRAMA 2011) or c) contains a crop that has at least one registered variety in Spain in the period 1973-2010, as an unequivocal sign of economic concern to the country. The resulting reduced list of crop genera was assessed by experts from institutions dealing with crop breeding in Spain. They validated all selected genera and proposed some additional genera valuable for breeding according to their expertise.

Spanish Checklist of Crop Wild Relatives

Wild species within the selected crop genera native to Spain were identified using *Flora Iberica* – the national flora of reference in Spain (Castroviejo 1986-2012) –, or the Anthos project –linked to *Flora Iberica* experts – (Anthos 2017). The wild species native to the Canary Islands were identified following Acebes Ginovés et al. (2010). When the genera were not yet published in any of these references, other bibliography was consulted (Romero Zarco 1996; Pascual 2004; Killian et al. 2011). The CWR checklist was set at the taxonomic level of species: infraspecific taxa levels were not included as separate entities.

Information regarding primary use category, IUCN threat category and number of infraspecific taxa belonging to the species included in the Red List of Spanish Vascular Flora (Moreno 2008), endemism, crossability potential with crops of reference and number of chromosomes, was gathered for each CWR species on the list. The content and source of each field is shown in Online Resource 2. Crossability potential was assessed according to the gene pool concept by Harlan and de Wet (1971) (possibility of gene transfer between wild and cultivated species) and the taxon group concept by Maxted et al. (2006) (assimilation of taxonomic hierarchy to the gene pool concept). Crossability information was obtained for each species following *Flora Iberica* chapters, the Harlan and de Wet Inventory (<https://www.cwrdiversity.org/checklist/>), the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA 2017) or additional references. Taxonomical information was obtained following *Flora Iberica* chapters, the Anthos project or additional references. All sources used for this assessment are indicated in Online Resource 3. To apply the crossability potential according to the gene pool concept, a complementary list of cultivated species was generated for each selected genus using the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA 2017) as reference. Each species was assigned to its corresponding gene pool or taxon group concept. Moreover, when available, information on confirmed or potential use of the species was registered in the database.

Prioritized Spanish Checklist of Crop Wild Relatives

To generate a manageable list of CWR species that could be subjected to conservation status assessment and conservation measures, the Spanish Checklist of Crop Wild Relatives was prioritized using the previously gathered information. Criteria used for prioritization included: crossability potential (gene pool and taxon group concepts), threat status and endemism for the Food and Forage & Fodder categories and only crossability potential for the Ornamental and Industrial & Other uses categories. Prioritized species were those found in gene pool categories 1 (primary gene pool: cultivated and wild forms of the crop) and 2 (secondary gene pool: gene transfer is possible using conventional breeding techniques) (Harlan & de Wet 1971) or taxon group category 2 (same series or section as a crop) and 3 (same subgenus as a crop) (Maxted et al. 2006). The gene pool concept always prevailed over the taxon group concept, but when information on crossability between species was unavailable, the taxon group concept criterion was applied. For the Food and Forage & Fodder use categories, species belonging to any of the IUCN threat categories (critically endangered, endangered, vulnerable and near threatened) or endemic to Spain were also included in the Prioritized Spanish Checklist of Crop Wild Relatives (Table 2).

Table 2. Criteria applied for the prioritization of CWRs in the Spanish Checklist.

Criterion	Source	Selected values	CWR categories for application
Gene pool concept (Harlan & de Wet 1971) Taxon group concept (Maxted et al. 2006)	The Harlan & de Wet Inventory (Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives Project) ^a Germplasm Resources Information Network (GRIN) Taxonomy database of the United States Department of Agriculture (GRIN-USDA 2017) Additional references ^b .	Categories 1 and 2 ^c Concepts 2 and 3 ^d	All categories
Threat (IUCN criteria)	Red List of Spanish Vascular Flora (Moreno 2008)	CR, EN, VU, NT ^e	Food Forage & Fodder
Endemicity	<i>Flora Iberica</i> (Castroviejo 1986-2012) List of wild species of Canary Islands. (Acebes Ginovés et al. 2010) Additional references ^b	Spain	Food Forage & Fodder
^a More than 80 references have been used. Additional references can be found in Online Resource 3. ^b The Harlan & de Wet Inventory (https://www.cwrdiversity.org/checklist/) is developed under the Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives financed by the Crop Diversity Trust. ^c Gene pool categories: 1 = Primary GP: cultivated and wild forms of the crop; 2 = Secondary GP: Gene transfer is possible using conventional breeding techniques ^d Taxon group categories: 2 = Same series or section as a crop; 3 = Same subgenus as a crop Gene pool and Taxon group definitions taken from Maxted et al. 2006. ^e Threat status: CR = Critically endangered; EN = Endangered; VU = Vulnerable; NT =Near Threatened			

The resulting list was checked against the National Catalogue of Threatened Species in Spain promoted under Law 42/2007, royal decree 139/2011 (B.O.E. 46, 2011). The inclusion of species in this catalogue provides them with legal protection. This entails the design and implementation of appropriate conservation plans and the commitment to regularly assess their conservation status. Furthermore, the European CWR threat assessment by Bilz et al. (2011) was used to identify the species on the checklist that are threatened in the European context.

Because the seventeen Autonomous Communities in Spain hold have responsibility for the conservation of biodiversity and genetic resources in their territories, the number of species on the priority CWR checklist present in each autonomous community was estimated using occurrence data. Data were downloaded from the data portal of the Global Biodiversity Information Facility (GBIF 2011-2013) (for 500 species out of the 578 taxa on the Prioritized Spanish Checklist of Crop Wild Relatives. Data were not available for the remaining 78 species at that moment. All data were subject to taxonomic harmonization and quality assessment to only select data of good quality. The criteria applied for quality selection were: 1) Geographic coordinates had at least two decimal digits of decimal degree (around 1 km resolution); 2) Data records also included the name of the locality, which was congruent with the given coordinates. Data records not complying with both requirements were eliminated. Duplicated records, according to the geographic coordinates, were also eliminated.

Finally, all prioritized species were checked against the databases of the Spanish Plant Genetic Resources Center which gathers information from 19 different seed banks related to plant genetic resources in Spain. Furthermore, ten germplasm banks belonging to the REDBAG network, i.e., the Spanish Network of Germplasm Banks for Wild Plants and Autochthonous Plant Genetic Resources, associated with the Ibero-Macaronesian Association of Botanical Gardens, were also consulted. The curators of the corresponding germplasm banks were individually contacted to gather this information. The EURISCO catalogue (EURISCO 2017), the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA 2017) and the GENESYS Global Portal on Plant Genetic Resources (GENESYS 2017) databases were also consulted to complete the dataset with worldwide data. The data from each institution included number of accessions for each species (only those collected in Spain) and the coordinates of the collecting sites, when available. The information on number of accessions was added to the database associated with the Prioritized Spanish Checklist of Crop Wild Relatives. To identify the species in the most urgent need of collection to improve CWR collections in germplasm banks, we selected species categorized in genepools 1b and 2, or taxon group 2, under any category of threat according to the International Union for Conservation of Nature (IUCN), endemic to Spain and with less than five accessions in germplasm banks. We used five accessions as a threshold, as this is considered the minimum number of populations needed to conserve the genetic diversity of a species (Brown & Briggs, 1991). Priority for collection was assigned as follows: 1) Urgent priority. Primary or secondary gene pool and taxon group, endemic and threatened. No representation in gene banks; 2) Urgent. Species not represented in gene banks; 3) Need collecting. Less than five populations represented in gene banks; 4) Non priority for collection. More than five accessions in gene banks for each species.

Results

Baseline List of Crop Genera and List of Selected Crop Genera

The baseline list of crops of importance and associated genera comprised 203 genera (Online Resource 4). The list of selected crop genera used to generate the CWR checklist contained 61 genera. This list is shown in Online Resource 5 together with the information on family, use category and reason for inclusion in the list.

When categorized by use, the Food category contained 33 genera in 13 families; the Forage & Fodder category 12 genera in 2 families; the Ornamental category 5 genera in 5 families and the Industrial & Other uses category 10 genera in 7 families. Fabaceae and Poaceae are the two most important families with 11 genera each, followed by Brassicaceae with 7 genera.

Spanish Checklist of Crop Wild Relatives

The Spanish Checklist of CWR species rendered a total of 929 species. The Food category included 223 species, the Forage & Fodder category had 260, the Ornamental category had 240 and the Industrial & Other uses category had 206 (Figure 2).

This checklist of species together with information gathered on priority use, taxonomic classification, gene pool or taxon group concepts, threat status, endemism, and number of chromosomes was compiled into the Spanish Checklist of Crop Wild Relatives database which is available at: <https://pgrsecurespain.weebly.com/crop-wild-relatives-in-spain-ndash-spanish-checklist-of-cwr.html>. As information on gene pool was only found for 243 of the 929 species, the rest of the species were assigned their corresponding taxon group category.

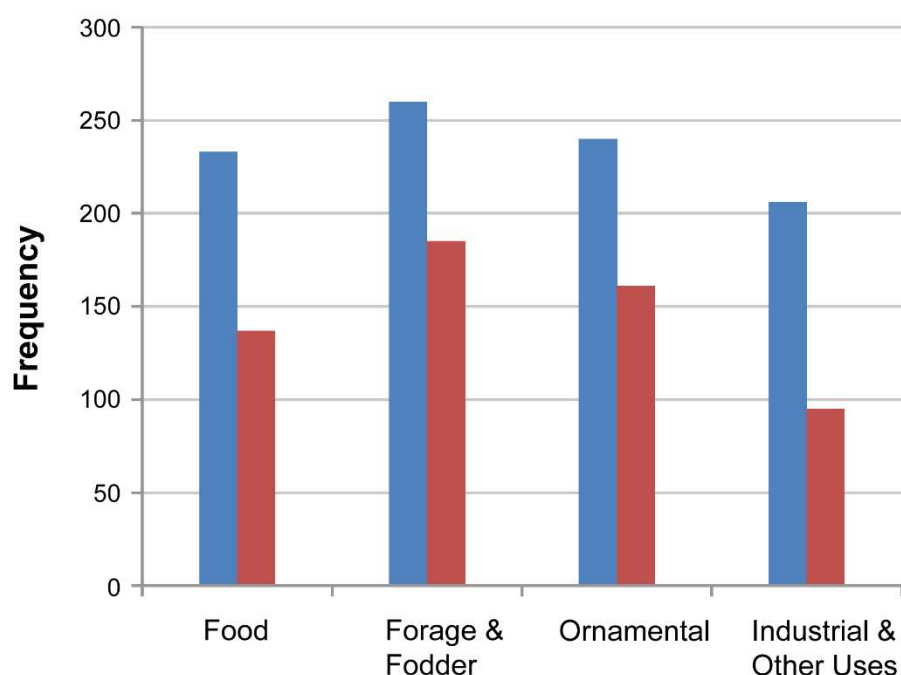


Figure 2: Number of species on the Spanish Checklist of crop wild relatives and the Prioritized Spanish Checklist of crop wild relatives, ordered by category.

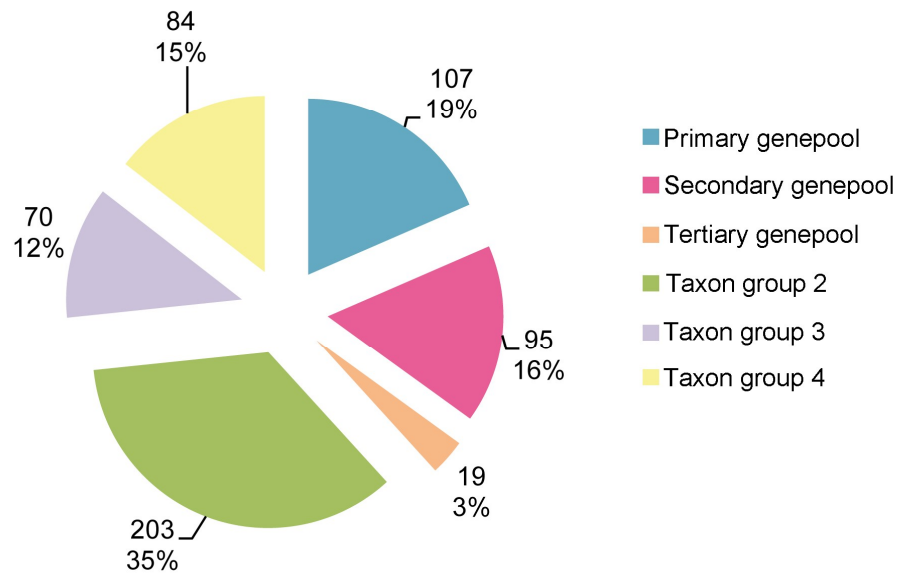
Prioritized Spanish Checklist of Crop Wild Relatives

Applying the agreed criteria to each use category reduced the number of species on the list to 578 (62% of the original checklist). All genera on the original checklist had species on the prioritized checklist except for *Fragaria* L. (strawberry relatives) whose species native to Spain did not meet the crossability criteria.

The Prioritized Spanish Checklist of Crop Wild Relatives contains 137 species related to Food crops, 185 to Forage & Fodder crops, 161 to Ornamental crops and 95 to Industrial & Other uses crops (Figure 2). All prioritized species, together with all gathered information during the process is available at the Prioritized Spanish Checklist of Crop Wild Relatives (<https://pgrsecurSpain.weebly.com/crop-wild-relatives-in-spain---prioritization-of-the-checklist.html>) and in the Supplementary Material Section of this Chapter (Annex 1).

In compliance with the prioritization criteria used, the selected species mainly belonged to the primary or secondary gene pools (107 and 95 species, respectively) or to taxon group 2 (203 species) (same section or subsection as the crop) (Figure 3a). Thus, over 70% of the selected species have a direct potential use in plant breeding. Forty percent of the prioritized species are endemic to Spain. Over one-fourth of the species (155 out of 578) are classified under one of the IUCN threat categories at the national level, including the Near Threatened category (Figure 3b), and 15 species are threatened at the European scale (Table 3). Furthermore, 43 species of the Prioritized Spanish checklist of Crop Wild Relatives are included in the National Catalogue of Protected Species under law 42/2007, royal decree 139/2011 (B.O.E. 46, 2011) (Online Resource 6).

a)



b)

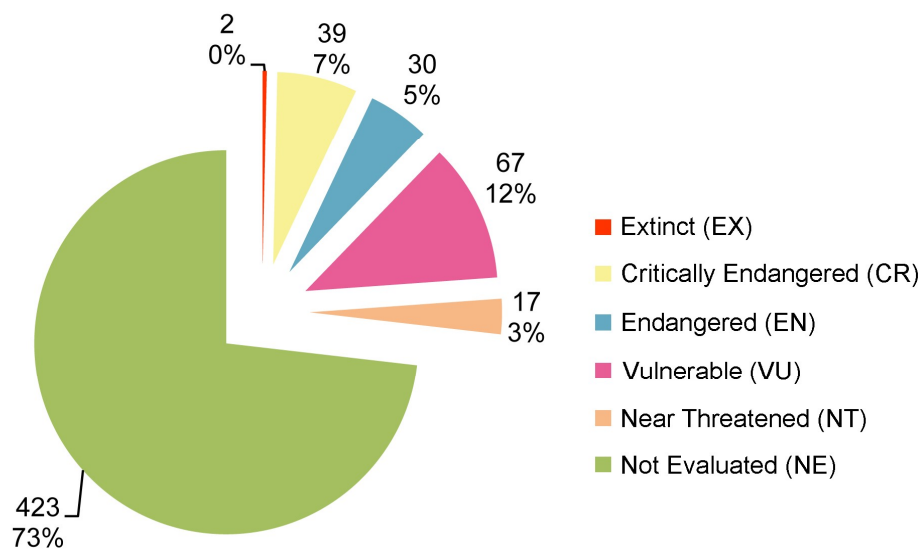


Figure 3: a) Classification of prioritized species according to the different gene pool or taxon group categories coined by Harlan & de Wet (1971) and Maxted et al. (2006). b) Number of species under any of the threat categories according to the Spanish Red List of Vascular Plants (Moreno 2008), following International Union for Conservation of Nature criteria (including Near Threatened). *Extinct: *Astragalus algerianus* E. Sheld. and *Astragalus baionensis* Loisel.

Table 3: CWR species on the Spanish Prioritized Checklist (Food and Forage & Fodder categories) threatened at the European level. Corresponding status in Spain is also shown.

(CR: critically endangered; EN=endangered; VU=vulnerable; NT=near threatened).

From the European Red List of Vascular Plants (Bilz et al. 2011) and the Spanish Red List of Vascular Flora (Moreno 2008)

Species	Family	Red List European Status	Red List Spanish Status
<i>Allium pyrenaicum</i> Costa & Vayr.	Liliaceae	VU	NT
<i>Allium schmitzii</i> Cout.	Liliaceae	VU	VU
<i>Asparagus arborescens</i> Willd.	Liliaceae	VU	Not Assessed
<i>Asparagus fallax</i> Svent.	Liliaceae	EN	EN
<i>Asparagus nesiotis</i> Svent.	Liliaceae	EN	EN
<i>Asparagus pastorianus</i> Webb & Berthel.	Liliaceae	VU	Not Assessed
<i>Asparagus plocamoides</i> Webb ex Svent.	Liliaceae	VU	Not Assessed
<i>Avena murphyi</i> Ladiz.	Poaceae	EN	EN
<i>Beta macrocarpa</i> Guss.	Chenopodiaceae	EN	Not Assessed
<i>Cicer canariense</i> A. Santos & G. P. Lewis	Fabaceae	EN	EN
<i>Lactuca singularis</i> Wilmott	Asteraceae	VU	Not Assessed
<i>Medicago citrina</i> (Font Quer) Greuter	Fabaceae	CR	CR
<i>Patellifolia nebbiana</i> (Moq.) A. J. Scott, Ford-Lloyd & J. T. Williams	Chenopodiaceae	CR	Not Assessed
<i>Prunus lusitanica</i> L.	Rosaceae	VU	VU
<i>Prunus ramburii</i> Boiss.	Rosaceae	VU	VU

Prioritized Spanish CWR were quite homogeneously distributed in all Autonomous Communities of Spain. Andalucía, the largest autonomous community, also had the greatest number of of priority CWR species, followed by Castilla-León and Castilla-La Mancha in Central Spain (Figure 4).

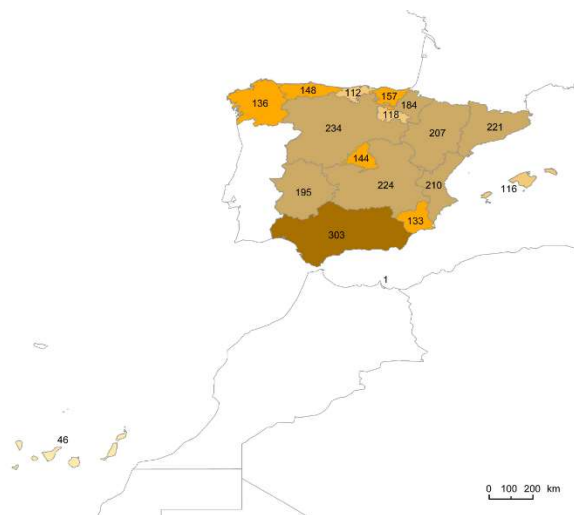


Figure 4: Number of different crop wild relative species in the Autonomous Communities of Spain

The *ex situ* assessment showed that germplasm banks hold accessions of approximately 70% of the prioritized CWR. Thus, 176 species are not represented in any of the institutions contacted. The Prioritized Spanish Checklist of Crop Wild Relatives (<https://pgrsecurSpain.weebly.com/crop-wild-relatives-in-spain---prioritization-of-the-checklist.html>) shows the number of accessions preserved in genebanks for all prioritized species. Less than 13% of the species represented in genebanks has 20 or more accessions in *ex situ* collections, while 34% has between only 1 and 4 accessions.

A total of 51 species of primary importance (because they were endemic to Spain, threatened according to IUCN, and belonged to the primary or secondary gene pools or taxon group 2) had less than five accessions in germplasm banks. Of these, twenty-three had no representation in genebanks (Priority 1). One hundred and fifty-three species are found to be in priority collecting category 2 (Urgent. Species not represented in gene banks), 195 in priority collecting category 3 (Need collecting. Less than five populations represented in gene banks) and finally 207 in priority collecting category 4 (Non priority for collection. More than five accessions in gene banks). All this information can be consulted for each species in the Prioritized Spanish Checklist of Crop Wild Relatives (<https://pgrsecurSpain.weebly.com/crop-wild-relatives-in-spain---prioritization-of-the-checklist.html>).

Discussion

Currently published CWR checklists and inventories provide a good background that exposes the regional and global importance of these species and the idiosyncrasy of each country in their development process. Our study fills an important gap in this area, as Spain has one of the largest and most diverse flora in the Euro-Mediterranean region (Médail and Quézel 1997; Molina-Venegas et al. 2015).

Crop list vs. floristic approach

The development of the Spanish Checklist of Crop Wild Relatives followed a “crop list” approach. We preferred this approach because the CWR European catalogue returns more than 6500 species for Spain (Kell et al. 2008), which represents almost 80% of Spanish flora. Such an extensive list is far too large to be operational for designing an effective strategy for CWR conservation, management and use. Furthermore, many species in this catalogue are already managed by other interest groups in the public administration, e.g. forestry species, which have their own National Inventory and conservation program (MIMA 2006). Thus, we generated the CWR checklist directly from a list of important crops to efficiently use economic resources and avoid duplication and overlap in the case of species that were already managed by the public administration.

The creation of a crop list according to global, national and regional socio-economic criteria and the subsequent identification of its CWR significantly simplified the procedure. It allowed us to focus on the most important CWR for Spain without neglecting any of the crops that contribute to the country's economy and to worldwide food security. This approach, also followed by Berlingeri and Crespo (2012) in Venezuela, and Idohou et al (2013) in Benin, may be a valid alternative for countries with large floras that require a manageable list in which the most important CWR are represented.

In most other cases, national CWR checklists have been developed following a “floristic” approach. Thus, the CWR checklists of the United Kingdom, Portugal, Italy, Cyprus, the Czech Republic, England and Scotland were generated by matching the corresponding floras against the Crop Wild Relative Catalogue for Europe and the Mediterranean (Kell et al. 2005), and this initial checklist was then prioritized. Similarly, in the generation of the CWR checklist of the United States (Khoury et al. 2013), the completed volumes of Flora of North America (FNA 1993+) and other sources of native flora were crossed against the Germplasm Resources Information Network database (GRIN-USDA 2017), based on Wiersema and León (1999). A similar approach was followed to generate the CWR checklist of China (Kell et al. 2014). However, the CWR Checklist of Israel was directly generated by consulting a multidisciplinary panel of experts including botanists, ecologists and plant breeders (Barazani et al. 2008).

Despite the differences in the approach to generating these national checklists, similar use categories are found in all lists consulted. The food and forages groups were considered in all of them, whereas the ornamental, environmental, medicinal or industrial uses were included in all inventories except for the Venezuelan checklist (Berlingeri and Crespo 2012). This broad perspective on plant genetic resources is important because different economic sectors could benefit from it.

Delimitation of Crop Wild Relatives in the Spanish Checklist

Non-native species were excluded from the Spanish Checklist of Crop Wild Relatives due to the large number of CWR species naturally occurring in Spain and the need for strict prioritization. Furthermore, introduced species growing far from their centers of diversity (“geographical areas where the botanical species shows a higher degree of variation and where there are significant genetic variants represented by alleles” (Corinto 2014)) may lack high genetic variability, which is fundamental for breeding purposes. However, according to Bossdorf et al. (2005), non-native species might be a source of genetic variation that should not be undervalued. These species were considered in all the consulted checklists except those for Italy and Cyprus. Our CWR checklist was equally restrictive from a taxonomical point of view, and only taxa at the species level were included. Consequently, the reference CWR checklist for Spain (929 species) is smaller than those generated for other countries, such as Finland (1905 taxa), UK (1955 species), Portugal (2261 taxa), USA (2495 taxa), the Czech Republic (3283 species) or China (almost 24500 species). In our opinion, national CWR checklists should be taxonomically robust, manageable, useful and dynamic, even if this means trading the exhaustiveness of the list for these properties. The generation of an initially delimited list of CWR also facilitates their subsequent prioritization to take direct actions. The maintenance of the checklist as a database available in the web allows for subsequent updates to adjust for potential taxonomic changes and variation in the assessment of the list of the most important crops.

Prioritized Spanish Checklist of Crop Wild Relatives

The criteria used to further prioritize the CWR of Spain was similar to those used by other authors in other countries and involved the concepts of crossability, threat and endemism. With regard to crossability, it is noteworthy that only 26% of the species on the checklist could be classified according to the gene pool concept. Information on crossability between CWR and cultivated species is generated (and held) by plant breeders. This type of valuable feedback needs to be captured by those documenting plant genetic resources, but this is not always easy because the information may be confidential and not available as published material. This lack of available information concerning direct crossability experiments is in consonance with the results found in previous studies (Kell et al. 2014; Fielder et al. 2015a, b). Although the taxon group concept can be a useful proxy to make decisions when genepool information is not available, these results clearly show that crossability

experiments between crops and their wild relatives are essential to assess and facilitate the potential use of CWR in plant breeding.

Threat assessment showed that 23% of the species on the prioritized list were under a threat category described by the IUCN. This percentage is higher compared to other countries like Cyprus (9%), Germany (16%), Lithuania (16%), Norway (13%), the UK (12%) and even compared to the large flora of China (17%), but lower compared to countries such as the Czech Republic (54%), Finland (71%), Jordan (32%) or Portugal (65%) (Kell et al. 2014, Iriondo et al. 2016). Regarding European threat assessment, 12% of the species on the list (66 of the 578 prioritized CWR) are classified in some category of threat at the European level. Some national or subnational strategies already include the identification of the level of threat of CWR species at the European level (Fielder et al. 2015a, b). However, if all national or subnational strategies included this objective, integrated preservation plans could be designed among countries, making better use of conservation resources and constructing effective conservation networks. Transboundary conservation efforts through protected areas complexes have been already reported as beneficial (Sheppard 1999), and some claim that would be the most sensible path to achieving real conservation success (Chester 2005). However, the final boost and distribution of efforts, considering both the European and national idiosyncrasy, should depend on the interested countries and their specific needs, efficiently managing national resources.

Despite the socio-economic importance of CWR and the large number of threatened CWR species (135 according to the IUCN criteria), only 43 CWR species on the prioritized list are under legal protection in Spain. We must underline that nine species are threatened at both the Spanish and European levels (see Table 3). Three of these species (endangered *Asparagus fallax* Svent. and *Cicer canariense* A. Santos & G. P. Lewis and critically endangered *Medicago citrina* (Font Quer) Greuter) are already protected in Spain by law and considered a priority for conservation (BOE 46, 2011). As a result, conservation plans are being designed and implemented for these species, and their conservation status is regularly assessed. However, the other six species require urgent conservation measures and should be included in the catalogue of protected species. Furthermore, species threatened at the European level which have not been assessed in the Spanish Red list should be evaluated in future editions of the Spanish Red List of Vascular Flora. The publication of the present list can also be very helpful in subsequent reviews of the national catalogue of protected species to include all endangered priority CWR species under legal protection. The specific mention of CWR in the Spanish National Strategy of Plant Conservation (MAGRAMA 2014) is an important step in recognising the importance of conserving CWR at the national level, even though implementing active conservation plans is the responsibility of the autonomous communities.

Regarding endemism data, the Prioritized Spanish Checklist of Crop Wild Relatives contains a notably higher percentage of endemics (35%) than the priority lists of other countries, except Portugal (65%). According to Iriondo et al. (2016), other European countries have far fewer endemics on their prioritized lists compared to the Iberian Peninsula: the priority Czech CWR list has 13% endemic species, followed by Germany with 10%. Even lower percentages are found in the priority lists of Cyprus (3%), the United Kingdom (1%), Lithuania (1%), Finland 0% and Norway 0%. These numbers highlight the value of Iberian biodiversity as an essential component of the Euro-Mediterranean region.

According to Brown and Briggs (1991) and Maxted et al. (2008), a minimum of five accessions from five different populations should be represented in germplasm banks to properly represent the genetic diversity of a species. Based on this premise, in addition to prioritizing the collection of the 176 species on the prioritized list that have no accessions in genebanks, CWR germplasm collections should also concentrate on improving the representation of conserved species to obtain the minimum number of sampled populations to represent their genetic diversity. From these two sets, the 51 species of the prioritized list that are endemic, threatened, and have less than five seed accessions preserved should have the highest priority. An important point to consider is that the coordinates of the accessions have not been evaluated in this study (unavailable in many cases), which may lead to duplicates in entries among the different institutions contacted. Hence, the representation of population diversity may be overestimated.

In contrast to the above-mentioned criterion of including seed accessions from a minimum of five populations in genebank collections, Whitlock et al. (2016) propose that over 35% percent of the populations should be

preserved to cover the recommendations of the Convention of Biological Diversity. Although this study was designed for the implementation of in situ conservation plans, it could also be applied to ex situ conservation, as its major objective was also to preserve enough genetic diversity to adequately represent the species. Considering that ex situ conservation aims to preserve as much genetic diversity as possible (Bachetta et al. 2008), the most adequate number of accessions to preserve should actually be estimated on a species-per-species basis, taking into account the breeding system of the species as well as the distribution and size of the populations along with their environmental conditions (Brown & Marshall 1995). In addition, the use of existing molecular data can also help to determine the minimum number of accessions required to properly represent the genetic diversity contained by a species (Camadro 2012). This suggests that the “minimum of five populations” criterion should be replaced in the long term by a more ambitious goal in which the number of accessions to be collected is estimated on a species-per-species basis, in a proportional way to the genetic diversity of the species. Recent publications (Parra-Quijano et al. 2012a, b, Phillips et al. 2014, 2016) advocate the inclusion of ecogeographic information when planning collecting missions and in situ conservation measures, making use of the ecogeographic characterization of the accessions or population data. This ecogeographic information can also be used as a proxy to estimate genetic diversity (Maxted et al. 2012) and infer possible genetic adaptation patterns that the species may contain (Parra-Quijano et al. 2012a).

Concluding remarks

Existing CWR species, along with the rest of biodiversity components, should be conserved using strategies based on the establishment and management of protected areas and the sustainable use by humans of the rest of the territory, as well as species-specific approaches. In this context, the proper identification of priority CWR is essential. The generated Spanish Checklist of Crop Wild Relatives should be coordinately managed by the agriculture and environment departments of the public administration, and continuously revised in a participatory way to include species with real potential that meet the needs of the changing trends in agriculture and plant breeding.

However, the mere generation of a CWR checklist does not assure proper conservation. The conservation status of priority CWR should be properly assessed, and species-specific in situ and ex situ conservation actions should be implemented when needed. In this sense, the Spanish Checklist and Prioritized Checklist of CWR are already being used to assess the *ex situ* conservation of CWR in the collections of the Spanish Plant Genetic Resources Center (De la Rosa et al. 2013). Furthermore, they are also being used to plan new CWR seed collecting campaigns to improve their *ex situ* conservation status (García et al. 2015).

References

- Acebes Ginovés JR, León Arencibia MC, Rodríguez Navarro ML, del Arco Aguilar M, García Gallo A, Pérez de Paz PL, Rodríguez Delgado O, Martín Osorio VE, Wildpret de la Torre W (2010) Spermatophyta. In: Arechavaleta M, Rodríguez S, Zurita N, García A (eds) Lista de especies silvestres de Canarias. Hongos, plantas y animales terrestres 2009. Gobierno de Canarias: Servicio de Biodiversidad, Dirección General del Medio Natural, Consejería de Medio Ambiente y Ordenación Territorial del Gobierno de Canarias, Tenerife, Spain, pp 122–172
- Aedo C, Medina L, Fernández-Albert M (2013) Species richness and endemism in the Spanish vascular flora. *Nord J Bot* 31:478–488. doi: 10.1111/j.1756-1051.2012.00009.x
- AEMET (2017) Agencia de Estatal de Meteorología. Informe climático del año 2016. www.aemet.es/en/noticias/2017/01/2016_muy_calido_y_humedo#enlaces_asociados. Accessed 30 April 2017
- Anthos (2017) Sistema de información de las plantas de España. Real Jardín Botánico, CSIC- Fundación Biodiversidad. www.anthos.es. Accessed 1 May 2017
- Arora RK, Nayar ER (1984) Wild Relatives of Crop Plants in India. New Delhi, India
- Bacchetta G, Bueno Sánchez A, Fenu G, et al (eds) (2008) Conservación ex situ de plantas silvestres. Principado de Asturias / La Caixa
- Barazani O, Perevolotsky A, Hadas R (2008) A problem of the rich: Prioritizing local plant genetic resources for ex situ conservation in Israel. *Biol Conserv*. 141:596–600. doi: 10.1016/j.biocon.2007.10.014

- Berlingeri C, Crespo MB (2012) Inventory of related wild species of priority crops in Venezuela. *Genet Resour Crop Evol* 59:655–681. doi: 10.1007/s10722-011-9709-2
- Bilz M, Kell SP, Maxted N, Lansdown R V (2011) European Red List of Vascular Plants. Publications Office of the European Union, Luxembourg
- Björn GK, Kristiansen K, Jacobsen LH (2011) Bevaring af plantegenetiske ressourcer I de vilde slægtninge til jordbrugets afgrøder. http://lfst.dk/fileadmin/user_upload/NaturErhverv/Filer/Landbrug/Genetiske_ressourcer/Planter/Publikationer/DJF-rapport__revideret_version_af_april_2011.pdf. Accessed 15 July 2017
- B.O.E - Boletín Oficial del Estado número 46, de 23 de Febrero de 2011. Referencia BOE-A-2011-3582. Real decreto 139/2011, de 4 de Febrero, para el desarrollo del Listado de Especies Silvestres en Régimen de Protección Especial y del Catálogo Español de Especies Amenazadas. Ministerio de Medio Ambiente, Medio Rural y Marino. Available at: <https://www.boe.es/buscar/pdf/2011/BOE-A-2011-3582-consolidado.pdf>. Accessed 16 July 2017
- Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144:1–11. doi: 10.1007/s00442-005-0070-z
- Brown AHD, Briggs JD (1991) Sampling strategies for genetic variation in ex situ collections of endangered plant species. In: Falk DA, Holsinger KE (eds.) *Genetics and Conservation of Rare Plants*. Oxford University Press, New York (USA), pp 99–199.
- Brown AHD, Marshall DR (1995) A basic sampling strategy: theory and practice. In: Guarino L, Ramanantha R V, Reid R (eds) *Collecting plant genetic diversity: Technical guidelines*. CAB International, Wallingford, UK, pp 75–91
- Brozynska M, Furtado A, Henry RJ (2016) Genomics of crop wild relatives: Expanding the gene pool for crop improvement. *Plant Biotechnol J* 14(4):1070–1085. doi: 10.1111/pbi.12454
- Camadro E (2012) Relevance of the genetic structure of natural populations, and sampling and classification approaches for conservation and use of wild crop relatives: potato as an example. *Botany* 90:1065-1072.
- Campos G, Gisbert C, Pérez-de-Castro A, Díez M (2017) Obtaining advanced generations from *Solanum peruvianum* PI 126944 in the genetic background of *S. lycopersicum* by immature seed culture. *Euphytica* 213:63.
- Caro M, Verlaan MG, Julián O et al. (2015) Assessing the genetic variation of Ty-1 and Ty-3 alleles conferring resistance to tomato yellow leaf curl virus in a broad tomato germplasm. *Mol Breeding* 35: 132. <https://doi.org/10.1007/s11032-015-0329-y>
- Castroviejo S (coord. gen.) (1986-2012) *Flora Iberica* 1-8, 10-15, 17, 18, 21. Real Jardín Botánico, CSIC, Madrid, Spain
- Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-analysis of crop yield under climate change and adaptation. *Nature Climate Change* 4(4):287–291. doi: 10.1038/NCLIMATE2153
- Chester C (2005) From Conservation Diplomacy to Transborder Landscapes : The Protection of Biodiversity Across North America ' s Borders. *George Wright Forum* 22:27–34
- Community Plant Variety Office (2011) Annual Report 2010. Publications Office of the European Union, Luxembourg
- Corinto GL (2014) Nikolai Vavilov ' s Centers of Origin of Cultivated Plants With a View to Conserving Agricultural Biodiversity. *Hum Evol* 29:1–17
- De Andrés MT, Benito A, Pérez-Rivera G, Ocete R, López MA, Gaforio L, Muñoz G, Cabello F, Martínez Zapater JM, Arroyo-García R (2012) Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. *Mol Ecol* 21:800–816. doi: 10.1111/j.1365-294X.2011.05395.x
- De la Rosa L, Aguiarino E, Mallor C, Rubio Teso ML, Parra-Quijano M, Torres E, Iriondo JM (2013) Prioritized crop wild relatives in Spain: status on the National Inventory of Plant Genetic Resources for Agriculture and Food. *Crop Wild Relat* 23–26
- Dempewolf H, Eastwood RJ, Guarino L, et al (2014) Adapting Agriculture to Climate Change: A Global Initiative to Collect, Conserve, and Use Crop Wild Relatives. *Agroecol Sustain Food Syst* 38:369–377. doi: <http://dx.doi.org/10.1080/21683565.2013.870629>
- EURISCO (2017) European Search Catalogue for Plant Genetic Resources. <https://eurisco.ipkgatersleben.de/apex/f?p=103:1:::..>. Accessed 28 November 2017
- FAO (2010) Food and Agriculture Organization of the United Nations. The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture
- FAO (2011) Food and Agriculture Organization of the United Nations. Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture.
- Fernández Martínez J, Melero Vara J, Muñoz-Ruz J, Ruso J, Domínguez J (2000) Selection of Wild and Cultivated Sunflower for Resistance to a New Broomrape Race that Overcomes Resistance of the Or5 Gene. *Crop Science* 40(2):550–555. doi:10.2135/cropsci2000.402550x

- Fielder H, Brotherton P, Hosking J, Hopkins JJ, Ford-Lloyd B, Maxted N (2015a) Enhancing the Conservation of Crop Wild Relatives in England. *PLoS One* 10:e0130804. doi: 10.1371/journal.pone.0130804
- Fielder H, Smith C, Ford-Lloyd B, Maxted N (2015b) Enhancing the conservation of crop wild relatives in Scotland. *J Nat Conserv* 29:51–61. doi: 10.1016/j.jnc.2015.11.002
- Fitzgerald H (2013) The National Crop Wild Relative Strategy Report for Finland. <http://www.mtt.fi/mttraportti/pdf/mttraportti121.pdf>. Accessed 01/01/2018
- FNA - Flora of North America Editorial Committee, eds. (1993+). *Flora of North America North of Mexico*. 20+ vols. New York and Oxford
- Ford-Lloyd BV, Schmidt M, Armstrong SJ, Barazani O, Engels J, Hadas R, Hammer K, Kell SP, Kang D, Khoshbakht K, Li Y, Long C, Lu B, Ma K, Nguyen VT, Qiu L, Ge S, Wei W, Zhang Z, Maxted N (2011) Crop Wild Relatives—Undervalued, Underutilized and under Threat? *Bioscience* 61:559–565. doi: 10.1525/bio.2011.61.7.10
- García RM, Parra-Quijano M, Fajardo J, de la Rosa L (2015) Use of Eco-geographic tools in planning an optimized collection of wild relatives of cultivated legumes and cereals in Spain. In: de Ron AM (ed) *EUCARPIA-PCWG International Symposium on Protein Crops*. Pontevedra, Spain, p 118
- García RM, Parra-Quijano M, Iriondo JM (2017) A multispecies collecting strategy for crop wild relatives based on complementary areas with a high density of ecogeographical gaps. *Crop Science* 57(3): 1059–1069. <https://doi.org/10.2135/cropsci2016.10.0860>
- GBIF.org (27th November 2011– 27th September 2013) GBIF Occurrence Download. 500 Datasets downloaded. www.gbif.org. Accessed 1 May 2017
- GENESYS (2017) GENESYS Global Portal on Plant Genetic Resources. Data downloaded for 136 species from the following institutions: Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research (<http://www.ipk-gatersleben.de>), International Livestock Research Institute (<http://www.ilri.cgiar.org>), Millennium Seed Bank Project, Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place (<http://www.rbgekew.org.uk/msbp/>), International Crop Research Institute for the Semi-Arid Tropics (<http://www.icrisat.org>), International Centre for Agricultural Research in Dry Areas (<http://www.icarda.cgiar.org/>), Northeast Regional Plant Introduction Station, Plant Genetic Resources Unit, USDA-ARS, New York State Agricultural Experiment Station, Cornell University (<http://www.nysaes.cornell.edu/>), Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USDA-ARS (<http://www.ars-grin.gov/ars/SoAtlantic/Griffin/pgrcu/>), North Central Regional Plant Introduction Station, USDA-ARS, NCRPIS (<http://www.ars-grin.gov/ars/MidWest/Ames/index.html>), Western Regional Plant Introduction Station, USDA-ARS, Washington State University (<http://www.ars-grin.gov/ars/PacWest/Pullman/>), National Small Grains Germplasm Research Facility, USDA-ARS (<http://www.ars-grin.gov/ars/PacWest/Aberdeen/>), Wheat Genetics Resource Center (<http://www.k-state.edu/wgrc>) and Ornamental Plant Germplasm Center, Ohio State University (<http://hcs.osu.edu/opgc/>). All intellectual property rights (including copyright) in the Data are owned and retained by the said institutions. Data accessed through GENESYS Global Portal on Plant Genetic Resources. <http://www.genesys-pgr.org>, Accessed 31 December 2017
- Gomez-Campo U, Aguinalgalde I, Ceresuela JL, Lázaro A, Martínez-Laborde JB, Parra-Quijano M, Simonetti E, Torres E, Tortosa ME (2005) An exploration of wild Brassica oleracea L. germplasm in northern Spain. *Genet Resour Crop Evol* 52:7–13
- GRIN-USDA (2017) Genetic Resources Information Network of United States Department of Agriculture – Agricultural Research Service. <https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomyquery.aspx>. Accessed 30 April 2017
- Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica* 156:1–13. doi: 10.1007/s10681-007-9363-0
- Harlan JR, de Wet MJM (1971) Toward a rational classification of cultivated plants. *Taxon* 20:506–517
- Heywood V (2011) Conservation strategies for species/populations occurring outside protected areas. In: Hunter D, Heywood V (eds) *Crop Wild Relatives. A manual of in situ conservation*. Earthscan, London, UK; Washington-DC, USA, pp 253–294
- Heywood V, Casas A, Ford-Lloyd B, Kell S, Maxted N (2007) Conservation and sustainable use of crop wild relatives. *Agric Ecosyst Environ* 121:245–255. doi: 10.1016/j.agee.2006.12.014
- Heywood VH, Zohary D (1995) A Catalogue of the Wild Relatives of Cultivated Plants Native to Europe. *Flora Mediterr* 5:375–415
- Honnay O, Jacquemyn H, Aerts R (2012) Crop wild relatives: More common ground for breeders and ecologists. *Front Ecol Environ* 10(3):121. doi: 10.1890/12.WB.007
- Howden SM, Soussana J-F, Tubiello FN, Chhetri N, Dunlop M, Meinke H (2007) Adapting agriculture to climate change. *Proc Natl Acad Sci USA* 104(50):19691–19696. doi:10.1073/pnas.0701890104
- Hunter D, Heywood V (2011) *Crop Wild Relatives. A manual of in situ conservation*. Earthscan, London, UK

- Idohou R, Assogbadjo A E, Fandohan B, Gouwakinnou GN, Glele Kakai RL, Sinsin B, Maxted N (2013) National inventory and prioritization of crop wild relatives: Case study for Benin. *Genet Resour Crop Evol* 60(4):1337–1352. <https://doi.org/10.1007/s10722-012-9923-6>
- Iriondo JM, Fielder H, Fitzgerald H, Kell SP, Labokas J, Negri V, Phillips J, Rubio Teso ML, Sensen S, Taylor N, Maxted N (2016) National Strategies for the Conservation of Crop Wild Relatives. In: Maxted N, Dulloo ME, Ford-Lloyd BV (eds) *Enhancing Crop Genepool Use. Capturing wild relative and landrace diversity for crop improvement*. CAB International, Wallingford, UK, pp 161–171
- Kell SP, Knupffer H, Jury SL, Ford-Lloyd BV, Maxted N (2008) Crops and wild relatives of the Euro-Mediterranean region: making and using a conservation catalogue. In: *Crop Wild Relative Conservation and Use*. CAB International, Wallingford, UK, pp 69–109
- Kell SP, Knüpffer H, Jury SL, Maxted N, Ford-Lloyd BV (2005) Catalogue of crop wild relatives for Europe and the Mediterranean. University of Birmingham, Birmingham, UK. Available online via the Crop Wild Relative Information System (CWRIS – <http://www.pgforum.org/cwriscwrisc.asp>) and on CD-ROM.
- Kell SP, Maxted N and Bilz M (2012) European Crop Wild Relative Threat Assessment: Knowledge gained and lessons learnt. In: Maxted N, Dulloo ME, Ford-Lloyd BV, et al (eds.). *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CAB International, Wallingford, UK, pp 218–242
- Kell S, Qin H, Chen B, Ford-Lloyd B, Wei W, Kang D, Maxted N (2014) China's crop wild relatives: Diversity for agriculture and food security. *Agric Ecosyst Environ* 209:138–154. doi: 10.1016/j.agee.2015.02.012
- Khoury CK, Greene S, Wiersema J, Maxted N, Jarvis A, Struik PC (2013) An inventory of crop wild relatives of the United States. *Crop Sci* 53:1496–1508. doi: 10.2135/cropsci2012.10.0585
- Khoury CK, Heider B, Castañeda-Álvarez NP, Achicanoy HA, Sosa CC, Miller RE et al. (2015) Distributions, ex situ conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato (*Ipomoea batatas* (L.) Lam., I. series Batatas). *Front Plant Sci* 6:251. doi: 10.3389/fpls.2015.00251
- Killian B, Mammen K, Millet E, Sharma R, Graner A, Salamini F, Hammer K, Hakan O (2011) *Aegilops*. In: Kole C (ed) *Wild Crop Relatives: Genomic and Breeding Resources, Cereals*. Springer-Verlag, Berlin, Heidelberg, pp 1–76
- Kole C (2011a) *Wild crop relatives: Genomic and breeding resources. Cereals*. Springer, Heidelberg, Germany
- Kole C (2011b) *Wild crop relatives: Genomic and breeding resources. Millets and grasses*. Springer, Heidelberg, Germany
- Kole C (2011c) *Wild crop relatives: Genomic and breeding resources. Oilseeds*. Springer, Heidelberg, Germany
- Kole C (2011d) *Wild crop relatives: Genomic and breeding resources. Legume crops and forages*. Springer, Heidelberg, Germany
- Kole C (2011e) *Wild crop relatives: Genomic and breeding resources. Temperate fruits*. Springer, Heidelberg, Germany
- Labokas J, Karpavičienė B, Šveistytė L, et al (2010) Towards in situ conservation of crop wild relatives in Lithuania. In: First meeting of the ECPGR Wild Species Conservation in Genetic Reserves Working Group and On-farm Conservation and Management Working Group jointly held with the final dissemination meeting of the EU project AGRI GENRES 057-AEGRO. Funchal, Madeira (Portugal)
- Lala S, Amri A, Maxted N (2017) Towards the conservation of crop wild relative diversity in North Africa: checklist, prioritisation and inventory. *Genet Resour Crop Evol*. doi: 10.1007/s10722-017-0513-5
- Lotze-Campen H (2011) Improved data for integrated modeling of global environmental change. *Environ Res Lett* 6:41002. doi: 10.1088/1748-9326/6/4/041002
- Magos-Brehm J, Maxted N, Ford-Lloyd BV, Martins-Loução MA (2008) National inventories of crop wild relatives and wild harvested plants: Case-study for Portugal. *Genet Resour Crop Evol* 55:779–796. doi: 10.1007/s10722-007-9283-9
- Magos-Brehm J, Maxted N, Martins-Loução MA, Ford-Lloyd BV (2010) New approaches for establishing conservation priorities for socio-economically important plant species. *Biodivers Conserv* 19:2715–2740. doi: 10.1007/s10531-010-9871-4
- MAGRAMA (2011) Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid, Spain. *Anuario de estadística agraria* 2010
- MAGRAMA (2014) Ministerio de Agricultura, Alimentación y Medio Ambiente. *Estrategia Española de Conservación Vegetal 2014-2020. Principios y orientaciones para la conservación de la diversidad vegetal silvestre en España*. http://www.mapama.gob.es/es/biodiversidad/planes-y-estrategias/estrategia_ce_vegetal_2014-2020_tcm7-332576.pdf. Accessed 16 July 2017
- Markkola H (2005) Regional Red List Assessment and Biodiversity Action Plans for Crop Wild Relatives in Ireland. Master Thesis. Faculty of Science of the University of Birmingham
- Martín-Sánchez JA, Gómez-Colmenarejo M, Del Moral J, et al (2003) A new Hessian fly resistance gene (H30) transferred from the wild grass *Aegilops triuncialis* to hexaploid wheat. *Theor Appl Genet* 106:1248–1255. doi: 10.1007/s00122-002-1182-z

- Maxted N (2003) Conserving the genetic resources of crop wild relatives in European protected areas. *Biol Conserv* 113:411–417. doi: 10.1016/S0006-3207(03)00123-X
- Maxted N, Brehm JM, Kell S (2013) Resource Book for the Preparation of National Plans for Conservation of Crop Wild Relatives and Landraces.
- Maxted N, Ford-Lloyd B V., Jury S, Kell S, Scholten M (2006) Towards a definition of a crop wild relative. *Biodivers Conserv* 15:2673–2685. doi: 10.1007/s10531-005-5409-6
- Maxted N, Kell S, Ford-Lloyd B, Dulloo E, Toledo Á (2012) Toward the systematic conservation of global crop wild relative diversity. *Crop Sci* 52:774–785. doi: 10.2135/cropsci2011.08.0415
- Maxted N, Scholten M, Codd R, Ford-Lloyd B (2007) Creation and use of a national inventory of crop wild relatives. *Biol Conserv* 140:142–159. doi: 10.1016/j.biocon.2007.08.006
- Maxted N, White K, Valkoun J, Konopka J, Hargreaves S (2008) Towards a conservation strategy for *Aegilops* species. *Plant Genet Resour* 6:126–141. doi: 10.1017/S147926210899314X
- Médail F, Quézel P (1997) Hot-spots analysis for conservation of plant biodiversity in the Mediterranean basin. *Ann Missouri Bot Gard* 84:112–127
- Medail F, Quezel P (1999) Biodiversity Hotspots in the Mediterranean Basin: Setting Global Conservation Priorities. *Conserv Biol* 13:1510–1513 . doi: 10.1046/j.1523-1739.1999.98467.x
- MIMA (2006) Ministerio de Industria y Medio Ambiente. Estrategia Española para la Conservación y el Uso Sostenible de los Recursos Genéticos Forestales. http://www.mapama.gob.es/en/biodiversidad/publicaciones/sfs_tcm11-30498.pdf. Accessed 28 November 2017
- Molina-Venegas R, Aparicio A, Lavergne S, Arroyo J (2015) The building of a biodiversity hotspot across a land-bridge in the Mediterranean. *Proc R Soc B Biol Sci*. doi: 10.1098/rspb.2015.1116
- Moreno JC coord. (2008) Lista Roja de la flora vascular española. Dirección General del Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid, España
- Müller C, Robertson RD (2014) Projecting future crop productivity for global economic modeling. *Agric Econ (United Kingdom)* 45:37–50. doi: 10.1111/agec.12088
- NOAA (2017) National Centers for Environmental Information, State of the Climate: Global Climate Report for Annual 2016, published online January 2017, retrieved on July 15, 2017 from <https://www.ncdc.noaa.gov/sotc/global/201613>
- Panella L, Landucci F, Torricelli R, Gigante D, Donnini D, Venanzoni R, Negri V (2014) The National Crop Wild Relative Strategy for Italy: First steps to be taken. 21 pp. doi: 10.2135/cropsci2013.05.0355
- Parra-Quijano M, Iriondo JM, Torres E (2012a) Improving representativeness of genebank collections through species distribution models, gap analysis and ecogeographical maps. *Biodivers Conserv* 21:79–96. doi: 10.1007/s10531-011-0167-0
- Parra-Quijano M, Iriondo JM, Torres E (2012b) Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genet Resour Crop Evol* 59:205–217. doi: 10.1007/s10722-011-9676-7
- Pascual H (2004) *Lupinus mariae-josephi* (Fabaceae), nueva y sorprendente especie descubierta en España. *An Jardín Botánico Madrid* 61:69–72
- Pautasso M (2012) Challenges in the conservation and sustainable use of genetic resources. *Biol Lett* 8:321–3. doi: 10.1098/rsbl.2011.0984
- Pérez de Castro A, Díez MJ, Nuez F (2005) Evaluation of breeding tomato lines partially resistant to Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus derived from *Lycopersicon chilense*. *Canadian Journal of Plant Pathology* 27(2): 268–275. doi: 10.1080/07060660509507224
- Phillips J, Asdal Å, Magos Brehm J, Rasmussen M, Maxted N (2016) In situ and ex situ diversity analysis of priority crop wild relatives in Norway. *Divers Distrib* 1–15. doi: 10.1111/ddi.12470
- Phillips J, Kyrtatzis A, Christoudoulou C, Kell S, Maxted N (2014) Development of a national crop wild relative conservation strategy for Cyprus. *Genet Resour Crop Evol* 61:817–827. doi: 10.1007/s10722-013-0076-z
- Pico B, Ferriol M, Díez MJ, Nuez F (1999) Developing tomato breeding lines resistant to tomato yellow leaf curl virus. *Plant Breeding* 118: 537–542. doi:10.1046/j.1439-0523.1999.00427.x
- Pinheiro de Carvalho MÂ, Nóbrega H, Freitas G, Fontinha S, Frese L (2012) Towards the establishment of a genetic reserve for *Beta patula* Aiton. In: Maxted N, Dulloo ME, Ford-Lloyd BV et al. (eds) *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. CAB International, Wallingford, UK, pp 36–44
- Prosperi JM, Jenczewski E, Angevain M, Ronfort J (2006) Morphologic and agronomic diversity of wild genetic resources of *Medicago sativa* L. collected in Spain. *Genet Resour Crop Evol* 53:843–856. doi: 10.1007/s10722-004-6476-3

- Romero Zarco C (1996) Sinopsis del género *Avena* L. (Poaceae, Aveneae) en España peninsular y Baleares. *Lagascalia* 18:171–198
- Ruiz M, Quemada M, García RM, et al (2016) Use of thermographic imaging to screen for drought-tolerant genotypes in *Brachypodium distachyon*. *Crop Pasture Sci* 67:99–108 . doi: 10.1071/CP15134
- Sheppard D (1999) Conservation without frontiers - The global view. In: EUROPARC 99. Transcending Borders - Parks for Europe. Zakopane, Poland, p 26
- Smekalova TN (2008) National Crop Wild Relative In Situ conservation Strategy for Russia. In: Maxted N, Ford-Lloyd BV, Kell SP, et al. (eds) *Crop Wild Relative Conservation and Use*. CAB International, Oxfordshire, UK, pp 143–151
- Soler C, Ruiz-Fernández J, Monte JV, de Bustos A, Jouve N (1997) The assessment of variability in Spanish populations of wild relatives of cereals. *Bocconea* 7:107–119
- Stamp P, Visser R (2012) The twenty-first century, the century of plant breeding. *Euphytica* 186:585–591. doi: 10.1007/s10681-012-0743-8
- Taylor NG, Kell SP, Holubec V, Parra-Quijano M, Chobot K, Maxted N (2017) A systematic conservation strategy for crop wild relatives in the Czech Republic. *Divers Distrib* 1–15. doi: 10.1111/ddi.12539
- Tester M, Langridge P (2010) Breeding Technologies to Increase Crop Production in a Changing World. *Science* 327:818–822. doi: 10.1126/science.1183700
- Tubiello FN, Fischer G (2007) Reducing climate change impacts on agriculture: Global and regional effects of mitigation, 2000–2080. *Technol Forecast Soc Change* 74:1030–1056. doi: 10.1016/j.techfore.2006.05.027
- Turner N, Meyer R (2011) Synthesis of regional impacts and global agricultural adjustments. In: Yadav S, Redden R, Hatfield J, et al. (eds) *Crop adaptation to climate change*. Wiley-Blackwell, Chichester, West Sussex, UK, pp 156–165
- UN (2015) United Nations General Assembly. Transforming our world: the 2030 Agenda for Sustainable Development. http://www.un.org/ga/search/view_doc.asp?symbol=A/RES/70/1&Lang=E. Accessed 1 December 2017
- UN CBD (2010) United Nations Convention on Biological Diversity Conference of the Parties 10 Decision X/2
- UPOV - International Union for the Protection of New Varieties of Plants (2011) List of the taxa protected by the members of the Union. Geneva, October 2011. http://www.upov.int/edocs/mdocs/upov/en/c/45/c_45_6.pdf. Accessed 27 December 2017
- van Treuren R, Hoekstra R, van Hintum TJJ (2017) Inventory and prioritization for the conservation of crop wild relatives in The Netherlands under climate change. *Biol Conserv* 216:123–139. <https://doi.org/10.1016/j.biocon.2017.10.003>
- Vincent H, Wiersema J, Kell S, et al (2013) A prioritized crop wild relative inventory to help underpin global food security. *Biol Conserv* 167:265–275 . doi: 10.1016/j.biocon.2013.08.011
- VMABCC-BIOVERSITY (2009) Libro rojo de parientes silvestres de cultivos de Bolivia. La Paz, Bolivia
- Whitlock R, Hipperson H, Thompson DBA, Butlin RK, Burke T (2016) Consequences of in-situ strategies for the conservation of plant genetic diversity. *Biol Conserv*. 203:134–142. doi: 10.1016/j.biocon.2016.08.006
- Wiersema JH, León B (1999) *World economic plants: A standard reference*. CRC Press LLC, Boca Raton, FL (USA)
- Zamir D (2001) Improving plant breeding with exotic genetic libraries. *Nat Rev Genet* 2:983–989. doi: 10.1038/35103589

Supplementary material

Online Resource 1: Selection of projects for the conservation of CWR developed over the last few years.

Available at: <https://link.springer.com/article/10.1007/s10722-018-0610-0>, Supplementary Material 1.

Online Resource 2: Information gathered for each CWR species listed for the generation of the Prioritized Spanish Checklist of Crop Wild Relatives. Available at: <https://link.springer.com/article/10.1007/s10722-018-0610-0>, Supplementary Material 2.

Online Resource 3: List of references used for the compilation of associated information of the Spanish crop wild relatives. Available at <https://link.springer.com/article/10.1007/s10722-018-0610-0>, Supplementary Material 3.

Online Resource 4: Database with the baseline list of crops of importance and associated information for each genus. Information on use of the crop, inclusion of the crop in the Annex 1 of the International Treaty of Plant Genetic Resources for Food and Agriculture, nativeness, economic importance and use in breeding is displayed. Available at <https://link.springer.com/article/10.1007/s10722-018-0610-0>, Supplementary Material 4

Online Resource 5: List of prioritized genera obtained for the generation of the Spanish Checklist of CWRs. Information on families, use categories to which they have been assigned and the reason for their inclusion on the list. Genera ordered by use category and alphabetically. Codes: 1 = Included in Annex 1 of the International Treaty for Food and Agriculture; 2 = Included in Spanish Annual Directory 2010; 3 = Included in the list of registered varieties, period 1973-2010; 4 = Experts in agrobiodiversity advice. Available at <https://link.springer.com/article/10.1007/s10722-018-0610-0>, Supplementary Material 5

Online Resource 6: List of protected species under law 2007/42, royal decree 139/2011 and on the Checklist or on the Prioritized Checklist of Spanish of Crop Wild Relatives. Codes for categories: 1 = Food; 2 = Forage & Fodder; 3 = Ornamental; 4 = Industrial & Other uses. * = The species are not prioritized but are listed on the Spanish Checklist of Crop Wild Relatives. ** = These species are not included on the Spanish Checklist of Crop Wild Relatives nor in the Prioritized Checklist because *Diplotaxis* was one of the genera recommended by experts and only those species suggested by them were included. *** = These species are not included on the Spanish Checklist of Crop Wild Relatives nor on the Prioritized Checklist because *Flora Iberica* does not include them in the publication of their respective chapters. Available at <https://link.springer.com/article/10.1007/s10722-018-0610-0>, Supplementary Material 6.

Annex 1: Prioritized Spanish Checklist of Crop Wild Relatives available at <https://pgrsecurespain.weebly.com/crop-wild-relatives-in-spain---prioritization-of-the-checklist.html>. Codes for priority collection: 1= Urgent priority. Primary or secondary gene pool and taxon group, endemic and threatened. No representation in gene banks; 2= Urgent. Species not represented in gene banks; 3= Need collecting. Less than five populations represented in gene banks; 4= Non priority for collection. More than five accessions in gene banks

Category	Species	Endemicity	Concept and Level Gene Pool or Taxon Group	Red List of Spanish Vascular Flora	Threat Category (IUCN)	European Red List of Vascular Plants and Category	No. of accessions in Germplasm banks	Priority for collection
Food	<i>Aegilops geniculata</i> Roth	NO	Gene pool 2	NO	NA	NO -	220	4
Food	<i>Aegilops lorentii</i> Hochst.	NO	Gene pool 2	NO	NA	NO -	1	3
Food	<i>Aegilops neglecta</i> Req. ex Bertol.	NO	Gene pool 2	NO	NA	NO -	75	4
Food	<i>Aegilops triuncialis</i> L.	NO	Gene pool 2	NO	NA	NO -	277	4
Food	<i>Aegilops ventricosa</i> Tausch	NO	Gene pool 2	NO	NA	NO -	46	4
Forage & Fodder	<i>Agrostis alpina</i> Scop.	NO	Taxon Group 3	NO	NA	NO -	0	2
Forage & Fodder	<i>Agrostis barceloi</i> L. Sáez & Rossellò	YES (SP)	Taxon Group 4	YES	CR	NO -	6	4
Forage & Fodder	<i>Agrostis canina</i> L.	NO*	Taxon Group 3	YES	VU	NO -	5	4
Forage & Fodder	<i>Agrostis capillaris</i> L.	NO	Gene pool 1b	NO	NA	NO -	56	4
Forage & Fodder	<i>Agrostis castellana</i> Boiss. & Reut.	NO	Taxon Group 2	NO	NA	NO -	8	4
Forage & Fodder	<i>Agrostis curtisii</i> Kerguelen	NO	Taxon Group 3	NO	NA	NO -	10	4
Forage & Fodder	<i>Agrostis hesperica</i> Romero García, Blanca & Morales Torres	YES (SP & PT)	Taxon Group 3	NO	NA	NO -	0	2
Forage & Fodder	<i>Agrostis nevadensis</i> Boiss.	YES (SP)	Taxon Group 3	NO	NA	NO -	5	4
Forage & Fodder	<i>Agrostis pourreti</i> Willd.	NO	Taxon Group 3	NO	NA	NO -	0	2
Forage & Fodder	<i>Agrostis rupestris</i> All.	NO	Taxon Group 3	NO	NA	NO -	3	3
Forage & Fodder	<i>Agrostis schleicheri</i> Jord. & Verl.	NO	Taxon Group 3	NO	NA	NO -	3	3
Forage & Fodder	<i>Agrostis stolonifera</i> L.	NO	Gene pool 1b	NO	NA	NO -	13	4
Forage & Fodder	<i>Agrostis tenerima</i> Trin.	NO	Taxon Group 3	NO	NA	NO -	0	2
Forage & Fodder	<i>Agrostis tileni</i> Nieto Feliner & Castroviejo	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Food	<i>Allium ampeloprasum</i> L.	NO	Gene pool 1B	NO	NA	NO -	35	4
Food	<i>Allium commutatum</i> Guss.	NO	Gene pool 1B	NO	NA	NO -	1	3
Food	<i>Allium grosii</i> Font Quer	YES (SP)	Taxon Group 4	NO	NA	NO -	5	4
Food	<i>Allium melananthum</i> Coincy	YES (SP)	Taxon Group 4	NO	NA	NO -	1	3
Food	<i>Allium palentinum</i> Losa & P. Montserrat	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Food	<i>Allium pruinatum</i> Link ex Spreng.	YES (SP & PT)	Taxon Group 4	YES	VU	NO -	6	4
Food	<i>Allium pyrenaicum</i> Costa & Vayr.	YES (SP)	Taxon Group 4	YES	NT	YES VU	9	4
Food	<i>Allium rouyi</i> Gaut.	NO	Taxon Group 4	YES	CR	NO -	4	3
Food	<i>Allium schmitzii</i> Cout.	YES (SP & PT)	Taxon Group 2	YES	VU	YES VU	0	1
Food	<i>Allium schoenoprasum</i> L.	NO	Taxon Group 1B	NO	NA	NO -	5	4
Food	<i>Allium sphaerocephalon</i> L.	NO	Taxon Group 4	NO*	VU	NO -	13	4
Food	<i>Allium stearnii</i> Pastor & Valdés	YES (SP & PT)	Taxon Group 4	NO	NA	NO -	1	3

Annex 1: Prioritized Spanish Checklist of Crop Wild Relatives available at <https://pgrsecurespain.weebly.com/crop-wild-relatives-in-spain---prioritization-of-the-checklist.html>. Codes for priority collection: 1= Urgent priority. Primary or secondary gene pool and taxon group, endemic and threatened. No representation in gene banks; 2= Urgent. Species not represented in gene banks; 3= Need collecting. Less than five populations represented in gene banks; 4= Non priority for collection. More than five accessions in gene banks

Category	Species	Endemicity	Concept and Level Gene Pool or Taxon Group	Red List of Spanish Vascular Flora	Threat Category (IUCN)	European Red List of Vascular Plants and Category	No. of accessions in Germplasm banks	Priority for collection
Food	<i>Allium subhirsutum</i> L.	NO	Taxon Group 4	NO*	NT	NO -	0	2
Food	<i>Apium bermejoi</i> L. Llorens	YES (SP)	Taxon Group 4	YES	CR	NO -	41	4
Food	<i>Apium graveolens</i> L.	NO	Gene pool 1	NO*	CR	NO -	1	3
Ornamental	<i>Argyranthemum broussonetii</i> (Pers.) Humphries	YES (CAN)	Gene pool 2	YES	VU	NO -	15	4
Ornamental	<i>Argyranthemum callichrysum</i> (Svent.) Humphries	YES (CAN)	Taxon Group 2	YES	VU	NO -	16	4
Ornamental	<i>Argyranthemum coronopifolium</i> (Willd.) Humphries	YES (CAN)	Gene pool 2	YES	VU	NO -	4	3
Ornamental	<i>Argyranthemum foeniculaceum</i> (Willd.) Webb ex Sch. Bip.	YES (CAN)	Taxon Group 2	YES	VU	NO -	13	4
Ornamental	<i>Argyranthemum frutescens</i> (L.) Sch. Bip.	YES (CAN)	Gene pool 1b	NO*	VU-NT-LC	NO -	72	4
Ornamental	<i>Argyranthemum gracile</i> Sch. Bip.	YES (CAN)	Taxon Group 2	NO	NA	NO -	10	4
Ornamental	<i>Argyranthemum haouarytheum</i> Humphries & Bramwell	YES (CAN)	Taxon Group 2	NO	NA	NO -	20	4
Ornamental	<i>Argyranthemum lemsii</i> Humphries	YES (CAN)	Taxon Group 2	YES	VU	NO -	1	3
Ornamental	<i>Argyranthemum lidii</i> Humphries	YES (CAN)	Taxon Group 2	YES	EN	NO -	9	4
Ornamental	<i>Argyranthemum maderense</i> (D. Don) Humphries	YES (CAN)	Taxon Group 2	YES	VU	NO -	10	4
Ornamental	<i>Argyranthemum sundingii</i> L. Borgen	YES (CAN)	Gene pool 2	YES	CR	NO -	16	4
Ornamental	<i>Argyranthemum sventenii</i> Humphries & Aldridge	YES (CAN)	Taxon Group 2	YES	VU	NO -	10	4
Ornamental	<i>Argyranthemum tenerifae</i> Humphries	YES (CAN)	Taxon Group 2	NO	NA	NO -	8	4
Ornamental	<i>Argyranthemum winteri</i> (Svent.) Humphries	YES (CAN)	Taxon Group 2	YES	CR	NO -	6	4
Food	<i>Asparagus acutifolius</i> L.	NO	Gene pool PU (3)	NO	NA	NO -	29	4
Food	<i>Asparagus albus</i> L.	NO	Gene pool PU (3)	NO	NA	NO -	3	3
Food	<i>Asparagus aphyllus</i> L.	NO	Gene pool PU (2)	NO	NA	NO -	1	3
Food	<i>Asparagus arborescens</i> Willd.	YES (CAN)	Taxon Group 4	NO	NA	YES VU	5	4
Food	<i>Asparagus fallax</i> Svent.	YES (CAN)	Taxon Group 4	YES	EN	YES EN	1	3
Food	<i>Asparagus maritimus</i> (L.) Mill.	NO	Taxon Group 3	YES	CR	NO -	0	2
Food	<i>Asparagus nesiotis</i> Svent.	YES (SP & PT)	Taxon Group 4	NO*	EN	YES EN	3	3
Food	<i>Asparagus officinalis</i> L.	NO	Taxon Group 1B	NO*	VU	NO -	8	4
Food	<i>Asparagus pastorianus</i> Webb & Berthel.	YES (CAN)	Taxon Group 4	NO	NA	YES VU	9	4
Food	<i>Asparagus plocamoides</i> Webb ex Svent.	YES (CAN)	Taxon Group 4	NO	NA	YES VU	10	4
Food	<i>Asparagus stipularis</i> Forssk.	NO	Taxon Group 3	NO	NA	NO -	1	3
Forage & Fodder	<i>Astragalus algerianus</i> E. Sheld.	NO	Taxon Group 4	YES	EX	NO -	0	2
Forage & Fodder	<i>Astragalus alopecuroides</i> L.	NO	Taxon Group 3	NO	NA	NO -	12	4
Forage & Fodder	<i>Astragalus alpinus</i> L.	NO	Taxon Group 3	NO	NA	NO -	1	3

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Forage & Fodder	<i>Astragalus australis</i> (L.) Lam	NO	Taxon Group 3	NO	NA	NO -	1	3
Forage & Fodder	<i>Astragalus baionensis</i> Loisel.	NO	Taxon Group 4	YES	EX	NO -	0	2
Forage & Fodder	<i>Astragalus balearicus</i> Chater	YES (SP)	Taxon Group 4	NO	NA	NO -	19	4
Forage & Fodder	<i>Astragalus bourgaeanus</i> Coss.	NO	Taxon Group 2	NO	NA	NO -	1	3
Forage & Fodder	<i>Astragalus cavanillesii</i> Podlech	YES (SP)	Taxon Group 3	YES	CR	NO -	0	2
Forage & Fodder	<i>Astragalus clusianus</i> Soldano	YES (SP)	Taxon Group 3	NO	NA	NO -	4	3
Forage & Fodder	<i>Astragalus danicus</i> Retz.	NO	Taxon Group 2	NO	LC	NO -	0	2
Forage & Fodder	<i>Astragalus depressus</i> L.	NO	Taxon Group 3	NO	NA	NO -	0	2
Forage & Fodder	<i>Astragalus edulis</i> Bunge	NO	Taxon Group 4	YES	EN	NO -	8	4
Forage & Fodder	<i>Astragalus ginez-lopezii</i> Talavera	YES (SP)	Taxon Group 4	YES	EN	NO -	0	2
Forage & Fodder	<i>Astragalus glaucus</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Astragalus glycyphyllos</i> L.	NO	Gene pool 1b	NO	NA	NO -	5	4
Forage & Fodder	<i>Astragalus granatensis</i> Lam.	NO	Taxon Group 3	NO	NA	NO -	4	3
Forage & Fodder	<i>Astragalus hispanicus</i> Coss. ex Bunge	YES (SP)	Taxon Group 4	NO	NA	NO -	4	3
Forage & Fodder	<i>Astragalus hypoglottis</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Astragalus longidentatus</i> Chater	NO	Taxon Group 4	YES	NT	NO -	4	3
Forage & Fodder	<i>Astragalus mareoticus</i> Delarb.	NO	Taxon Group 4	YES	VU	NO -	1	3
Forage & Fodder	<i>Astragalus nevadensis</i> Boiss.	YES (SP)	Taxon Group 3	NO	NA	NO -	6	4
Forage & Fodder	<i>Astragalus nitidiflorus</i> Jiménez Mun. & Pau	YES (SP)	Taxon Group 4	YES	CR	NO -	0	2
Forage & Fodder	<i>Astragalus oxyglottis</i> M. Bieb.	NO	Taxon Group 4	YES	VU	NO -	4	3
Forage & Fodder	<i>Astragalus penduliflorus</i> Lam.	NO	Taxon Group 3	YES	EN	NO -	1	3
Forage & Fodder	<i>Astragalus sempervirens</i> Lam.	NO	Taxon Group 3	NO	NA	NO -	9	4
Forage & Fodder	<i>Astragalus tremolsianus</i> Pau	YES (SP)	Taxon Group 3	YES	CR	NO -	2	3
Forage & Fodder	<i>Astragalus turolensis</i> Pau	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Food	<i>Avena canariensis</i> R. Baum, Rajhathy & D. R. Sampson	YES (CAN)	Gene pool 3	YES	VU	NO -	12	4
Food	<i>Avena fatua</i> L.	NO	Gene pool 1B	NO	NA	NO -	30	4
Food	<i>Avena lusitanica</i> (Tab. Morais) R. Baum	YES (SP)	Gene pool 3	NO	NA	NO -	0	2
Food	<i>Avena murphyi</i> Ladiz.	YES (SP)	Gene pool 2	YES	EN	YES EN	20	4
Food	<i>Avena sterilis</i> L.	NO	Gene pool CU (1b)	NO	NA	NO -	560	4
Food	<i>Beta macrocarpa</i> Guss.	NO	Gene pool 1B	NO	NA	YES EN	36	4
Food	<i>Beta maritima</i> L.	NO	Gene pool 1b	NO*	VU	NO -	4	3

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Food	<i>Borago officinalis</i> L.	NO	Taxon Group 1B	NO	NA	NO -	14	4
Industrial & Other Us	<i>Brachypodium distachyon</i> (L.) P. Beauv.	NO	Gene pool 1b	NO	NA	NO -	7	4
Industrial & Other Us	<i>Brachypodium stacei</i> Catalán, Joch. Mull, Hasterok & Jenkins	NO	Gene pool 2	NO	NA	NO -	2	3
Food	<i>Brassica balearica</i> Pers.	YES (SP)	Gene pool 3	NO	NA	NO -	7	4
Food	<i>Brassica barrelieri</i> (L.) Janka	NO	Gene pool 2	NO	NA	NO -	11	4
Food	<i>Brassica bourgeau</i> (Webb ex Christ) Kuntze	YES (CAN)	Gene pool 2	YES	EN	NO -	8	4
Food	<i>Brassica montana</i> Pourr.	NO	Gene pool 2	NO	NA	NO -	2	3
Food	<i>Brassica napus</i> L.	NO	Gene pool 1B	NO	NA	NO -	1	3
Food	<i>Brassica nigra</i> (L.) W.D.J. Koch	NO	Gene pool 1B	NO	NA	NO -	11	4
Food	<i>Brassica oleracea</i> L.	NO	Gene pool 1B	NO	NA	NO -	38	4
Food	<i>Brassica repanda</i> (Wind.) DC.	YES (Some)	Gene pool 3	NO*	VU	NO -	75	4
Food	<i>Brassica tournefortii</i> Gouan	NO	Gene pool CU (3)	NO	NA	NO -	3	3
Food	<i>Capsella bursa-pastoris</i> (L.) Medik.	NO	Gene pool PU (3)	NO	NA	NO -	7	4
Industrial & Other Us	<i>Carthamus creticus</i> L.	NO	Gene pool 2	NO	NA	NO -	0	2
Food	<i>Cicer canariense</i> A. Santos & G. P. Lewis	YES (CAN)	Taxon Group 4	YES	EN	YES EN	11	4
Food	<i>Cichorium intybus</i> L.	NO	Gene pool 1b	NO	NA	NO -	33	4
Food	<i>Cichorium spinosum</i> L.	NO	Gene pool 2	NO	NA	NO -	2	3
Food	<i>Cynara alba</i> Boiss. ex DC.	YES (SP)	Gene pool 2	YES	VU	NO -	3	3
Food	<i>Cynara algarbiensis</i> Coss. ex Mariz	NO	Gene pool 2	YES	VU	NO -	9	4
Food	<i>Cynara cardunculus</i> L.	NO	Gene pool 1B	NO	NA	NO -	10	4
Food	<i>Cynara humilis</i> L.	NO	Gene pool 2	NO	NA	NO -	13	4
Food	<i>Cynara tournefortii</i> Boiss. & Reut.	YES (SP & PT)	Taxon Group 4	YES	CR	NO -	16	4
Forage & Fodder	<i>Dactylis glomerata</i> L.	NO	Gene pool 1b	NO	NA	NO -	947	4
Forage & Fodder	<i>Dactylis metlesicsii</i> Schonfelder & Ludwig	YES (CAN)	Taxon Group 4	YES	EN	NO -	0	2
Forage & Fodder	<i>Dactylis smithii</i> Link	YES (CAN)	Taxon Group 4	NO	DD	NO -	3	3
Food	<i>Daucus arcanus</i> García Martín & Silvestre	YES (SP)	Taxon Group 4	YES	EN	NO -	1	3
Food	<i>Daucus carota</i> L.	YES (SP & PT)	Gene pool 1B	NO	NA	NO -	133	4
Forage & Fodder	<i>Deschampsia cespitosa</i> (L.) Beauv.	NO	Gene pool 1b	NO	NA	NO -	8	4
Forage & Fodder	<i>Deschampsia setacea</i> (Huds.) Hack.	NO	Taxon Group 4	YES	EN	NO -	2	3
Ornamental	<i>Dianthus algetanus</i> Graells ex F. N. Williams	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Dianthus anticarius</i> Boiss. & Reut.	NO	Taxon Group 3	NO*	VU	NO -	0	2

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Ornamental	<i>Dianthus armeria</i> L.	NO	Taxon Group 2	NO	NA	NO -	3	3
Ornamental	<i>Dianthus barbatus</i> L.	NO	Gene pool 1b	NO*	NT	NO -	2	3
Ornamental	<i>Dianthus benearnensis</i> Loret	NO	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Dianthus boissieri</i> Willk.	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Dianthus broteri</i> Boiss. & Reut.	YES (SP & PT)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Dianthus carthusianorum</i> L.	NO	Gene pool 1b	NO	NA	NO -	0	2
Ornamental	<i>Dianthus costae</i> Willk.	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Dianthus crassipes</i> R. Roem.	YES (SP & PT)	Taxon Group 2	NO	NA	NO -	2	3
Ornamental	<i>Dianthus deltoides</i> L.	NO	Gene pool 1b	NO	NA	NO -	3	3
Ornamental	<i>Dianthus hyssopifolius</i> L.	NO	Gene pool 2	NO	NA	NO -	4	3
Ornamental	<i>Dianthus langedanus</i> Willk.	YES (SP & PT)	Taxon Group 3	NO	NA	NO -	9	4
Ornamental	<i>Dianthus laricifolius</i> Boiss. & Reut.	YES (SP & PT)	Taxon Group 2	NO*	VU	NO -	2	3
Ornamental	<i>Dianthus legionensis</i> (Willk.) F. N. Williams	YES (SP)	Taxon Group 3	NO	NA	NO -	1	3
Ornamental	<i>Dianthus multiceps</i> Costa ex Willk.	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Dianthus pungens</i> L.	NO	Taxon Group 3	NO	NA	NO -	3	3
Ornamental	<i>Dianthus pyrenaicus</i> Pourr.	NO	Taxon Group 2	NO	NA	NO -	4	3
Ornamental	<i>Dianthus rupicola</i> Biv.	NO	Taxon Group 3	NO*	VU	NO -	0	2
Ornamental	<i>Dianthus seguieri</i> subsp. <i>requienii</i> (Godr.) Bernal, M. Laínz & Muñoz Garm.	NO	Gene pool 2	NO	NA	NO -	0	2
Ornamental	<i>Dianthus toletanus</i> Boiss. & Reut.	YES (SP)	Taxon Group 2	YES	NT	NO -	0	1
Food	<i>Diplotaxis erucoides</i> (L.) DC.	NO	Gene pool PU (3)	NO	NA	NO -	10	4
Food	<i>Diplotaxis muralis</i> (L.) DC.	NO	Gene pool PU (3)	NO	NA	NO -	0	2
Food	<i>Diplotaxis tenuifolia</i> (L.) DC.	NO	Gene pool PU (2)	NO	NA	NO -	2	3
Food	<i>Erucastrum canariense</i> Webb & Berthel.	YES (CAN)	Gene pool CU (3)	NO	NA	NO -	2	3
Food	<i>Erucastrum gallicum</i> (Willd.) O. E. Schulz	NO	Gene pool 2	YES	NT	NO -	0	2
Forage & Fodder	<i>Festuca agustinii</i> Linding.	YES (CAN)	Taxon Group 4	NO	NA	NO -	4	3
Forage & Fodder	<i>Festuca altopyreanaica</i> Fuente & Ortúñez	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca aragonensis</i> (Willk.) Fuente & Ortúñez	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca arundinacea</i> Schreb.	NO	Gene pool 1b	NO	NA	NO -	178	4
Forage & Fodder	<i>Festuca borderi</i> (Hack.) K.Richt.	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca brigantina</i> (Markgr. -Dann.) Markgr.-Dann.	YES (SP & PT)	Taxon Group 4	YES	VU	NO -	1	3

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Forage & Fodder	<i>Festuca burnatii</i> St. -Yves	YES (SP)	Taxon Group 4	NO	NA	NO -	1	3
Forage & Fodder	<i>Festuca clementei</i> Boiss.	YES (SP)	Taxon Group 4	YES	VU	NO -	4	3
Forage & Fodder	<i>Festuca cordubensis</i> Devesa	YES (SP)	Taxon Group 4	NO	DD	NO -	0	2
Forage & Fodder	<i>Festuca curvifolia</i> Lag. ex Lange	YES (SP)	Taxon Group 4	NO	NA	NO -	8	4
Forage & Fodder	<i>Festuca elegans</i> Boiss.	YES (SP & PT)	Taxon Group 4	NO	NA	NO -	7	4
Forage & Fodder	<i>Festuca frigida</i> (Hack.) K.Richt.	YES (SP)	Taxon Group 4	YES	VU	NO -	1	3
Forage & Fodder	<i>Festuca gigantea</i> (L.) Vill.	NO	Gene pool 1b	NO	NA	NO -	5	4
Forage & Fodder	<i>Festuca glacialis</i> (Miégev. ex Hack.) K.Richt.	YES (SP)	Taxon Group 4	NO	NA	NO -	2	3
Forage & Fodder	<i>Festuca graniticola</i> Kerguelen & Morla	YES (SP)	Taxon Group 4	YES	VU	NO -	0	2
Forage & Fodder	<i>Festuca iberica</i> (Hack.) K.Richt.	YES (SP & PT)	Taxon Group 4	NO	NA	NO -	4	3
Forage & Fodder	<i>Festuca lasto</i> Boiss.	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca longiauriculata</i> Fuente, Ortúñez & Ferrero	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca paucispicula</i> Fuente & Sánchez Mata	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca picoeuropeana</i> Nava	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca pratensis</i> Huds.	NO	Gene pool 1b	NO	NA	NO -	3	3
Forage & Fodder	<i>Festuca pseudeskia</i> Boiss.	YES (SP)	Taxon Group 4	NO	NA	NO -	1	3
Forage & Fodder	<i>Festuca quadrifolia</i> Honck.	YES (SP)	Taxon Group 4	YES	VU	NO -	0	2
Forage & Fodder	<i>Festuca querana</i> Litard.	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca reverchonii</i> Hack.	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca rivas-martinezii</i> Fuente & Ortúñez	YES (SP & PT)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca rothmaleri</i> (Litard.) Markgr.-Dann.	YES (SP)	Taxon Group 4	NO	NA	NO -	1	3
Forage & Fodder	<i>Festuca segimonensis</i> Fuente, Ortúñez & Müller	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Forage & Fodder	<i>Festuca summilusitana</i> Franco & Rocha Afonso	YES (SP & PT)	Taxon Group 4	NO	NA	NO -	1	3
Forage & Fodder	<i>Festuca vettonica</i> Fuente, Ortúñez & Ferrero	YES (SP)	Taxon Group 4	NO	NA	NO -	0	2
Industrial & Other Us	<i>Gentiana burseri</i> Lapeyr.	NO	Gene pool 2	NO	NA	NO -	3	3
Industrial & Other Us	<i>Gentiana lutea</i> L.	NO*	Gene pool 1b	NO	RE	NO -	127	4
Forage & Fodder	<i>Hedysarum coronarium</i> L.	NO	Gene pool 1b	NO	NA	NO -	21	4
Forage & Fodder	<i>Hedysarum flexuosum</i> L.	NO	Taxon Group 2	NO	DD	NO -	0	2
Forage & Fodder	<i>Hedysarum glomeratum</i> F. Dietr.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Hedysarum spinosissimum</i> L.	NO	Taxon Group 2	NO	NA	NO -	1	3
Food	<i>Hordeum bulbosum</i> L.	NO	Gene pool 2	NO	NA	NO -	10	4

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Food	<i>Hordeum distichon</i> L.	NO	Gene pool 1B	NO	NA	NO -	0	2
Food	<i>Hordeum zeocriton</i> L.	NO	Gene pool 1B	NO	NA	NO -	0	2
Industrial & Other Us	<i>Hypericum maculatum</i> Crantz	NO	Gene pool 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Hypericum perforatum</i> L.	NO	Gene pool 1b	NO	NA	NO -	169	4
Industrial & Other Us	<i>Hypericum richeri</i> Vill.	NO	Gene pool 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Hypericum tetrapterum</i> Fr.	NO	Gene pool 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Hypericum undulatum</i> Schousb. ex Willd.	NO	Taxon Group 2	NO	NA	NO -	4	3
Food	<i>Lactuca livida</i> Boiss. & Reut.	NO	Gene pool 2	NO	NA	NO -	0	2
Food	<i>Lactuca palmensis</i> Bolle	YES (CAN)	Taxon Group 4	YES	NT	NO -	7	4
Food	<i>Lactuca perennis</i> L.	NO	Taxon Group 4	NO*	VU	NO -	2	3
Food	<i>Lactuca saligna</i> L.	NO	Gene pool 2	NO	NA	NO -	10	4
Food	<i>Lactuca serriola</i> L.	NO	Gene pool 1B	NO	NA	NO -	192	4
Food	<i>Lactuca singularis</i> Wilmott	YES (SP)	Taxon Group 4	NO	NA	YES VU	0	2
Food	<i>Lactuca virosa</i> L.	NO	Gene pool 2	NO	NA	NO -	86	4
Food	<i>Lathyrus annuus</i> L.	NO	Taxon Group 2	NO	NA	NO -	14	4
Food	<i>Lathyrus bahini</i> Genty	NO	Taxon Group 4	NO	LC	NO -	1	3
Food	<i>Lathyrus cirrhosus</i> Ser.	NO	Taxon Group 2	NO	NA	NO -	0	2
Food	<i>Lathyrus clymenum</i> L.	NO	Taxon Group 2	NO	NA	NO -	20	4
Food	<i>Lathyrus latifolius</i> L.	NO	Taxon Group 2	NO	NA	NO -	17	4
Food	<i>Lathyrus nudicaulis</i> (Willk.) Amo	YES (SP & PT)	Taxon Group 4	NO	NA	NO -	0	2
Food	<i>Lathyrus ochrus</i> (L.) DC.	NO	Taxon Group 1b	NO	NA	NO -	7	4
Food	<i>Lathyrus pisiformis</i> L.	NO	Taxon Group 4	YES	VU	NO -	0	2
Food	<i>Lathyrus pulcher</i> J. Gay	NO	Taxon Group 2	NO	NA	NO -	0	2
Food	<i>Lathyrus sylvestris</i> L.	NO	Taxon Group 2	NO	NA	NO -	2	3
Food	<i>Lathyrus tingitanus</i> L.	NO	Taxon Group 2	NO	NA	NO -	15	4
Food	<i>Lathyrus tuberosus</i> L.	NO	Taxon Group 2	NO	NA	NO -	10	4
Food	<i>Lathyrus vivanti</i> P. Monts	NO	Taxon Group 4	YES	VU	NO -	0	2
Industrial & Other Us	<i>Lavandula angustifolia</i> Mill.	NO	Gene pool 1b	NO	NA	NO -	14	4
Industrial & Other Us	<i>Lavandula dentata</i> L.	NO	Gene pool 2	NO	NA	NO -	6	4
Industrial & Other Us	<i>Lavandula lanata</i> Boiss.	YES (SP)	Taxon Group 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Lavandula latifolia</i> Medik.	NO	Gene pool 2	NO	NA	NO -	291	4

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Industrial & Other Us	<i>Lavandula pedunculata</i> (Mill.) Cav.	NO	Gene pool 2	NO	NA	NO -	6	4
Industrial & Other Us	<i>Lavandula stoechas</i> L.	NO*	Gene pool 1b	NO	NA	NO -	93	4
Industrial & Other Us	<i>Lavandula viridis</i> L'Hér.	NO	Gene pool 2	NO	NA	NO -	1	3
Food	<i>Lens ervoides</i> (Brign)	NO	Gene pool 2	NO	NA	NO -	1	3
Food	<i>Lens nigricans</i> (M. Bieb.) Godr.	NO	Gene pool 2	NO	NA	NO -	77	4
Ornamental	<i>Limonium album</i> (Coincy) Sennen	YES (SP)	Taxon Group 3	YES	VU	NO -	0	2
Ornamental	<i>Limonium algarvense</i> Erben	NO	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Limonium aragonense</i> (Debeaux) Font Quer	YES (SP)	Taxon Group 3	YES	CR	NO -	0	2
Ornamental	<i>Limonium arborescens</i> (Brouss) Kuntze	YES (CAN)	Gene pool 1b	YES	EN	NO -	0	1
Ornamental	<i>Limonium augustebrateatum</i> Erben	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium auriculae-ursifolium</i> (Pourr.) Druce	NO	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium bellidifolium</i> (Gouan) Dumort.	NO	Gene pool 1b	NO	NA	NO -	1	3
Ornamental	<i>Limonium benmageci</i> Marrero Rodr. in Marrero Rodr. & Almeida	YES (CAN)	Taxon Group 2	YES	CR	NO -	2	3
Ornamental	<i>Limonium biflorum</i> (Pignatti) Pignatti	YES (SP)	Taxon Group 2	NO	NA	NO -	1	3
Ornamental	<i>Limonium binervosum</i> (G.E. Sm.) C.E. Salmon	NO	Taxon Group 2	NO	NA	NO -	2	3
Ornamental	<i>Limonium bourgeau</i> (Webb ex Boiss.) Kuntze	YES (CAN)	Taxon Group 2	NO	NA	NO -	5	4
Ornamental	<i>Limonium brassicifolium</i> (Webb & Berthel.) Kuntze	YES (CAN)	Taxon Group 2	YES	EN	NO -	2	3
Ornamental	<i>Limonium camposanum</i> Erben	YES (SP)	Taxon Group 2	YES	NT	NO -	4	3
Ornamental	<i>Limonium carthaginense</i> (Rouy) C. E. Hubb. & Sandwith	YES (SP)	Taxon Group 2	YES	VU	NO -	0	1
Ornamental	<i>Limonium cataunicum</i> Willk. & Costa) Pignatti	YES (SP)	Taxon Group 2	YES	CR	NO -	2	3
Ornamental	<i>Limonium cavanillesii</i> Erben	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium cossonianum</i> Kuntze	YES (SP)	Taxon Group 2	NO	NA	NO -	3	3
Ornamental	<i>Limonium costae</i> (Willk.) Pignatti	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Limonium delicatulum</i> (Girard) Kuntze	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium dendroides</i> Svent.	YES (CAN)	Taxon Group 3	YES	CR	NO -	1	3
Ornamental	<i>Limonium densissimum</i> (Pignatti) Pignatti	NO	Taxon Group 2	YES	VU	NO -	0	2
Ornamental	<i>Limonium dichotomum</i> (Cav.) Kuntze	YES (SP)	Taxon Group 2	NO	NA	NO -	5	4
Ornamental	<i>Limonium dodartii</i> (Girard) Kuntze	NO	Taxon Group 2	YES	CR	NO -	0	2
Ornamental	<i>Limonium dufourii</i> (Girard) Kuntze	YES (SP)	Taxon Group 2	YES	CR	NO -	1	3
Ornamental	<i>Limonium echioides</i> (L.) Mill.	NO	Taxon Group 3	NO	NA	NO -	0	2

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Ornamental	<i>Limonium emarginatum</i> (Willd.) Kuntze	NO	Taxon Group 2	YES	VU	NO -	1	3
Ornamental	<i>Limonium erectum</i> Erben	YES (SP)	Taxon Group 3	YES	EN	NO -	3	3
Ornamental	<i>Limonium estevei</i> Fern. Casas	YES (SP)	Taxon Group 2	YES	CR	NO -	0	1
Ornamental	<i>Limonium fruticans</i> (Webb) Kuntze	YES (CAN)	Gene pool 1b	YES	EN	NO -	1	3
Ornamental	<i>Limonium furfuraceum</i> (Lag.) Kuntze	YES (SP)	Gene pool 2	NO	NA	NO -	1	3
Ornamental	<i>Limonium girardianum</i> (Guss.) Fourr.	NO	Taxon Group 2	NO	NA	NO -	1	3
Ornamental	<i>Limonium grosii</i> L. Llorens	YES (SP)	Taxon Group 2	YES	VU	NO -	5	4
Ornamental	<i>Limonium gymnesicum</i> Erben	YES (SP)	Taxon Group 2	NO	NA	NO -	1	3
Ornamental	<i>Limonium humile</i> Mill.	NO	Taxon Group 2	YES	VU	NO -	0	2
Ornamental	<i>Limonium imbricatum</i> (Webb ex Girard) C. F. Hubb.	YES (CAN)	Taxon Group 2	YES	EN	NO -	7	4
Ornamental	<i>Limonium interjectum</i> Soler & Roselló	YES (SP)	Taxon Group 2	YES	EN	NO -	0	1
Ornamental	<i>Limonium latebracteatum</i> Erben	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Limonium lobatum</i> (L. fil.) Chaz.	NO	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium macrophyllum</i> (Brouss.) Kuntze	YES (CAN)	Taxon Group 2	YES	VU	NO -	1	3
Ornamental	<i>Limonium minutum</i> (L.) Chaz.	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium narbonense</i> Mill.	NO	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium ovalifolium</i> (Poir.) Kuntze	NO*	Taxon Group 2	NO*	CR	NO -	0	2
Ornamental	<i>Limonium papillatum</i> (Webb & Berthel.) Kuntze	NO	Taxon Group 3	YES	NT	NO -	3	3
Ornamental	<i>Limonium parvibracteatum</i> Pignatti	YES (SP)	Taxon Group 2	NO	LC	NO -	0	2
Ornamental	<i>Limonium pectinatum</i> (Aiton) Kuntze	NO	Taxon Group 3	NO	NA	NO -	4	3
Ornamental	<i>Limonium perezii</i> (Stapf) C. F. Hubb.	YES (CAN)	Taxon Group 2	YES	CR	NO -	1	3
Ornamental	<i>Limonium perplexum</i> L. Sáez & Roselló	YES (SP)	Taxon Group 2	YES	CR	NO -	0	1
Ornamental	<i>Limonium preauxii</i> (Webb & Berthel.) Kuntze	YES (CAN)	Taxon Group 2	YES	EN	NO -	7	4
Ornamental	<i>Limonium puberulum</i> (Webb) Kuntze	YES (CAN)	Taxon Group 2	YES	EN	NO -	5	4
Ornamental	<i>Limonium redivivum</i> (Svent.) G. Kunkel & Sunding	YES (CAN)	Taxon Group 2	YES	EN	NO -	2	3
Ornamental	<i>Limonium relicticum</i> R. Mesa & A. Santos	YES (CAN)	Taxon Group 2	YES	CR	NO -	0	1
Ornamental	<i>Limonium revolutum</i> Erben	YES (SP)	Taxon Group 3	YES	VU	NO -	4	3
Ornamental	<i>Limonium rigualii</i> M.B. Crespo & Erben	YES (SP)	Taxon Group 2	YES	VU	NO -	0	1
Ornamental	<i>Limonium ruizii</i> (Font Quer) Fen. Casas	YES (SP)	Taxon Group 3	YES	VU	NO -	0	2
Ornamental	<i>Limonium santapolense</i> Erben	YES (SP)	Taxon Group 2	YES	VU	NO -	0	1
Ornamental	<i>Limonium sinuatum</i> (L.) Mill.	NO	Gene pool 1b	NO	NA	NO -	0	2

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Ornamental	<i>Limonium spectabile</i> (Svent.) G. Kunkel & Sunding	YES (CAN)	Taxon Group 2	YES	CR	NO -	2	3
Ornamental	<i>Limonium subglabrum</i> Erben	YES (SP)	Taxon Group 3	YES	EN	NO -	0	2
Ornamental	<i>Limonium sventenii</i> A. Santos & M. Fernández	YES (CAN)	Taxon Group 2	YES	CR	NO -	4	3
Ornamental	<i>Limonium tabernense</i> Erben	YES (SP)	Taxon Group 3	YES	VU	NO -	1	3
Ornamental	<i>Limonium tenuicaule</i> Erben	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium thiniense</i> Erben	YES (SP)	Taxon Group 2	YES	VU	NO -	0	1
Ornamental	<i>Limonium thouinii</i> (Viv.) Kuntze	NO	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Limonium tournefortii</i> (Boiss.) Erben	YES (SP)	Taxon Group 2	NO	DD	NO -	1	3
Ornamental	<i>Limonium tremolsii</i> (Rouy) Erben	YES (SP)	Taxon Group 2	YES	NT	NO -	4	3
Ornamental	<i>Limonium tuberculatum</i> (Boiss.) Kuntze	NO	Taxon Group 2	YES	CR	NO -	5	4
Ornamental	<i>Limonium viciosoi</i> (Pau) Erben	YES (SP)	Taxon Group 3	NO	DD	NO -	0	2
Ornamental	<i>Limonium vigaroense</i> Marrero Rodr. & Almeida	YES (CAN)	Taxon Group 2	YES	CR	NO -	1	3
Ornamental	<i>Limonium vigoii</i> L. Sáez, Curcó & Rosselló	YES (SP)	Taxon Group 2	YES	CR	NO -	2	3
Ornamental	<i>Limonium virgatum</i> (Willd.) Fourr.	NO	Taxon Group 2	NO	NA	NO -	4	3
Ornamental	<i>Limonium vulgare</i> Mill.	NO	Taxon Group 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Linum bienne</i> Miller	NO	Gene pool 2	NO	NA	NO -	4	3
Industrial & Other Us	<i>Linum narbonense</i> L.	NO	Gene pool 2	NO	NA	NO -	11	4
Industrial & Other Us	<i>Linum tenue</i> Desf.	NO	Gene pool 2	NO	NA	NO -	1	3
Foragege & Fodder	<i>Lolium edwardii</i> H. Scholz, Stierstorfer & v. Gaisberg	YES (CAN)	Taxon Group 4	YES	VU	NO -	1	3
Foragege & Fodder	<i>Lolium multiflorum</i> Lam.	NO	Gene pool 1b	NO	NA	NO -	82	4
Foragege & Fodder	<i>Lolium perenne</i> L.	NO	Gene pool 1b	NO	NA	NO -	293	4
Foragege & Fodder	<i>Lolium saxatile</i> H. Scholz & S. Scholz	YES (CAN)	Taxon Group 4	YES	EN	NO -	2	3
Foragege & Fodder	<i>Lupinus angustifolius</i> L.	NO	Gene pool 1b	NO	NA	NO -	805	4
Foragege & Fodder	<i>Lupinus consentinii</i> Guss.	NO	Gene pool 1b	NO	NA	NO -	8	4
Foragege & Fodder	<i>Lupinus gredensis</i> Gand.	YES (SP & PT)	Taxon Group 4	NO	NA	NO -	139	4
Foragege & Fodder	<i>Lupinus hispanicus</i> Boiss. & Reut.	YES (SP & PT)	Gene pool 2	NO	NA	NO -	227	4
Foragege & Fodder	<i>Lupinus luteus</i> L.	NO	Gene pool 1b	NO	NA	NO -	184	4
Foragege & Fodder	<i>Lupinus mariae-josephae</i> H. Pascual	YES (SP)	Taxon Group 4	YES	CR	NO -	10	4
Foragege & Fodder	<i>Lupinus micranthus</i> Guss.	NO	Gene pool 2	NO	NA	NO -	16	4
Foragege & Fodder	<i>Lupinus pilosus</i> L.	NO	Gene pool 2	NO	NA	NO -	0	2
Food	<i>Malus sylvestris</i> (L.) Mill.	NO	Taxon Group 2	NO	NA	NO -	5	4

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Foragege & Fodder	<i>Medicago arabica</i> (L.) Huds.	NO	Gene pool 1b	NO	NA	NO -	33	4
Foragege & Fodder	<i>Medicago citrina</i> (Font Quer) Greuter	YES (SP)	Taxon Group 2	YES	CR	YES CR	7	4
Foragege & Fodder	<i>Medicago coronata</i> (L.) Bartal.	NO	Taxon Group 2	NO	NA	NO -	0	2
Foragege & Fodder	<i>Medicago disciformis</i> DC.	NO	Taxon Group 2	NO	NA	NO -	2	3
Foragege & Fodder	<i>Medicago doliata</i> Carmign.	NO	Taxon Group 2	NO	NA	NO -	61	4
Foragege & Fodder	<i>Medicago falcata</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Foragege & Fodder	<i>Medicago hybrida</i> (Pourr.) Trautv.	NO	Gene pool PU (3)	NO	NA	NO -	0	2
Foragege & Fodder	<i>Medicago intertexta</i> (L.) Mill.	NO	Taxon Group 2	NO	NA	NO -	7	4
Foragege & Fodder	<i>Medicago italica</i> (Mill.) Fiori	NO	Taxon Group 2	NO	NA	NO -	15	4
Foragege & Fodder	<i>Medicago laciniata</i> (L.) Mill.	NO	Taxon Group 2	NO	NA	NO -	99	4
Foragege & Fodder	<i>Medicago littoralis</i> Rohde ex Loisel.	NO	Taxon Group 2	NO	NA	NO -	76	4
Foragege & Fodder	<i>Medicago lupulina</i> L.	NO	Gene pool 1b	NO	NA	NO -	21	4
Foragege & Fodder	<i>Medicago minima</i> (L.) L.	NO	Taxon Group 2	NO	NA	NO -	85	4
Foragege & Fodder	<i>Medicago murex</i> Willd.	NO	Taxon Group 2	NO	NA	NO -	10	4
Foragege & Fodder	<i>Medicago polymorpha</i> L.	NO	Taxon Group 2	NO	NA	NO -	435	4
Foragege & Fodder	<i>Medicago praecox</i> DC.	NO	Taxon Group 2	NO	NA	NO -	5	4
Foragege & Fodder	<i>Medicago rigidula</i> (L.) All.	NO	Gene pool 1b	NO	NA	NO -	52	4
Foragege & Fodder	<i>Medicago sativa</i> L.	NO	Gene pool 1b	NO	NA	NO -	75	4
Foragege & Fodder	<i>Medicago scutellata</i> (L.) Mill.	NO	Gene pool PU (1b)	NO	NA	NO -	7	4
Foragege & Fodder	<i>Medicago secundiflora</i> Durieu	NO	Taxon Group 2	NO	NA	NO -	0	2
Foragege & Fodder	<i>Medicago soleirollii</i> Duby	NO	Taxon Group 2	NO	NA	NO -	1	3
Foragege & Fodder	<i>Medicago suffruticosa</i> Ramond ex DC.	NO	Taxon Group 2	NO	NA	NO -	14	4
Foragege & Fodder	<i>Medicago truncatula</i> Gaertn.	NO	Gene pool 1b	NO	NA	NO -	174	4
Foragege & Fodder	<i>Medicago turbinata</i> (L.) All.	NO	Taxon Group 2	NO	NA	NO -	11	4
Food	<i>Moricandia arvensis</i> (L.) DC.	NO	Gene pool PU (3)	NO	NA	NO -	14	4
Ornamental	<i>Narcissus alcaracensis</i> Ríos & al.	YES (SP)	Taxon Group 2	YES	EN	NO -	0	1
Ornamental	<i>Narcissus assoanus</i> Dufour ex Schult. & Schult.	NO	Gene pool 2	NO	NA	NO -	0	2
Ornamental	<i>Narcissus bicolor</i> L.	NO	Gene pool 2	NO	NA	NO -	0	2
Ornamental	<i>Narcissus bugei</i> (Fern. Casas) Fern. Casas	YES (SP)	Taxon Group 2	YES	VU	NO -	0	1
Ornamental	<i>Narcissus bulbocodium</i> L.	NO	Gene pool 1b	NO	NA	NO -	5	4
Ornamental	<i>Narcissus cantabricus</i> DC.	NO	Gene pool 2	NO	NA	NO -	0	2

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Ornamental	<i>Narcissus cavanillesii</i> Barra & G. López	NO	Taxon Group 3	NO	NA	NO -	1	3
Ornamental	<i>Narcissus conspicuus</i> (Haw.) Sweet	¿?	Gene pool 2	YES	VU	NO -	0	2
Ornamental	<i>Narcissus cuatrecasasii</i> Fern. Casas, M. Laínz & Ruiz Rejón	NO	Gene pool 2	YES	VU	NO -	0	2
Ornamental	<i>Narcissus cyclamineus</i> DC.	YES (SP & PT)	Gene pool 1b	NO	LC	NO -	0	2
Ornamental	<i>Narcissus dubius</i> Gouan	NO	Taxon Group 2	NO	NA	NO -	2	3
Ornamental	<i>Narcissus elegans</i> (Haw.) Spach	NO	Taxon Group 3	NO	DD	NO -	2	3
Ornamental	<i>Narcissus eugeniae</i> Fern. Casas	YES (SP)	Gene pool 2	YES	VU	NO -	0	1
Ornamental	<i>Narcissus gaditanus</i> Boiss. & Reut. in Boiss., Diagn.	YES (SP & PT)	Taxon Group 2	YES	VU	NO -	0	1
Ornamental	<i>Narcissus hedraeanthus</i> (Webb & Heldr.) Colmeiro	YES (SP)	Gene pool 2	NO	NA	NO -	1	3
Ornamental	<i>Narcissus jonquilla</i> L.	YES (SP & PT)	Gene pool 1b	NO	NA	NO -	2	3
Ornamental	<i>Narcissus longispathus</i> Pugsley	YES (SP)	Taxon Group 2	YES	EN	NO -	1	3
Ornamental	<i>Narcissus minor</i> L.	NO*	Gene pool 2	NO	NA	NO -	0	2
Ornamental	<i>Narcissus moschatus</i> L.	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Narcissus munozii-garmediae</i> Fern. Casas	YES (SP)	Taxon Group 2	YES	VU	NO -	1	3
Ornamental	<i>Narcissus nevadensis</i> Pugsley	YES (SP)	Taxon Group 2	YES	CR	NO -	0	1
Ornamental	<i>Narcissus obsoletus</i> (Haw.) Steud.	NO	Taxon Group 3	NO	NA	NO -	1	3
Ornamental	<i>Narcissus pachybolbus</i> Durieu	NO	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Narcissus pallidiflorus</i> Pugsley	NO	Gene pool 2	YES	NT	NO -	0	2
Ornamental	<i>Narcissus papyraceus</i> Ker Gawl.	NO	Gene pool 1b	NO	NA	NO -	0	2
Ornamental	<i>Narcissus perez-chiscanoi</i> Fern. Casas	YES (SP)	Taxon Group 2	NO	DD	NO -	0	2
Ornamental	<i>Narcissus poeticus</i> L.	NO	Gene pool 1b	NO	NA	NO -	3	3
Ornamental	<i>Narcissus pseudonarcissus</i> L.	NO	Gene pool 1b	NO	NA	NO -	3	3
Ornamental	<i>Narcissus radinganorum</i> Fern. Casas	YES (SP)	Taxon Group 2	YES	EN	NO -	0	1
Ornamental	<i>Narcissus rupicola</i> Dufour ex Schult. & Schult.	YES (SP & PT)	Gene pool 2	NO	NA	NO -	1	3
Ornamental	<i>Narcissus scaberulus</i> Henrig.	YES (SP)	Taxon Group 3	NO	NA	NO -	0	2
Ornamental	<i>Narcissus segurenensis</i> Ríos & al.	YES (SP)	Taxon Group 2	NO	DD	NO -	0	2
Ornamental	<i>Narcissus serotinus</i> Loefl. ex L.	NO	Taxon Group 3	NO	NA	NO -	3	3
Ornamental	<i>Narcissus tazetta</i> L.	NO	Gene pool 1b	NO	NA	NO -	0	2
Ornamental	<i>Narcissus tortifolius</i> Fern. Casas	YES (SP)	Taxon Group 2	YES	VU	NO -	3	3
Ornamental	<i>Narcissus tortuosus</i> Haw.	NO	Taxon Group 2	YES	EN	NO -	0	2
Ornamental	<i>Narcissus triandrus</i> L.	NO*	Gene pool 1b	NO	NA	NO -	0	2

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Ornamental	<i>Narcissus viridiflorus</i> Schousb.	NO	Taxon Group 3	YES	VU	NO -	1	3
Ornamental	<i>Narcissus yepesii</i> Ríos & al.	YES (SP)	Taxon Group 2	YES	VU	NO -	1	3
Food	<i>Olea europaea</i> L.	NO	Gene pool 1B	NO	NA	NO -	10	4
Forage & Fodder	<i>Ornithopus compressus</i> L.	NO	Gene pool 1b	NO	NA	NO -	530	4
Forage & Fodder	<i>Ornithopus perpusillus</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Ornithopus sativus</i>	NO	Gene pool 1b	NO	NA	NO -	10	4
Industrial & Other Us	<i>Papaver dubium</i> L.	NO	Gene pool 2	NO	NA	NO -	3	3
Industrial & Other Us	<i>Papaver rhoeas</i> L.	NO	Gene pool 2	NO	NA	NO -	18	4
Industrial & Other Us	<i>Papaver somniferum</i> L.	NO	Gene pool 1b	NO	NA	NO -	12	4
Food	<i>Patellifolia patellaris</i> (Moq.) A. J. Scott, Ford-Lloyd & J.T. Williams	NO	Gene pool PU (3)	NO	NA	NO -	36	4
Food	<i>Patellifolia procumbens</i> (C. Sm. ex Hornem.) A. J. Scott, Ford-Lloyd & J. T. Williams	NO	Gene pool PU (3)	NO	NA	NO -	5	4
Food	<i>Patellifolia webbiana</i> (Moq.) A. J. Scott, Ford-Lloyd & J. T. Williams	YES (CAN)	Gene pool PU (3)	NO	NA	YES CR	0	2
Food	<i>Pisum sativum</i> L.	NO	Gene pool 1B	NO	NA	NO -	12	4
Forage & Fodder	<i>Poa alpina</i> L.	NO	Gene pool 1b	NO	NA	NO -	3	3
Forage & Fodder	<i>Poa angustifolia</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Poa annua</i> L.	NO	Taxon Group 3	NO	NA	NO -	29	4
Forage & Fodder	<i>Poa bulbosa</i> L.	NO	Gene pool 1b	NO	NA	NO -	6	4
Forage & Fodder	<i>Poa compressa</i> L.	NO	Gene pool 1b	NO	NA	NO -	1	3
Forage & Fodder	<i>Poa glauca</i> Vahl.	NO	Taxon Group 3	NO	NA	NO -	1	3
Forage & Fodder	<i>Poa laxa</i> Haenke	NO	Taxon Group 3	NO	NA	NO -	0	2
Forage & Fodder	<i>Poa pitardiana</i> H. Scholz	YES (CAN)	Taxon Group 4	YES	VU	NO -	0	2
Forage & Fodder	<i>Poa pratensis</i> L.	NO	Gene pool 1b	NO	NA	NO -	12	4
Forage & Fodder	<i>Poa supina</i> Schrad.	NO	Taxon Group 3	NO	NA	NO -	1	3
Food	<i>Prunus avium</i> L.	NO	Gene pool 1B	NO	NA	NO -	8	4
Food	<i>Prunus insititia</i> L.	NO	Taxon Group 3	NO	NA	NO -	15	4
Food	<i>Prunus lusitanica</i> L.	NO	Taxon Group 4	YES	VU	YES VU	5	4
Food	<i>Prunus mahaleb</i> L.	NO	Gene pool 2	NO	NA	NO -	19	4
Food	<i>Prunus prostrata</i> Labill.	NO	Gene pool 2	NO	NA	NO -	12	4
Food	<i>Prunus ramburii</i> Boiss.	YES (SP)	Gene pool 2	YES	VU	YES VU	16	4

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Food	<i>Prunus spinosa</i> L.	NO	Gene pool 1B	NO	NA	NO -	27	4
Food	<i>Pyrus bourgaeana</i> Decne.	NO	Taxon Group 2	NO	NA	NO -	8	4
Food	<i>Pyrus cordata</i> Desv.	NO	Taxon Group 2	NO	NA	NO -	0	2
Food	<i>Pyrus spinosa</i> Forssk.	NO	Taxon Group 2	NO	NA	NO -	0	2
Food	<i>Raphanus sativus</i> L.	NO	Gene pool CU (3)	NO	NA	NO -	1	3
Ornamental	<i>Rosa agrestis</i> Savi	NO	Taxon Group 2	NO	NA	NO -	4	3
Ornamental	<i>Rosa arvensis</i> Huds.	NO	Taxon Group 2	NO	NA	NO -	4	3
Ornamental	<i>Rosa canina</i> L.	NO	Gene pool 1b	NO	NA	NO -	18	4
Ornamental	<i>Rosa dumalis</i> Bechst.	NO	Gene pool 2	NO	NA	NO -	0	2
Ornamenta	<i>Rosa elliptica</i> Tausch.	NO	Gene pool 2	NO	NA	NO -	0	2
Ornamental	<i>Rosa glauca</i> Pourr.	NO	Gene pool 2	NO	NA	NO -	2	3
Ornamental	<i>Rosa micrantha</i> Borrer ex Sm.	NO	Gene pool 2	NO	NA	NO -	3	3
Ornamental	<i>Rosa pendulina</i> L.	NO	Gene pool 2	NO	NA	NO -	7	4
Ornamental	<i>Rosa pimpinellifolia</i> L.	NO	Taxon Group 2	NO	NA	NO -	4	3
Ornamental	<i>Rosa pouzinii</i> Tratt.	NO	Taxon Group 2	NO	NA	NO -	4	3
Ornamental	<i>Rosa rubiginosa</i> L.	NO	Gene pool 1b	NO	NA	NO -	0	2
Ornamental	<i>Rosa sempervirens</i> L.	NO	Taxon Group 2	NO	NA	NO -	2	3
Ornamental	<i>Rosa sicula</i> Tratt.	NO	Taxon Group 2	NO	NA	NO -	1	3
Ornamental	<i>Rosa stylosa</i> Desv.	NO	Taxon Group 2	NO	NA	NO -	0	2
Ornamental	<i>Rosa tomentosa</i> Sm.	NO	Gene pool 2	NO	NA	NO -	0	2
Ornamental	<i>Rosa villosa</i> L.	NO	Gene pool 1b	NO	NA	NO -	6	4
Industrial & Other Us	<i>Salvia lavandulifolia</i> Vahl	NO*	Gene pool 1b	NO	NA	NO -	187	4
Industrial & Other Us	<i>Salvia sclarea</i> L.	NO	Gene pool 1b	NO	NA	NO -	4	3
Food	<i>Secale montanum</i> Guss.	NO	Taxon Group 3	NO	NA	NO -	5	4
Industrial & Other Us	<i>Sideritis arborescens</i> Salzm. ex Benth.	NO	Gene pool 2	NO*	NT	NO -	3	3
Industrial & Other Us	<i>Sideritis borgiae</i> Andrés	NI	Taxon Group 3	NO	RE	NO -	0	2
Industrial & Other Us	<i>Sideritis bourgaeana</i> Boiss. & Reut.	YES (SP)	Taxon Group 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Sideritis bubanii</i> Font Quer	YES (SP)	Gene pool 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Sideritis calduchi</i> Cirujano & al.	YES (SP)	Taxon Group 3	NO	RE	NO -	1	3
Industrial & Other Us	<i>Sideritis carbonellii</i> Socorro	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Sideritis chamaedryfolia</i> Cav.	YES (SP)	Taxon Group 2	YES	VU	NO -	2	3

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Industrial & Other Us	<i>Sideritis dianica</i> D. Rivera, Obón, De la Torre & A. Barber	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Sideritis endressii</i> Willk.	NO	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Sideritis fruticulosa</i> Pourr.	NO	Gene pool 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Sideritis glacialis</i> Boiss.	YES (SP)	Gene pool 2	NO	NA	NO -	5	4
Industrial & Other Us	<i>Sideritis glauca</i> Cav.	YES (SP)	Taxon Group 2	YES	VU	NO -	2	3
Industrial & Other Us	<i>Sideritis grandiflora</i> Salzm. ex Benth.	NO	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Sideritis hirsuta</i> L.	NO	Gene pool 1b	NO	NA	NO -	18	4
Industrial & Other Us	<i>Sideritis hyssopifolia</i> L.	NO	Taxon Group 2	NO*	NT	NO -	15	4
Industrial & Other Us	<i>Sideritis ibanyezii</i> Pau	YES (SP)	Taxon Group 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Sideritis ilicifolia</i> Willd.	YES (SP)	Gene pool 2	NO	NA	NO -	3	3
Industrial & Other Us	<i>Sideritis incana</i> L.	NO	Gene pool 2	NO	NA	NO -	16	4
Industrial & Other Us	<i>Sideritis lacaitae</i> Font Quer	YES (SP)	Taxon Group 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Sideritis lasiantha</i> Pers.	YES (SP)	Taxon Group 2	YES	NT	NO -	1	3
Industrial & Other Us	<i>Sideritis laxepicata</i> (Degen & Debeaux) Socorro, I. Tárrega & M.L. Zafra	YES (SP)	Taxon Group 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Sideritis leucantha</i> Cav.	YES (SP)	Gene pool 2	NO	NA	NO -	9	4
Industrial & Other Us	<i>Sideritis lurida</i> J. Gay ex Lacaita	YES (SP)	Taxon Group 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Sideritis montana</i> L.	NO	Taxon Group 3	NO	NA	NO -	1	3
Industrial & Other Us	<i>Sideritis montserratiana</i> Stübing, R. Roselló, Olivares & Peris	YES (SP)	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Sideritis osteoxylla</i> (Pau ex Vicioso) Alcaraz, Peinado, Mart. Parras, J.S. Carrión & Sánchez Gómez	YES (SP)	Taxon Group 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Sideritis ovata</i> Cav.	YES (SP)	Taxon Group 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Sideritis paulii</i> Pau	YES (SP)	Taxon Group 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Sideritis pungens</i> Benth.	YES (SP)	Taxon Group 2	NO	NA	NO -	4	3
Industrial & Other Us	<i>Sideritis pusilla</i> (Lange) Pau	NO	Taxon Group 2	NO	RE	NO -	3	3
Industrial & Other Us	<i>Sideritis reverchonii</i> Willk.	YES (SP)	Taxon Group 2	YES	EN	NO -	0	1
Industrial & Other Us	<i>Sideritis romana</i> L.	NO	Taxon Group 3	NO	NA	NO -	0	2
Industrial & Other Us	<i>Sideritis serrata</i> Lag.	YES (SP)	Taxon Group 2	YES	CR	NO -	2	3
Industrial & Other Us	<i>Sideritis spinulosa</i> Barnades ex Asso	YES (SP)	Gene pool 2	NO	NA	NO -	6	4
Industrial & Other Us	<i>Sideritis stachydioides</i> Willk.	YES (SP)	Taxon Group 2	YES	VU	NO -	2	3
Industrial & Other Us	<i>Sideritis tragoriganum</i> Lag.	YES (SP)	Gene pool 2	NO	NA	NO -	5	4
Food	<i>Sinapis alba</i> L.	NO	Gene pool PU (3)	NO	NA	NO -	19	4

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Food	<i>Sinapis arvensis</i> L.	NO	Gene pool PU (3)	NO	NA	NO -	9	4
Food	<i>Solanum lidii</i> Sunding	YES (CAN)	Gene pool 2	YES	CR	NO -	16	4
Food	<i>Solanum vesperitio</i> Aiton	YES (CAN)	Taxon Group 4	YES	CR	NO -	28	4
Industrial & Other Us	<i>Thymus albicans</i> Hoffmanns. & Link	YES (SP & PT)	Taxon Group 2	YES	CR	NO -	0	1
Industrial & Other Us	<i>Thymus bacticus</i> Boiss. ex Lacaita	YES (SP)	Gene pool 2	NO	NA	NO -	5	4
Industrial & Other Us	<i>Thymus bracteatus</i> Lange ex Cutanda	YES (SP)	Gene pool 2	NO	NA	NO -	12	4
Industrial & Other Us	<i>Thymus caespititius</i> Brot.	NO	Gene pool 1b	NO	NA	NO -	1	3
Industrial & Other Us	<i>Thymus carnosus</i> Boiss.	YES (SP & PT)	Gene pool 2	YES	EN	NO -	0	1
Industrial & Other Us	<i>Thymus fontqueri</i> (Jalas) Molero & Rovira	YES (SP)	Gene pool 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Thymus froelichianus</i> Opiz	NO	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Thymus funkii</i> Coss.	YES (SP)	Gene pool 2	NO*	VU-NT	NO -	2	3
Industrial & Other Us	<i>Thymus granatensis</i> Boiss.	YES (SP)	Gene pool 2	NO	NA	NO -	3	3
Industrial & Other Us	<i>Thymus herba-barona</i> Loisel.	YES (SP)	Gene pool 1b	YES	CR	NO -	0	1
Industrial & Other Us	<i>Thymus hyemalis</i> Lange	NO	Gene pool 2	NO*	CR	NO -	2	3
Industrial & Other Us	<i>Thymus lacaitae</i> Pau	YES (SP)	Gene pool 2	NO	NA	NO -	12	4
Industrial & Other Us	<i>Thymus leptophyllus</i> Lange	YES (SP)	Gene pool 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Thymus longicaulis</i> C. Presl	NO	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Thymus longiflorus</i> Boiss.	YES (SP)	Gene pool 2	NO	NA	NO -	8	4
Industrial & Other Us	<i>Thymus loscosii</i> Willk. in Willk. & Lange	YES (SP)	Gene pool 2	NO	NA	NO -	5	4
Industrial & Other Us	<i>Thymus mastichina</i> (L.) L.	YES (SP & PT)	Gene pool 1b	NO	NA	NO -	139	4
Industrial & Other Us	<i>Thymus mastigophorus</i> Lacaita	YES (SP)	Gene pool 2	NO	NA	NO -	3	3
Industrial & Other Us	<i>Thymus membranaceus</i> Boiss.	YES (SP)	Gene pool 2	NO	NA	NO -	4	3
Industrial & Other Us	<i>Thymus moroderi</i> Pau ex Mart. Mart.	YES (SP)	Gene pool 2	YES	NT	NO -	0	1
Industrial & Other Us	<i>Thymus nervosus</i> J. Gay ex Willk.	NO	Taxon Group 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Thymus origanoides</i> Webb & Berthel.	YES (CAN)	Taxon Group 2	YES	VU	NO -	2	3
Industrial & Other Us	<i>Thymus orospedanus</i> Villar	YES (SP)	Gene pool 2	NO	NA	NO -	2	3
Industrial & Other Us	<i>Thymus piperella</i> L.	YES (SP)	Gene pool 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Thymus praecox</i> Opiz	NO	Gene pool 2	NO	NA	NO -	15	4
Industrial & Other Us	<i>Thymus pulegioides</i> L.	NO	Gene pool 1b	NO	NA	NO -	2	3
Industrial & Other Us	<i>Thymus richardii</i> Pers.	NO*	Taxon Group 2	YES	VU	NO -	4	3
Industrial & Other Us	<i>Thymus serpylloides</i> Bory	YES (SP)	Gene pool 2	NO	NA	NO -	8	4

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Industrial & Other Us	<i>Thymus villosus</i> L.	YES (SP & PT)	Gene pool 2	NO	NA	NO -	1	3
Industrial & Other Us	<i>Thymus vulgaris</i> L.	NO	Gene pool 1b	NO	NA	NO -	87	4
Industrial & Other Us	<i>Thymus webbianus</i> Rouy	YES (SP)	Taxon Group 2	YES	CR	NO -	0	1
Industrial & Other Us	<i>Thymus wilddenowii</i> Boiss.	NO	Taxon Group 2	NO	NA	NO -	0	2
Industrial & Other Us	<i>Thymus willkommii</i> Ronniger	YES (SP)	Taxon Group 2	YES	NT	NO -	2	3
Industrial & Other Us	<i>Thymus zygis</i> Loebl. ex L.	NO	Gene pool 1b	NO	NA	NO -	86	4
Forage & Fodder	<i>Trifolium alpinum</i> L.	NO	Taxon Group 2	NO	NA	NO -	9	4
Forage & Fodder	<i>Trifolium angustifolium</i> L.	NO	Gene pool 1b	NO	NA	NO -	91	4
Forage & Fodder	<i>Trifolium arvense</i> L.	NO	Gene pool 1b	NO	NA	NO -	34	4
Forage & Fodder	<i>Trifolium aureum</i> Pollich	NO	Gene pool 1b	NO	NA	NO -	4	3
Forage & Fodder	<i>Trifolium badium</i> Schreb	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium bocconeii</i> Savi	NO	Taxon Group 2	NO	NA	NO -	31	4
Forage & Fodder	<i>Trifolium boissieri</i> Guss.	NO	Taxon Group 2	NO	NA	NO -	5	4
Forage & Fodder	<i>Trifolium campestre</i> Schreb.	NO	Gene pool 1b	NO	NA	NO -	68	4
Forage & Fodder	<i>Trifolium cernuum</i> Brot.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium cherleri</i> L.	NO	Taxon Group 2	NO	NA	NO -	366	4
Forage & Fodder	<i>Trifolium diffusum</i> Ehrh.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium dubium</i> Sibth.	NO	Gene pool 1b	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium fragiferum</i> L.	NO	Taxon Group 2	NO	NA	NO -	4	3
Forage & Fodder	<i>Trifolium gemellum</i> Pourr. ex Willd.	NO	Taxon Group 2	NO	NA	NO -	28	4
Forage & Fodder	<i>Trifolium glomeratum</i> L.	NO	Taxon Group 2	NO	NA	NO -	685	4
Forage & Fodder	<i>Trifolium hirtum</i> All.	NO	Taxon Group 2	NO	NA	NO -	6	4
Forage & Fodder	<i>Trifolium hybridum</i> L.	NO	Gene pool 1b	NO	NA	NO -	1	3
Forage & Fodder	<i>Trifolium incarnatum</i> L.	NO	Gene pool 1b	NO	NA	NO -	3	3
Forage & Fodder	<i>Trifolium isthmocarpum</i> Brot.	NO	Taxon Group 2	NO	NA	NO -	3	3
Forage & Fodder	<i>Trifolium laplaceum</i> L.	NO	Gene pool 1b	NO	NA	NO -	13	4
Forage & Fodder	<i>Trifolium leucanthum</i> M. Bieb.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium ligusticum</i> Balb. ex Loisel.	NO	Taxon Group 2	NO	NA	NO -	1	3
Forage & Fodder	<i>Trifolium lucanicum</i> Gasparr. ex Guss.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium medium</i> L.	NO	Gene pool 1b	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium michelianum</i> Savi	NO	Gene pool 1b	NO	NA	NO -	0	2

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Forage & Fodder	<i>Trifolium micranthum</i> Viv.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium montanum</i> L.	NO	Taxon Group 2	NO	NA	NO -	3	3
Forage & Fodder	<i>Trifolium mutabile</i> Port.	NO	Taxon Group 2	NO	NA	NO -	1	3
Forage & Fodder	<i>Trifolium nigrescens</i> Viv.	NO	Gene pool 1b	NO	NA	NO -	4	3
Forage & Fodder	<i>Trifolium obscurum</i> Savi	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium occidentale</i> Coombe	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium ochroleucon</i> Huds.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium ornithopodioides</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium pallescens</i> Schreb.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium pallidum</i> Waldst. & Kit.	NO	Taxon Group 2	NO	NA	NO -	1	3
Forage & Fodder	<i>Trifolium patens</i> Schreb.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium phleoides</i> Pourr. ex Willd.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium physodes</i> M. Bieb.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium pratense</i> L.	NO	Gene pool 1b	NO	NA	NO -	82	4
Forage & Fodder	<i>Trifolium repens</i> L.	NO	Gene pool 1b	NO	NA	NO -	125	4
Forage & Fodder	<i>Trifolium resupinatum</i> L.	NO	Gene pool 1b	NO	NA	NO -	26	4
Forage & Fodder	<i>Trifolium retusum</i> L.	NO	Taxon Group 2	NO	NA	NO -	1	3
Forage & Fodder	<i>Trifolium rubens</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium scabrum</i> L.	NO	Taxon Group 2	NO	NA	NO -	34	4
Forage & Fodder	<i>Trifolium spadiceum</i> L.	NO	Taxon Group 2	NO	NA	NO -	2	3
Forage & Fodder	<i>Trifolium spumosum</i> L.	NO	Taxon Group 2	NO	NA	NO -	4	3
Forage & Fodder	<i>Trifolium squamosum</i> L.	NO	Gene pool 1b	NO	NA	NO -	5	4
Forage & Fodder	<i>Trifolium squarrosum</i> L.	NO	Gene pool 1b	NO	NA	NO -	3	3
Forage & Fodder	<i>Trifolium stellatum</i> L.	NO	Taxon Group 2	NO	NA	NO -	81	4
Forage & Fodder	<i>Trifolium striatum</i> L.	NO	Gene pool 1b	NO	NA	NO -	287	4
Forage & Fodder	<i>Trifolium subterraneum</i> L.	NO	Gene pool 1b	NO	NA	NO -	2078	4
Forage & Fodder	<i>Trifolium suffocatum</i> L.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium sylvaticum</i> Gérard ex Loisel.	NO	Taxon Group 2	NO	NA	NO -	0	2
Forage & Fodder	<i>Trifolium thalii</i> Vill.	NO	Taxon Group 2	NO	NA	NO -	4	3
Forage & Fodder	<i>Trifolium tomentosum</i> L.	NO	Taxon Group 2	NO	NA	NO -	26	4
Forage & Fodder	<i>Trifolium vesciculosum</i> Savi	NO	Gene pool 1b	NO	NA	NO -	8	4

Annex 1: Prioritized Spanish Checklist of Crop Wild Relatives available at <https://pgrsecurespain.weebly.com/crop-wild-relatives-in-spain---prioritization-of-the-checklist.html>. Codes for priority collection: 1= Urgent priority. Primary or secondary gene pool and taxon group, endemic and threatened. No representation in gene banks; 2= Urgent. Species not represented in gene banks; 3= Need collecting. Less than five populations represented in gene banks; 4= Non priority for collection. More than five accessions in gene banks

Category	Species	Endemicity	Concept and Level Gene Pool or Taxon Group	Red List of Spanish Vascular Flora	Threat Category (IUCN)	European Red List of Vascular Plants and Category	No. of accessions in Germplasm banks	Priority for collection
Food	<i>Vicia altissima</i> Desf.	NO	Taxon Group 4	YES	CR	NO -	1	3
Food	<i>Vicia amphicarpa</i> L.	NO	Taxon Group 3	NO	NA	NO -	1	3
Food	<i>Vicia angustifolia</i> (L.) Amoen.	NO	Taxon Group 3	NO	NA	NO -	1	3
Food	<i>Vicia argentea</i> Lapeyr.	NO	Taxon Group 4	YES	VU	NO -	1	3
Food	<i>Vicia articulata</i> Hornem.	NO	Gene pool 1B	NO	NA	NO -	6	4
Food	<i>Vicia bifoliolata</i> J. J. Rodr.	YES (SP)	Taxon Group 4	YES	CR	NO -	3	3
Food	<i>Vicia bithynica</i> (L.) L.	NO	Gene pool 2	NO	NA	NO -	6	4
Food	<i>Vicia cirrhosa</i> C. Sm. ex Webb & Berthel.	YES (CAN)	Taxon Group 4	NO	NA	NO -	7	4
Food	<i>Vicia cordata</i> Hoppe	NO	Taxon Group 3	NO	NA	NO -	3	3
Food	<i>Vicia filicaulis</i> Webb & Berthel.	YES (CAN)	Taxon Group 4	NO	NA	NO -	4	3
Food	<i>Vicia glauca</i> C. Presl	NO	Taxon Group 4	NO*	VU	NO -	3	3
Food	<i>Vicia hybrida</i> L.	NO	Taxon Group 2	NO	NA	NO -	23	4
Food	<i>Vicia lathyroides</i> L.	NO	Taxon Group 2	NO	NA	NO -	2	3
Food	<i>Vicia leucantha</i> Biv.	NO	Taxon Group 4	YES	VU	NO -	0	2
Food	<i>Vicia lutea</i> L.	NO	Taxon Group 2	NO*	VU	NO -	44	4
Food	<i>Vicia narbonensis</i> L.	NO	Gene pool 1B	NO	NA	NO -	10	4
Food	<i>Vicia nataliae</i> U. Reifenger & Reifenger	YES (CAN)	Taxon Group 4	YES	EN	NO -	0	2
Food	<i>Vicia pannonica</i> Crantz	NO	Taxon Group 1B	NO	NA	NO -	10	4
Food	<i>Vicia peregrina</i> L.	NO	Taxon Group 3	NO	NA	NO -	22	4
Food	<i>Vicia pyrenaica</i> Pourr.	NO	Taxon Group 3	NO	NA	NO -	13	4
Food	<i>Vicia sativa</i> L.	NO	Taxon Group 1B	NO	NA	NO -	175	4
Food	<i>Vicia scandens</i> R. P. Murray	YES (CAN)	Taxon Group 4	YES	NT	NO -	6	4
Food	<i>Vicia sepium</i> L.	NO	Taxon Group 3	NO	NA	NO -	21	4
Food	<i>Vicia chaetocalyx</i> Webb & Berthel.	YES (CAN)	Taxon Group 4	NO	DD	NO -	0	2
Food	<i>Vitis vinifera</i> L.	NO	Gene pool 1B	NO	NA	NO -	623	4

CAPÍTULO 2:

Identification and assessment of the crop wild relatives of Spain that require most urgent conservation actions

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Abstract

Crop Wild Relatives (CWR) are receiving significant attention over the last decades. Numerous conservation plans and guidelines to better manage these resources have been developed lately at both national and international levels. In this sense, Spain is following a similar path to that followed by other countries and has included CWR in the National Strategy for Plant Conservation of Spain and invested in scientific projects dealing with their conservation. In this work, we present a preliminary assessment of the conservation status (both *in situ* and *ex situ*) of the Spanish CWR that are in a most urgent need of conservation. Crossability to crops, endemism, threat status according to IUCN standards and high-quality georeferenced occurrence data were the criteria applied to select the target species, generating a list of 47 CWR species. Eleven of them, classified as Critically Endangered or Endangered by IUCN criteria are not, and should be, included in the National Catalogue of Threatened Species of Spain; however 35 of them are included in at least one autonomous catalogue. Seventy-five per cent of the species are represented in protected areas, but if a minimum of five populations inside protected areas is sought the representation decreases to a 37%. The preliminary assessment of *ex situ* conservation shows that a high percentage of the species (81%) has at least one accession in national or international germplasm banks. However, additional studies are needed to determine if the accessions included in germplasm banks provide an adequate representation of the genetic variability of the species.

Keywords: Threatened; endemic; conservation status; crop wild relatives.

Introduction

Crop Wild Relatives (CWR) are species closely related to crops (Heywood & al., 2007) and their utilization as useful gene donors in crop breeding is well recognized (Ford-Lloyd & al., 2011; Hajjar and Hodgkin, 2007). Their evolution in natural conditions makes them really valuable, as natural selection pressures may have provided them with adaptation traits to different conditions (Hawtin & al., 1996). Thus, CWR are not only worth for conservation as components of biodiversity but should be also seen as inexorable future starring elements for food security under the climate change context (Maxted & al., 2010). Their conservation has attracted the interest of scientists, institutions and governments over the last years. Thus, they are specifically mentioned in the targets of the Global Strategy for Plant Conservation outlined by the Convention on Biological Diversity (UN CBD). The generation and publication of multiple lists and inventories of CWR all over the world endorses this assertion; among others, there are inventories for the United Kingdom (Maxted & al., 2007), Venezuela (Berlingeri & Crespo, 2012), the United States (Khoury & al., 2013), China (Kell & al. 2014), Italy (Landucci & al., 2014), Cyprus (Phillips & al., 2014), England (Fielder & al., 2015a); Scotland (Fielder & al., 2015b), Norway (Phillips & al., 2016), The Netherlands (van Treuren & al., 2017), the Czech Republic (Taylor & al., 2017), Spain (Rubio Teso & al., 2018) and even a global one (Vincent & al., 2013). As any other wild species, CWR populations are threatened by habitat fragmentation, loss of habitat or genetic erosion (Heywood, 2011; Kell & al., 2012; Maxted & al., 2010, 2012), therefore conservation measures are needed to maintain their genetic diversity and avoid extinction.

In Spain, the creation of the National Catalogue of Threatened Species promoted by Royal Decree 139/2011 (BOE n. 46, 23/02/2011), provides the ultimate framework to design and implement a conservation plan for endangered species. Additionally, the autonomous communities in which Spain is structured have enacted legislation comprising Regional Catalogues of Threatened Species that confer protection within their territorial limits. The inclusion of a species in these catalogues implies legal protection and the commitment by the administrations to elaborate periodic assessments of its conservation status and implement conservation measures. The *in situ* conservation of Spanish CWR could be approached using the Natura 2000 network. This network was designed in 1992 under the Habitats Directive (Council Directive 92/43/EEC) seeking the creation of a transnational system in Europe to protect both species and their habitats. Consequently, it may provide an effective way to confer passive conservation to CWR populations in Spain. In addition, it could facilitate the drafting of CWR genetic reserves in which their genetic diversity could be more actively preserved (Iriondo & al., 2008; Maxted & al., 2008). On the other hand, the *ex situ* conservation of seeds in germplasm

collections can prevent the loss of genetic diversity of plant species (Bacchetta & al., 2008). Thus, it should be considered as a complementary system to the *in situ* conservation. The compilation of CWR information on threat status, endemism and crossability with crops can help in implementing conservation plans and directing efforts in the right way. In this sense, Rubio Teso & al. (2018) generated a prioritized CWR list for Spain containing 578 species. Still 578 species is a large number of species to consider for the implementation of conservation measures. Hence, it arises the need of identifying the CWR species which most urgently need conservation actions and assessing their conservation status. The aim of this paper is to generate information that may help in ordering priorities for CWR conservation in Spain and implementing conservation actions. Thus, we pose the following questions: Which are the CWR in most urgent need of conservation? Are these species legally protected? What is the *in situ* conservation status of their populations? Are these species conveniently represented in germplasm banks?

Material and Methods

Selection of species

The selection of species aimed at identifying the crop wild relatives that were in most urgent need of knowing their conservation status and of implementing conservation actions. Thus, using as reference the Prioritized Spanish Checklist of Crop Wild Relatives (578 species; Rubio Teso & al., 2018), a strict filtering was made to include just those species which simultaneously: a) were threatened under any of the IUCN categories according to the Spanish Red List of Vascular Flora (Moreno, 2008), b) were endemic to Spain, and c) had high crossability potential with crops of reference, belonging to genepool concept levels 1 or 2 (Harlan & de Wet, 1971) or taxon group concept levels 2 or 3 (Maxted & al., 2006). These three criteria, call attention to three key factors: threat, uniqueness and facility of use for breeding purposes.

Distribution data for the resulting species were downloaded from the GBIF data portal (GBIF, 2011-2013), filtering by scientific name and country (Spain). Synonyms were taken into account and included in the search. Quality of the georeferencing data was evaluated to be able to provide an accurate estimate on whether the populations of the target species fell within limits of protected areas. Consequently, data lacking locality description, geographic coordinates or with geographic coordinates with less than two decimals of decimal degrees (around 1 km accuracy) were eliminated from the analysis. Duplicates based on geographic coordinates were also eliminated. Only species with distribution data with the minimum quality standards established were selected and taken into account for further analysis.

Legal protection of the target species

To assess whether any of the target species were under legal protection in Spain, the Spanish National Catalogue of Threatened Species promoted by Royal Decree 139/2011 (BOE n 46, 23/02/2011) was checked. In addition, the Regional Catalogues of Threatened Species from all seventeen autonomous communities in Spain were consulted in order to verify their protection at the subnational level.

In situ and ex situ conservation preliminary assessment of the target species

A gap analysis (Scott & al., 1993) is a useful approach used to assess the representation of biological components in protected areas. This analysis provides a rough estimation of the *in situ* conservation status of a given species. However, it must be noted that while occurrence data confirms the presence of a species in a given territory, the lack of occurrence data does not necessarily mean the absence of the species. Once this premise was established, a gap analysis was performed using the distribution data of the selected species and the layer of Sites of Community Importance constituting Natura 2000 network in Spain. The analysis was performed using ArcGIS software, v. 10.1 (ESRI, USA). The number of populations for each species after georeferencing quality data assessment and of those within the Sites of Community Importance were added to the database of the study.

Brown & Briggs (1991) considered that the adequate preservation of the genetic diversity of an endangered species requires conservation of a minimum of five populations. On the other hand, Whitlock & al. (2016)

established that 35% of the populations of a species are needed to conserve 70% of its genetic diversity. Consequently, these two thresholds were considered for the conservation assessment of this study.

Simultaneously, *ex situ* conservation status was assessed consulting different national and international databases. Again, the absence of data in the searched databases does not necessarily mean that there are no accessions preserved anywhere else, but that data are not available or public. Databases consulted were: I) the Spanish network of autochthonous plant genetic resources and wild plant germplasm banks (REDBAG), which belongs to the Iberian Macaronesian Association of Botanical Gardens; II) the European Search Catalogue for Plant Genetic Resources (EURISCO), and III) the GRIN-USDA database belonging to the United States National Plant Germplasm System (GRIN-USDA). Information on number of accessions were not accessible in all sources consulted. Thus, the assessment focused on the presence/absence of accessions of the target species in germplasm collections.

Results

Selection of target species

The selection of species according to the established criteria, including the georeferencing quality criterion, generated a list of 47 species. If this last criterion had not been taken into account, 26 additional species would have been included. Results indicate that the CWR species in most urgent need of conservation assessment belong predominantly to the ornamental category use (Table 1) and to the Plumbaginaceae (40%), Lamiaceae (19%) and Amaryllidaceae (15%) families. The species were not evenly distributed among the three most endangered IUCN categories, as almost half of the species belonged to the Vulnerable category (22 species), followed by the Critically Endangered category (12 species), the Endangered category (eight species) and finally by the Near Threatened category (five species).

Table 1. First two columns show the distribution of target CWR genera and species across use categories. In last two columns, number of genera and species per category of use in the Prioritized Spanish Checklist of Crop Wild Relatives (Rubio Teso et al., 2018) is shown.

Category	N. genera	N. species	N. genera CWR	N. species CWR
Food	3	3	32	137
Forage & fodder	2	3	12	185
Ornamental	4	32	5	161
Industrial	2	9	10	95
TOTAL	11	47	59	578

A database was generated containing information on the scientific name of the each of the target CWR species, taxonomic family, use category, IUCN threat category, number of populations recorded with minimum georeferencing quality data, number of these populations within the Sites of Community Importance of the Natura 2000 network, presence of accessions in germplasm collections, category of legal protection according to the Spanish National Catalogue of Threatened Species and categories of legal protection according to the Regional Catalogues of Threatened Species of the 17 autonomous communities. All this detailed information is shown in Table 2.

Table 2: List of species associated to category, family and threat category according to IUCN standards (Th.), number of populations (NP), number of populations inside the network of Sites of Community Importance of Natura 2000 (NP SCI) and percentage in relation to total number of populations (%SCI), presence of accessions preserved in national and international germplasm banks (Germ. banks), inclusion of the species in the National Catalogue of Threatened Species (Nat. Cat.; R.D. 139/2011) and inclusion of the species in the catalogues of the autonomous communities of Spain (Aut. Cat.). Abbreviations are: CR: Critically Endangered; EN: Endangered; VU: Vulnerable; NT: Near Threatened; DE: Danger of Extinction; PR: Protected; IE: Interest for Ecosystems of Canary Islands; MS: Monitored Species; RP: Included in Regime of Protection; SH: Sensitive to Habitat Alteration; SI: Special Interest; SP: Special Protection; AND: Andalucía; ARA: Aragón; BAL: Baleares; CAN: Canarias; CAT: Cataluña; CLM: Castilla-La Mancha; MUR: Región de Murcia; VAL: Comunidad Valenciana.

Category	Family	Species	Threat ¹	Num of populations	Num of populations in SCI	% SCI	Germplasm banks	National Catalogue ²	Autonomous Communities Catalogues ³
Ornamental	Asteraceae	<i>Argyranthemum broussonetii</i> (Pers.) Humphries	VU	3	0	0	YES	NO	NO
Ornamental	Asteraceae	<i>Argyranthemum callichrysium</i> (Svent.) Humphries	VU	3	2	66.7	YES	NO	NO
Ornamental	Asteraceae	<i>Argyranthemum foeniculaceum</i> (Willd.) Webb ex Sch. Bip.	VU	1	0	0	YES	NO	NO
Ornamental	Asteraceae	<i>Argyranthemum maderense</i> (D. Don) Humphries	VU	2	1	50	YES	NO	YES (CAN - IE)
Ornamental	Asteraceae	<i>Argyranthemum winteri</i> (Svent.) Humphries	CR	1	0	0	YES	YES - VU	YES (CAN - VU)
Forage & fodder	Fabaceae	<i>Astragalus cavanillesii</i> Podlech	CR	3	0	0	NO	NO	YES (CLM - VU) (MUR - VU)
Forage & fodder	Fabaceae	<i>Astragalus tremolsianus</i> Pau	CR	6	6	100	YES	YES - PR	YES (AND - DE)
Food	Asteraceae	<i>Cynara alba</i> Boiss. ex DC.	VU	21	9	42.9	YES	NO	NO
Ornamental	Caryophyllaceae	<i>Dianthus toletanus</i> Boiss. & Reut.	NT	10	5	50	NO	NO	NO
Ornamental	Plumbaginaceae	<i>Limonium album</i> (Coincy) Sennen	VU	12	6	50	NO	NO	YES (MUR - VU)
Ornamental	Plumbaginaceae	<i>Limonium aragonense</i> (Debeaux) Font Quer	CR	5	2	40	NO	NO	YES (ARA - SH)
Ornamental	Plumbaginaceae	<i>Limonium arborescens</i> (Brouss) Kuntze	EN	1	0	0	YES	YES - PR	YES (CAN - IE)
Ornamental	Plumbaginaceae	<i>Limonium carthaginense</i> (Rouy) C. E. Hubb. & Sandwith	VU	2	0	0	YES	NO	YES (MUR - VU)
Ornamental	Plumbaginaceae	<i>Limonium catalaunicum</i> (Willk. & Costa) Pignatti	CR	28	3	10.7	YES	NO	YES (ARA - SI) (CAT - DE)
Ornamental	Plumbaginaceae	<i>Limonium dufourei</i> (Girard) Kuntze	CR	6	1	16.7	YES	NO	YES (VAL - DE)
Ornamental	Plumbaginaceae	<i>Limonium erectum</i> Erben	EN	3	2	66.7	YES	NO	YES (CLM - DE)
Ornamental	Plumbaginaceae	<i>Limonium estevei</i> Fern. Casas	CR	12	10	83.3	YES	NO	YES (AND - DE)
Ornamental	Plumbaginaceae	<i>Limonium fruticosum</i> (Webb) Kuntze	EN	2	0	0	YES	YES - PR	YES (CAN - IE)
Ornamental	Plumbaginaceae	<i>Limonium grosii</i> L. Llorens	VU	2	1	50	YES	NO	NO
Ornamental	Plumbaginaceae	<i>Limonium puberulum</i> (Webb) Kuntze	EN	1	0	0	YES	NO	YES (CAN - IE)
Ornamental	Plumbaginaceae	<i>Limonium revolutum</i> Erben	VU	1	0	0	YES	NO	YES (CAT - VU)
Ornamental	Plumbaginaceae	<i>Limonium rigualii</i> M.B. Crespo & Erben	VU	4	1	25	YES	NO	YES (VAL - MS)
Ornamental	Plumbaginaceae	<i>Limonium ruizii</i> (Font Quer) Fen. Casas	VU	44	16	36.4	NO	NO	YES (ARA -VU)
Ornamental	Plumbaginaceae	<i>Limonium santapolense</i> Erben	VU	9	1	11.1	YES	NO	YES (VAL - MS)
Ornamental	Plumbaginaceae	<i>Limonium subglabrum</i> Erben	EN	5	0	0	YES	NO	YES (AND - SP)
Ornamental	Plumbaginaceae	<i>Limonium tabernense</i> Erben	VU	15	11	73.3	YES	NO	YES (AND - RP)
Ornamental	Plumbaginaceae	<i>Limonium thiniense</i> Erben	VU	12	5	42	YES	NO	YES (VAL - MS)
Ornamental	Plumbaginaceae	<i>Limonium tremolsii</i> (Rouy) Erben	NT	4	3	75	YES	NO	YES (CAT - VU)

Table 2: List of species associated to category, family and threat category according to IUCN standards (Th.), number of populations (NP), number of populations inside the network of Sites of Community Importance of Natura 2000 (NP SCI) and percentage in relation to total number of populations (%SCI), presence of accessions preserved in national and international germplasm banks (Germ. banks), inclusion of the species in the National Catalogue of Threatened Species (Nat. Cat.; R.D. 139/2011) and inclusion of the species in the catalogues of the autonomous communities of Spain (Aut. Cat.). Abbreviations are: CR: Critically Endangered; EN: Endangered; VU: Vulnerable; NT: Near Threatened; DE: Danger of Extinction; PR: Protected; IE: Interest for Ecosystems of Canary Islands; MS: Monitored Species; RP: Included in Regime of Protection; SH: Sensitive to Habitat Alteration; SI: Special Interest; SP: Special Protection; AND: Andalucía; ARA: Aragón; BAL: Baleares; CAN: Canarias; CAT: Cataluña; CLM: Castilla-La Mancha; MUR: Región de Murcia; VAL: Comunidad Valenciana.

Category	Family	Species	Threat ¹	Num of populations	Num of populations in SCI	% SCI	Germplasm banks	National Catalogue ²	Autonomous Communities Catalogues ³
Forage & fodder	Fabaceae	<i>Medicago citrina</i> (Font Quer) Greuter	CR	4	2	50	YES	YES - VU	YES (VAL - VU)
Ornamental	Amaryllidaceae	<i>Narcissus alcaracensis</i> Ríos & al.	EN	4	3	75	YES	NO	YES (CLM - VU)
Ornamental	Amaryllidaceae	<i>Narcissus bugei</i> (Fern. Casas) Fern. Casas	VU	10	1	10	YES	NO	YES (AND - RP)
Ornamental	Amaryllidaceae	<i>Narcissus eugeniae</i> Fern. Casas	VU	2	1	50	YES	NO	NO
Ornamental	Amaryllidaceae	<i>Narcissus longispathus</i> Pugsley	EN	10	6	60	YES	YES - DE	YES (AND - DE)
Ornamental	Amaryllidaceae	<i>Narcissus nevadensis</i> Pugsley	CR	5	5	100	YES	YES - DE	YES (AND - DE) (MU - DE*)
Ornamental	Amaryllidaceae	<i>Narcissus tortifolius</i> Fern. Casas	VU	16	10	62.5	YES	NO	YES (AND - VU) (MU - VU)
Ornamental	Amaryllidaceae	<i>Narcissus yepesii</i> Ríos & al.	VU	5	4	80	YES	NO	NO
Food	Rosaceae	<i>Prunus ramburii</i> Boiss.	VU	17	13	76.5	YES	NO	NO
Industrial & other us	Lamiaceae	<i>Sideritis chamaedryfolia</i> Cav.	VU	34	11	32.4	YES	NO	YES (CLM - VU) (VAL - VU**)
Industrial & other us	Lamiaceae	<i>Sideritis glauca</i> Cav.	VU	21	6	28.6	YES	YES - PR	YES (MUR - VU)
Industrial & other us	Lamiaceae	<i>Sideritis lasiantha</i> Pers.	NT	200	119	59.5	YES	NO	YES (MUR - VU)
Industrial & other us	Lamiaceae	<i>Sideritis reverchonii</i> Willk.	EN	17	0	0	NO	NO	NO
Industrial & other us	Lamiaceae	<i>Sideritis serrata</i> Lag.	CR	6	0	0	YES	YES - DE	NO
Industrial & other us	Lamiaceae	<i>Sideritis stachydioides</i> Willk.	VU	20	16	80	YES	NO	NO
Food	Solanaceae	<i>Solanum lidii</i> Sunding	CR	2	1	50	YES	YES - DE	YES (CAN - DE)
Industrial & other us	Lamiaceae	<i>Thymus herba-barona</i> Loisel.	CR	2	1	50	NO	NO	YES (BAL - DE***)
Industrial & other us	Lamiaceae	<i>Thymus moroderi</i> Pan ex Mart. Mart.	NT	97	11	11.3	YES	NO	YES (MUR - VU)
Industrial & other us	Lamiaceae	<i>Thymus Willkommii</i> Ronniger	NT	9	7	77.8	YES	NO	YES (CAT - VU) (VAL - MS)

Legal protection of target species

Ten of the 47 target CWR species are included in the Spanish National Catalogue of Threatened Species, which represent around 21% of the species of this study. Four of them are classified in this catalogue as “in danger of extinction”, four as “protected” and two as “vulnerable”. Thirty-five species (around 74% of the species of this study) are included in at least one of the regional catalogues. From these, six species are present in two regional catalogues. Eleven species from our list are classified into the highest IUCN threat categories (Critically Endangered and Endangered) but not included in the National Catalogue of Threatened Species; however, all of them except for *Sideritis reverchonii* Willk., are included in the regional catalogues (see Table 2).

Preliminary in situ and ex situ conservation assessment of target species

The application of the georeferencing data quality criteria produced a final occurrence dataset for 47 species, with 699 records in total. Figure 1 depicts the distribution of these occurrences in Spain.

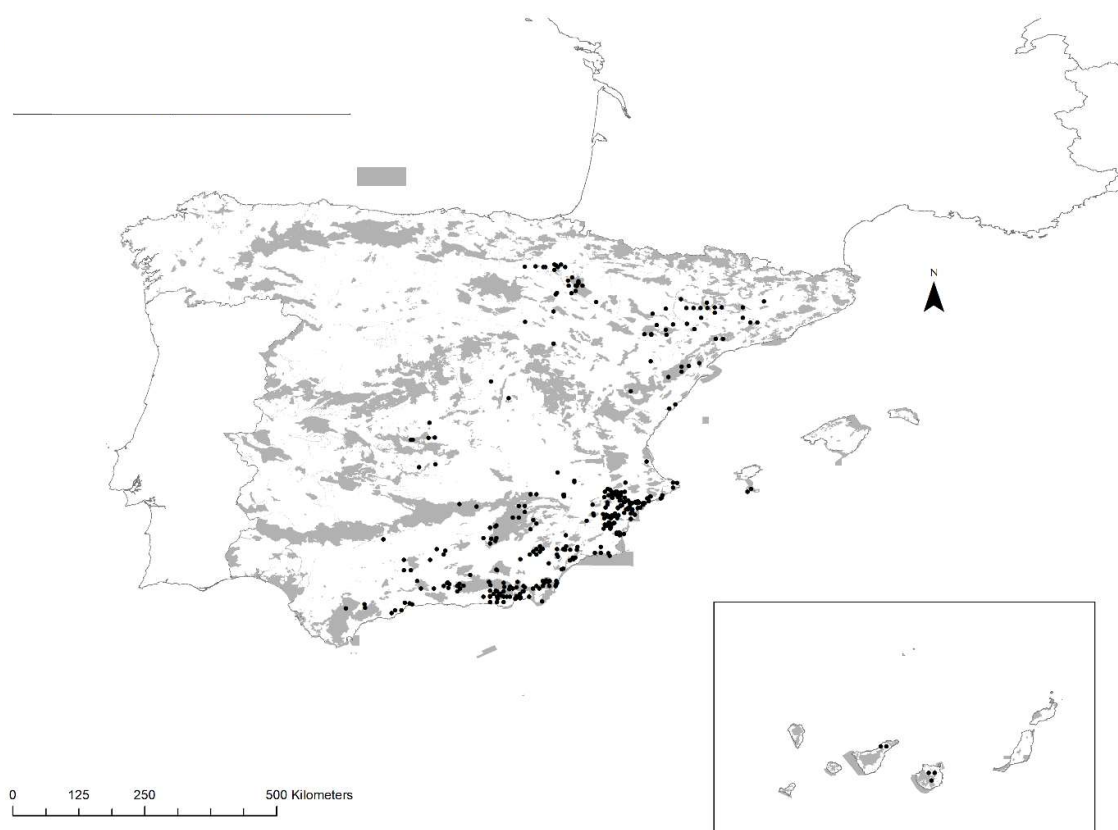


Figure 1: Distribution of the occurrence data of target threatened and endemic CWR of Spain. Grey areas correspond to Sites of Community Interest – Natura 2000

The *in situ* gap analysis showed that 39% of the recorded populations of the target species were inside protected areas (Table 3). On the other hand, 36 target species (74%) have at least one of their populations within the limits of the Sites of Community Importance, and 18 species (38%) five or more populations. The application of the threshold involving the conservation of 35% of the populations showed that 27 species (57%) would comply with this requisite.

Regarding *ex situ* conservation, 40 species (85%) have at least one accession preserved in national and international germplasm collections (Table 3). The coverage of the *ex situ* conserved species was quite akin along the IUCN categories. Finally, two species (*Astragalus cavanillesii* Podlech and *Sideritis reverchonii* Willk.) have no populations within the limits of the Sites of Community Importance of the Natura 2000 network, nor accessions in germplasm banks. It is remarkable that these two species classified respectively as Critically Endangered and Endangered in the Spanish Red List of Vascular Flora are not included into the National

Catalogue of Threatened Species of Spain, although *A. cavanillesii* is included in the catalogues of Castilla La Mancha and Región de Murcia (see Table 2).

Table 3. In situ, ex situ and legal conservation status of target CWR species of Spain (T CWR). For abbreviations on the rest of variables see Table 2.

Th.	T CWR	NP SCI	% SCI	Germ. banks	Nat. Cat	Aut. Cat
CR	12	9	42	9	6	11
EN	8	4	25	7	3	7
VU	22	18	39	20	1	13
NT	5	5	55	4	0	4
TOTAL	47	36	39	40	10	35

Discussion

Selection of species

The Mediterranean area is a region with high speciation rates and endemism (Medail & Quezel, 1999; Thompson, 2005) and the Iberian Peninsula shelters more than 30% of European endemic species (Araújo & al., 2007). Thus, it is not surprising that 13% of the prioritized CWR of Spain (Rubio Teso & al., 2018) fulfilled the targeted criteria of being both threatened and endemic. The lack of available high-quality data for 26 of these species reduced this percentage to 8%. This lack of publicly available data does not mean it does not exist and can be simply explained by the zeal of some administrations in sharing sensitive data that could menace the survival of the populations. Thus, it has been a common procedure that projects focused on the study of threatened plants provide low-resolution occurrence data to chorological databases to preserve the location from unwanted visits (e.g. AFA project (Bañares & al., 2001)). We must also highlight that some of the occurrence data may be outdated as sources used include very old records. Nevertheless, the selection carried out of georeferencing data of high quality eliminated most of the old records. In any case, the work with endemic and threatened species requires up-to-date data that reflect the real distribution status of the analysed species. Thus, records over ten-years old should be revisited and their occurrence confirmed, at least for the most endangered species.

The selection of target species in this study clearly favours species included in the Ornamental category (32 species). This can be explained by the fact that genera selected from this category (particularly *Limonium* Mill. and *Narcissus* L.) are highly diversified and narrowly distributed and thus, with higher number of endemics and threatened species. In addition, these genera have their centers of diversity in the Mediterranean basin (Crespo, 2009; Raimondo, 1993; Roselló & al., 1994; SantosGally & al., 2012; Simón & al., 2010), and are probably responsible for the Eastern distribution of the occurrences shown in Figure 1.

Legal protection of target species

Thirty-seven target species considered to be in different levels of threat according to the Spanish Red List of Vascular Flora (Moreno, 2008) are not included in the National Catalogue as scientific information concerning threatened species is made available. However, results show that more than 74% of the CWR target species are included in the regional catalogues which provide further protection. On the other hand, eleven of the CWR species classified in the two highest IUCN threat categories are not found in the National Catalogue although 10 of them are in the regional catalogues. The inclusion of these species into regional catalogues demonstrate the engagement of the autonomous administrations in preserving their autochthonous flora, as a first step to start protecting our flora. Still, the inclusion of these species into the regional catalogues does not suppress the need to include them in the National Catalogue. The national administration is also giving steps in this sense and committed to increment knowledge about threatened plants, as manifested through the concatenation of

different projects dealing with the study of threatened plant species in Spain, *i.e.*, the Atlas and Red Book of Vascular Flora of Spain (Bañares & al., 2004), the collection of germplasm and development of management protocols for protected plants of Spain project (ref: TEC0004223-TRAGSATEC) or the SEFA project (<http://www.conservacionvegetal.org/proyectos.php>). Results from our study stress the need of implementing conservation actions for the eleven species in the highest IUCN categories and not included in the National Catalogue, particularly focusing on *Astragalus cavanillesii* Podlech and *Sideritis reverchonii* Willk., which have no known populations within protected areas or seed accessions in germplasm banks. Special attention should be given to *S. reverchonii*, which is not included in any of the regional catalogues of threatened flora either.

In situ and ex situ conservation preliminary assessment of target species

Different assessments of the Natura 2000 network have been reported concerning the conservation of different biological entities in Spain (Martínez & al., 2006; Araújo & al., 2007; Rubio-Salcedo & al., 2013). Whilst Araújo & al. (2007) found acceptable representation (73-98% depending on the used criteria) of plant and animal species (pteridophytes, gymnosperms, dicotyledons, monocotyledons, reptiles, amphibians, birds and mammals), Rubio-Salcedo & al. (2013) concluded a poor coverage of lichen species based on the percentage of the potential distribution area present in the network. The representation of the target CWR of our study in the Natura 2000 network was around the lower range of values presented by Araújo & al. (2007). This can be explained by the much higher resolution of the data we have used (1 km vs. 50 km) and the stricter criterion applied to assign a grid cell to a protected area. On the other hand, the percentage of distribution area present in the network of our target species was in the same range of values found by Rubio-Salcedo & al. (2013) for lichens. Determining whether the actual coverage of endangered and endemic CWR in the Natura 2000 network is acceptable depends on which thresholds are set as a reference base. Thus, if the threshold is the species representation in the network by at least one population, the assessment is favourable, as that reported by Araújo & al. (2007). When the criteria are based on having in the network a higher number of populations, *e.g.*, five (Brown & Briggs, 1991), or a substantial representation of its populations, *e.g.* 35% (Whitlock & al., 2016), the percentage of targeted CWR species that comply with these requirements is much lower and the appropriateness of the Natura 2000 network for their passive in situ conservation becomes arguable.

Concerning the choice of the optimal threshold to assess the conservation status of endangered endemic CWR in Spain, it is clear that the drafting of conservation measures should not stop with the simple representation of targeted CWR species in protected areas networks or in germplasm banks. Following this approach, it is likely that the genetic diversity component of threatened species will be neglected, being this especially serious in the case of CWR. Attempts to incorporate this component to conservation efforts have been made over the last decades (Brown & Briggs, 1991; Hamilton, 1994; Whitlock & al., 2016) and recently implemented in CWR conservation through the use of ecogeographical land characterization maps as a proxy to estimate genetic diversity (Maxted & al., 2012; Parra-Quijano & al., 2012; Phillips & al., 2016; Taylor & al., 2017). Thus, the problem of assessing the conservation status of the genetic diversity of a species could be approached by following Whitlock et al. (2016) and including 35% of known populations or proportionally representing populations from each of the ecogeographical units where the species is found (Parra-Quijano & al., 2012).

The high percentage of target CWR species found in national and international germplasm collections (81%) highlights the concern of Spanish conservationists in preserving threatened and endemic flora, and the high activity of seed collecting that has taken place by the REDBAG network in order to ex situ preserve at least 60% of Spanish threatened plant species (REDBAG) as targeted by the Global Strategy for Plant Conservation. The latest update of the objectives of the Global Strategy for Plant Conservation raises to 75% the percentage of threatened species to be ex situ preserved (UN CBD, 2010), a goal which is still met for the targeted CWR species. In any case, the nine target CWR species without representation in germplasm banks should be a priority for *ex situ* collecting missions.

In order to assess whether the genetic diversity component of the target species is being conserved, it is essential to gather information including the number of accessions of each species preserved in germplasm collections. Their origin and collection dates are also important data that should be retrieved. All this information would allow a more precise assessment of the *ex situ* conservation status of the species and the design of collecting actions to improve the quality of germplasm collections holdings. García & al. (2017) provide an example of

this approach. In this study, they identified 88 Spanish CWR species from legumes and cereals crops and assessed their *ex situ* conservation status, proposing an optimized harvesting design for their collection.

Conclusions

To integrate these species into the national conservation programmes, we suggest an expert conservation assessment for the 11 species that are Critically Endangered and Endangered according to the IUCN criteria but not included in the National Catalogue of Threatened Species of Spain. The case of *Sideritis reverchonii* Willk., which is not included in any regional catalogue either, should be immediately addressed. These particular assessments would require gathering detailed information on field occurrences, exact number of accessions and their origin in germplasm collections and an ecogeographical evaluation. Authorities in Spain should be informed of results from these assessments and encouraged to design and implement the corresponding conservation plans. The threatened and endemic CWR of Spain are adequately represented at the species level both *in situ*, in the Natura 2000 network, and *ex situ*, by national and international genebanks. However, the *in situ* conservation of their genetic diversity by the Natura 2000 network is deficient, while additional information is needed to be able to make the assessment at the *ex situ* level.

References

- Araújo, M.B., Lobo, J.M. & Moreno, J.C. 2007. The effectiveness of Iberian protected areas in conserving terrestrial biodiversity. *Conserv. Biol.* 21: 1423-1432.
- Bacchetta, G., Bueno, Á., Fenu, G., Jiménez-Alfaro, B., Mattana, E., Piotto, B. & Virevaire, M. (Eds.). 2008. Conservación ex situ de plantas silvestres. Principado de Asturias. La Caixa. 378 p.
- Bañares, A., Bermejo, E., Blanca, G., Domínguez, F., Güemes, J., Moreno, J.C. & Ortiz, S. 2001. La conservación de la flora amenazada en España y el Atlas de Flora Amenazada. In: S.E.C.F.-Junta de Andalucía (Eds.). *Actas III Congreso Forestal Español* 3. Pp. 161-168. Granada.
- Bañares, A., Blanca, G., Güemes, J., Moreno, J. & Ortiz, S. 2004. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Dirección General de Conservación de la Naturaleza, Madrid. 1069 pp
- Berlingeri, C. & Crespo, M.B. 2012. Inventory of related wild species of priority crops in Venezuela. *Genet. Resour. Crop Evol.* 59(5): 655-681.
- Anonymous. 2011. Desarrollo del Listado de Especies Silvestres en Régimen de Protección Especial y del Catálogo Español de Especies Amenazadas. BOE 46, Sec. I, Pp 20912-20951. Real Decreto 139/2011, 4 de febrero (in Spanish).
- Brown, A.H.D. & Briggs, J.D. 1991. Sampling strategies for genetic variation in ex situ collections of endangered plant species. In: Falk, D.A. & Holsinger, K.E. (Eds.). *Genetics and Conservation of Rare Plants*. Pp. 99-199. Oxford Univ. Press, New York.
- Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities No L 206/7.
- Crespo, M.B. 2009. A new coastal species of *Limonium* (Plumbaginaceae) from Southeastern Spain. *Folia Geobot.* 44: 177-190.
- Fielder, H., Brotherton, P., Hosking, J., Hopkins, J.J., Ford-Lloyd, B. & Maxted, N. 2015a. Enhancing the Conservation of Crop Wild Relatives in England. *PLoS One* 10(6): e0130804.
- Fielder, H., Smith, C., Ford-Lloyd, B. & Maxted, N. 2015b. Enhancing the conservation of crop wild relatives in Scotland. *J. Nat. Conserv.* 29: 51-61.
- Ford-Lloyd, B. V., Schmidt, M., Armstrong, S.J., Barazani, O., Engels, J., Hadas, R., Hammer, K., Kell, S.P., Kang, D., Khoshbakht, K., Li, Y., Long, C., Lu, B.-R., Ma, K., Nguyen, V.T., Qiu, L., Ge, S., Wei, W., Zhang, Z. & Maxted, N. 2011. Crop Wild Relatives Undervalued, Underutilized and under Threat? *Bioscience* 61: 559-565.
- García, R.M., Parra-Quijano, M. & Iriondo, J.M. 2017. A multispecies collecting strategy for crop wild relatives based on complementary areas with a high density of ecogeographical gaps. *Crop Sci.* 57: 1059-1069.
- Hajjar, R. & Hodgkin, T. 2007. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica* 156: 1-13.
- Hamilton, M.B. 1994. Ex situ conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks. *Conserv. Biol.* 8: 39-49.

- Harlan, J.R. & de Wet, J.M.J. 1971. Toward a rational classification of cultivated plants. *Taxon* 20: 506-517.
- Hawtin, G., Iwanaga, M. & Hodgkin, T. 1996. Genetic resources in breeding for adaptation. *Euphytica* 92: 255- 256.
- Heywood, V. 2011. Conservation strategies for species/populations occurring outside protected areas. In: Hunter, D. & Heywood, V. (Eds.). *Crop Wild Relatives. A Manual of In Situ Conservation*. Pp. 253-294. Earthscan, London.
- Heywood, V., Casas, A., Ford-Lloyd, B., Kell, S. & Maxted, N. 2007. Conservation and sustainable use of crop wild relatives. *Agric. Ecosyst. Environ.* 121, 245-255.
- Iriondo, J.M., Ford-Lloyd, B., De Hond, L., Kell, S.P., Lefèvre, F., Korpelainen, H. & Lane, A. 2008. Plant Population Methodologies for the In Situ Genetic Conservation of CWR. In: Iriondo J.M. Maxted, N. & Dulloo, M.E. (Eds.). *Conserving Plant Genetic Diversity in Protected Areas*. Pp. 88-123. CAB International, Wallingford.
- Kell S.P., Maxted, N. & Bilz, M. 2012 European Crop Wild Relative Threat Assessment: Knowledge gained and lessons learnt. In: Maxted, N., Ford-Lloyd, B., Kell, S., Iriondo, J., Dulloo, M.E. & Turok, J. (Eds.). *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. Pp. 218-242. CAB International, Wallingford.
- Kell, S., Qin, H., Chen, B., Ford-Lloyd, B., Wei, W., Kang, D. & Maxted, N. 2014. China's crop wild relatives: Diversity for agriculture and food security. *Agric. Ecosyst. Environ.* 209: 138-154.
- Khoury, C.K., Greene, S., Wiersema, J., Maxted, N., Jarvis, A. & Struik, P.C. 2013. An inventory of crop wild relatives of the United States. *Crop Sci.* 53: 1496-1508.
- Landucci, F., Panella, L., Lucarini, D., Gigante, D., Donnini, D., Kell, S., Maxted, N., Venanzoni, R. & Negri, V. 2014. A prioritized inventory of crop wild relatives and wild harvested plants of Italy. *Crop Sci.* 54: 1628-1644.
- Martínez, I., Carreño, F., Escudero, A., & Rubio, A. 2006. Are threatened lichen species well-protected in Spain? Effectiveness of a protected areas network. *Biol. Conserv.* 133: 500-511.
- Maxted, N., Ford-Lloyd, B.V., Jury, S., Kell, S. & Scholten, M. 2006. Towards a definition of a crop wild relative. *Biodivers. Conserv.* 15: 2673-2685.
- Maxted, N. Kell, S.P. & Ford-Lloyd, B.V. 2008. Crop wild relative conservation and use: Establishing the context. In: Maxted, N., Ford-Lloyd, B., Kell, S., Iriondo, J., Dulloo, M.E. & Turok, J. (Eds.). *Crop Wild Relative Conservation and Use*. Pp. 1-30. CAB International, Wallington.
- Maxted, N., Kell, S., Ford-Lloyd, B., Dulloo, E. & Toledo, Á. 2012. Toward the systematic conservation of global crop wild relative diversity. *Crop Sci.* 52: 774-785.
- Maxted, N., Kell, S., Toledo, Á., Dulloo, E., Heywood, V., Hodgkin, T., Hunter, D., Guarino, L., Jarvis, A. & Ford-Lloyd, B. 2010. A global approach to crop wild relative conservation: Securing the gene pool for food and agriculture. *Kew Bull.* 65: 561-576.
- Maxted, N., Scholten, M., Codd, R. & Ford-Lloyd, B. 2007. Creation and use of a national inventory of crop wild relatives. *Biol. Conserv.* 140(1-2): 142-159.
- Medail, F. & Quezel, P. 1999. Biodiversity Hotspots in the Mediterranean Basin: Setting Global Conservation Priorities. *Conserv. Biol.* 13: 1510-1513.
- Moreno, J.C. coord. 2008. Lista Roja 2008 de la Flora Vascular Española. Dirección General del Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid. 86 p.
- Parra-Quijano, M., Iriondo, J.M. & Torres, E. 2012. Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genet. Resour. Crop Evol.* 59: 205-217.
- Phillips, J., Asdal, Å., Magos Brehm, J., Rasmussen, M. & Maxted, N. 2016. In situ and ex situ diversity analysis of priority crop wild relatives in Norway. *Divers. Distrib.* 22: 1112-1126.
- Phillips, J., Kyrtzsis, A., Christoudoulou, C., Kell, S. & Maxted, N. 2014. Development of a national crop wild relative conservation strategy for Cyprus. *Genet. Resour. Crop Evol.* 61: 817-827.
- Raimondo, F.M. 1993. *Limonium optimae*, a new species from central Sicily. *Flora Mediterr.* 3: 13-18.
- Roselló, J.A., Mus, M. & Soler, J.X. 1994. *Limonium ejulabilis*, a new endangered endemic species from Majorca (Balearic Islands, Spain). *An. Jard. Bot. Madrid* 51: 199-204.
- Rubio-Salcedo, M., Martínez, I., Carreño, F. & Escudero, A. 2013. Poor effectiveness of the Natura 2000 network protecting Mediterranean lichen species. *J. Nat. Conserv.* 21: 1-9.
- Rubio Teso, M.L., Torres Lamas, E., Parra-Quijano, M., de la Rosa, L., Fajardo, J. & Iriondo J.M. 2018. National inventory and prioritization of Crop Wild Relatives in Spain. *Genet. Resour. Crop Evol.* 65 (4): 1327-1253.
- Santos-Gally, R., Vargas, P. & Arroyo, J. 2012. Insights into Neogene Mediterranean biogeography based on phylogenetic relationships of mountain and lowland lineages of *Narcissus* (Amaryllidaceae). *J. Biogeogr.* 39: 782-798.

- Scott, J.M., Davis, F., Csuti, B., Noss, R., Butterfield, B., Anderson, H., Caicco, S., Erchia, F.D., Edwards, T.C. & Ulliman, J. 1993. Gap analysis: A Geographic approach to protection of Biological Diversity. Wildl. Monogr. 123: 3-41.
- Simón, V.I., Xavier Picó, F. & Arroyo, J. 2010. New microsatellite loci for *Narcissus papyraceus* (Amarillydaceae) and cross-amplification in other congeneric species. *Am. J. Bot.* 97: 10-13.
- Taylor, N.G., Kell, S.P., Holubec, V., Parra-Quijano, M., Chobot, K. & Maxted, N. 2017. A systematic conservation strategy for crop wild relatives in the Czech Republic. *Divers. Distrib.* 23(4): 448-462.
- Thompson, J.D. 2005. *Plant Evolution in the Mediterranean*. Oxford Univ. Press, New York. 293 p.
- van Treuren, R., Hoekstra, R. & van Hintum, T.J.L. 2017. Inventory and prioritization for the conservation of crop wild relatives in The Netherlands under climate change. *Biol. Conserv.* 216: 123-139.
- Vincent, H., Wiersema, J., Kell, S., Fielder, H., Dobbie, S., Castañeda-Álvarez, N.P., Guarino, L., Eastwood, R., Lén, B. & Maxted, N. 2013. A prioritized crop wild relative inventory to help underpin global food security. *Biol. Conserv.* 167: 265-275.
- Whitlock, R., Hipperson, H., Thompson, D.B.A., Butlin, R.K. & Burke, T. 2016. Consequences of in situ strategies for the conservation of plant genetic diversity. *Biol. Conserv.* 203: 134-142.

CAPÍTULO 3:

***In situ* Conservation Assessment of Forage and Fodder CWR in Spain Using Phytosociological Associations**

Manuscript published

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Abstract

Crop wild relatives (CWR) can be used to mitigate the negative effects of climate change on crops, but their genetic diversity conservation has not been properly addressed. We propose a new target unit for conservation (Asso-EcoU) based on the occurrence of phytosociological associations in different environments. This approach involves using ecogeographical information and distribution data of associations to identify an optimized set of locations for *in situ* genetic diversity conservation. Thirty-nine CWR species, grouped in 15 associations, generated 165 Asso-EcoUs. Using the Sites of Community Importance (SCI) of Natura 2000 in Spain, we performed three analyses: (1) gap analysis, (2) coverage of the network, and (3) complementarity analysis. Analyses were performed with both target conservation units, associations, or Asso-EcoUs. The SCI network includes 100% of the associations and 69% of the Asso-EcoUs. The coverage assessment showed that 8.8% of the network is enough to encompass all the networks' target conservation units. Complementarity analysis showed that seven and 52 SCI areas are needed to contain at least one site of the 15 associations and the 114 Asso-EcoUs, respectively. These results highlight the value of Asso-EcoUs to potentially incorporate the genetic diversity component into conservation plans, while increasing the number of species covered.

Keywords: CWR; optimized conservation; phytosociological associations; Ecogeographical Land Characterization (ELC) maps; genetic diversity; Sites of Community Importance (SCI) sites; Natura 2000.

Introduction

Crop Wild Relatives (CWR) are wild species that are closely related to crop species to which they contribute genetic material [1]. According to Lidder & Sonnino [2], CWR have more genetic diversity than crops, which are known to have a narrow genetic base as a result of domestication and genetic breeding, especially in recent decades [3]. This greater diversity and the environmental pressures to which they are subjected provide them with specific adaptive traits [4,5] that can be used to improve crop characteristics. In fact, they have already been successfully used in breeding programs of many crops [6–8]. Thus, CWR should be considered essential actors in sustaining food security and providing the adaptations needed to face the challenges brought about by climate change in the coming years. CWR genetic diversity conservation has traditionally been neglected [8]. Genetic erosion as well as habitat fragmentation including changes in land use and competition with alien species have been identified as the main agents threatening their integrity [9]. Nevertheless, in recent years, important steps have been taken to protect CWR as a valuable component of plant diversity. CWR have been included in National Plans for Plant Conservation [10] and national prioritized checklists of CWR and strategies for their conservation have been developed [11]. However, the listing of CWR species for their conservation may be insufficient if the main target is to preserve their genetic diversity. *Ex situ* conservation of CWR in gene banks is the most straightforward approach already pursued by several countries [11]. Nevertheless, it needs to be complemented with the *in situ* conservation of natural populations, where genetic diversity is dynamically evolving in response to an environmental change.

Although some studies have proposed certain areas for establishing CWR genetic reserves [12,13], there are still few comprehensive approaches to conserve *in situ* the genetic diversity of a targeted set of priority CWR species in a territory [see 14]. Adequately assessing genetic diversity by DNA characterization of all populations of a set of targeted CWR species is currently unfeasible due to limited economic resources. Consequently, alternative methods that capture the specific environmental attributes of the area under study can be used as a proxy of genetic diversity in order to identify possible adaptation patterns of plant populations [15,16]. One of them involves the use of ecogeographical land characterization maps (ELC maps) [15]. Heterogeneous environmental conditions are expected to generate diverging selective pressures and, therefore, generate genetic differentiation of adaptive value. Thus, the classification of the variety of habitats found in a territory with ELC maps can be used to identify natural populations with potentially different adaptations [17]. In any case, when specific traits are sought, the use of ecogeographical information as a proxy for adaptive genetic differentiation

among populations must be subsequently validated by appropriate phenotype evaluation and/or molecular marker characterization of the target populations.

Recent research has explored the use of ELC maps to study the distribution of a group of given CWR species along the ecogeographical categories of a given territory [18–20]. However, these studies do not explicitly link each ecogeographical category (hereafter, ELC categories) to each population. A new conservation unit based on each of the different ELC categories in which a species is distributed could be considered a valid approach to identify the genetic differentiation of adaptive values among populations that can be found in a species. However, this approach leads to a considerable increase in the number of conservation targets (target species x ecogeographical categories in which they are found). Thus, in this sense, it would be an advantage in terms of time, cost, and resources to manage the conservation of genetic diversity of various CWR species simultaneously.

The study of plant communities and aggregation of species in repetitive patterns considering their relationships and environmental dependence [21] may provide an alternative approach to reduce the number of target conservation units. The classification of phytosociological associations, known as syntaxonomy, is based on inventories listing all plant species co-occurring in an area. Thus, the basic syntaxonomical units – associations – are identified by comparing the inventories and fidelity of the species to a given plant community. In this context, the use of the association as a management unit could be used to conserve several CWR species that co-occur in a given set of environmental conditions. In this way, although the incorporation of ecogeographical categories as a proxy for genetic diversity conservation would increase the number of target units for conservation, the use of associations rich in target species could be helpful to manage several species at the same time. Forage and fodder CWR is an important group of CWR formed by species that commonly occur together in natural grasslands. Thus, they are especially suited for testing an association mediated *in situ* conservation approach.

The objective of this paper is to assess the *in situ* conservation of forage and fodder CWR in Spain by taking into account the ecogeographic heterogeneity of their populations. The assessment is based on the protection provided by the Sites of Community Interest (SCI) of Spain belonging to the Natura 2000 network. The conservation strategy proposed is built on the use of selected phytosociological associations containing CWR species. It is further based on the use of ELC categories (ecogeographical units in which a territory is divided) as a proxy of genetic diversity. For this purpose, we generated a new target conservation unit composed of the combination of phytosociological associations that contain targeted CWR and ELC categories of a territory where these associations occur, which we named Asso-EcoU. As part of the assessment, we posed the following questions: 1) Are phytosociological associations a valid approach for *in situ* conservation of fodder and forage CWR? 2) How well are the target Asso-EcoUs and associations of fodder and forage CWR protected by the SCI of the N2000 network? 3) Which would be the priority sites to establish genetic reserves to actively conserve *in situ* this group of CWR? To answer these questions, we carried out gap, coverage, and complementarity analyses and used the concept of effectiveness, as described by Caro [22]. One of our main concerns was to use a proxy to incorporate the genetic differentiation between the populations component when planning conservation actions for multiple species in protected areas. We found that the use of phytosociological associations combined with ecogeographical information of the territory could be an efficient way to manage and conserve *in situ* various target forage and fodder CWR species at the same time, including a representative sample of their genetic differentiation of adaptive value among populations. This makes an efficient use of existing conservation resources.

Material and Methods

Selection of Species and Phytosociological Associations

Target CWR species were selected from the Prioritized Spanish Checklist of crop wild relatives [23], among the subgroup category Fodder & Forage. According to the genepool concept of Harlan and de Wet [24], which

classifies wild plant species according to their crossability to crops. We selected Fodder & Forage CWR species belonging to genepool 1B (same species as the crop species).

The resulting 45 species belonging to GP 1B were then introduced into the SIVIM database (<http://www.sivim.info/sivi/>, last accessed 05/07/2019) to obtain a list of the phytosociological associations where at least two of the targeted CWR were found. The first three associations or sub-associations, to which each target CWR species was more prevalent (higher fidelity), were selected. Those associations that were repeated two or more times in the previous selection were chosen as target associations to conserve and their corresponding distribution data were downloaded from SIVIM (Iberian and Macaronesian Vegetation Information System) (<http://www.sivim.info/sivi/>, last accessed 25/08/2019). Occurrence data were transformed from Military Grid Reference System coordinates into geographic decimal coordinates using MSP Geotrans software v. 3.5, developed by the National Geospatial-Intelligence Agency of the United States (<https://earth-info.nga.mil/GandG/update/index.php?action=home>, last accessed 30/07/2019). To ensure that the target species were present in the selected association, we only used inventories of the association while explicitly citing the occurrence of the target species. In addition, we only used data records that provided an accuracy of at least 1x1 km.

Ecogeographical Analyses

Creation of the Ecogeographical Land Characterization Map and Representativeness Analysis

An Ecogeographical Land Characterization map was created using the ELCmap tool of Capfitogen software v. 2.0 [25]. Its extent was restricted to Peninsular Spain and the Balearic Islands because none of the selected associations were found in the Canary Islands. The kmeanbasic function was used to define the optimal number of ELC categories for the ELC map. This function uses a clustering algorithm determining the cut-off points based on the decrease in the sum of the intra-group squares. When the decrease in the intra-group sum of squares in a range of n and $n+1$ groups is less than 50%, the algorithm reaches the optimal number of categories [26]. It has been successfully used for the creation of other ELC maps, as described in Reference [17]. The territory was structured using a grid of 1x1 km cells. The ecogeographical variables used to create the ELC map were: (i) climatic variables: annual mean temperature, annual precipitation, temperature seasonality, temperature annual range, (ii) geophysics variables: elevation, slope degrees, (iii) soil variables: topsoil clay fraction, topsoil salinity, topsoil sodicity, topsoil organic carbon, topsoil pH, topsoil sand fraction, topsoil silt fraction, and (iv) other variables: latitude and longitude, which were included to create spatially aggregated categories. All these variables were chosen, in agreement with expert advice, to consider relevant ecogeographical factors in species distribution, which aim to create a generalist ELC map that would discriminate different adaptive environments for species with diverse ecological requirements.

The Capfitogen Representa tool of Capfitogen software v 2.0 was used to incorporate into the association occurrences, where the corresponding ELC category was derived from the ELC map of Spain with a 1x1 km resolution. The Asso-EcoU conservation unit represents those sites where a specific phytosociological association occurs under a particular ELC category. Thus, each combination of a selected phytosociological association with each of the ELC categories in which it is distributed becomes a unique Asso-EcoU.

Gap, Coverage, and Complementarity Analyses

Gap analyses [27] were carried out to estimate the protection provided by the SCI network of the Natura 2000 network. Thus, through gap analyses, we determined the effectiveness of the network, i.e., what percentage of the targeted associations and Asso-EcoUs were found at least once within the limits of the Sites of Community Importance of the Natura 2000 network in Spain. On the other hand, through coverage analysis, we determined the efficiency of the SCI network. The ratio of the number of protected areas containing the targeted conservation units versus the total number of protected areas of the network [25].

A complementarity analysis was carried out to identify the set of areas that encompass the maximum number of target conservation units in the minimum number of sites, following an iterative process [28]. First, the area containing the greatest number of different target conservation units is selected. Then, those target conservation units already present in the first area are excluded from the analysis and a second area containing the greatest

number of possible different target conservation units is chosen. This process is repeated until all target conservation units under analysis are covered.

All the analyses were performed with the associations and Asso-EcoUs, using the SCI network of Spain (Natura 2000 network) as target protected areas. The latter were downloaded from: https://www.miteco.gob.es/es/biodiversidad/servicios/banco-datos-naturaleza/informacion-disponible/rednatura_2000_lic_descargas.aspx# (last accessed 22/08/2019). The Complementa tool developed under Capfitogen software v 2.0 was used to perform all these analyses.

Results

Selection of Species and Phytosociological Associations

The selection of three associations for each of the 45 target forage and fodder CWR species resulted in a total of 84 different associations or sub-associations. Two species (*Lupinus consentinii* Guss. and *Trifolium vesiculosum* Savi) were not found in any of the inventories of the phytosociological associations recorded in the SIVIM database. Table S1 shows the list containing the three associations per target CWR species, the number of inventories of each association, the number of inventories in which the species is cited, and the fidelity of the target CWR species of the association. Of these 84 associations, 21 were initially selected for analyses since they were repeated at least two times (see Table S2). The data quality filtering procedure caused the exclusion of six phytosociological associations. This resulted in 15 associations containing 39 of the initial 45 species (Table 1).

Table 1. List of the 15 phytosociological associations chosen as a conservation target containing 39 priority forage and fodder crop wild relative (CWR) species of Spain. The number of inventories in which each CWR species is present in the association and the fidelity to the association are shown. Inventories: Number of inventories in which the species is present. Fidelity (%): percentage of inventories of the association in which the species is present.

	Target Associations	Priority CWR Species	Inventories	Fidelity (%)
1	<i>Agrimonia- subass. primuletosum columnae</i>	<i>Astragalus glycyphyllos</i> L.	10	19.6
		<i>Trifolium medium</i> L.	44	86.3
2	<i>Euphrasia-Plantaginatum mediae</i>	<i>Poa alpina</i> L.	32	11.9
		<i>Poa compressa</i> L.	12	4.5
		<i>Agrostis capillaris</i> L.	121	45.0
3	<i>Festuca amplexae-Agrostietum castellanae</i>	<i>Deschampsia cespitosa</i> (L.) P. Beauv.	6	1.9
		<i>Trifolium dubium</i> Sibth.	87	27.9
4	<i>Gaudinia verticillata- Hordeetum bulbosi</i>	<i>Hedysarum coronarium</i> L.	21	95.5
		<i>Trifolium lappaceum</i> L.	1	4.5
		<i>Trifolium squamosum</i> L.	9	40.9
		<i>Trifolium squarrosum</i> L.	2	9.1
5	<i>Helianthemum guttati</i>	<i>Medicago truncatula</i> Gaertn.	9	8.7
		<i>Trifolium nigrescens</i> Viv.	9	8.7
6	<i>Holoschoenetum vulgaris</i>	<i>Agrostis stolonifera</i> L.	214	41.2
7	<i>Linario eleganti- Anthoxanthetum aristati</i>	<i>Lupinus luteus</i> L.	7	14.3
		<i>Medicago arabica</i> (L.) Huds.	1	2.0
		<i>Ornithopus sativus</i> Brot.	5	10.2
		<i>Lupinus angustifolius</i> L.	8	16.3
8	<i>Lolio-Plantaginatum majoris</i>	<i>Lolium perenne</i> L.	308	88.3
9	<i>Medicagini rigidulae- Aegilopetum geniculatae</i>	<i>Medicago rigidula</i> (L.) All.	150	38.4
		<i>Dactylis glomerata</i> L.	117	95.9
10	<i>Rhinantho mediterranei- Trisetetum flavescens</i>	<i>Medicago lupulina</i> L.	70	57.4
		<i>Trifolium incarnatum</i> L.	2	1.6
		<i>Trifolium pratense</i> L.	113	92.6
		<i>Festuca arundinacea</i> Schreb.	16	13.1
		<i>Festuca pratensis</i> Huds.	39	32.0
		<i>Medicago sativa</i> L.	23	18.9

Table 1. List of the 15 phytosociological associations chosen as a conservation target containing 39 priority forage and fodder crop wild relative (CWR) species of Spain. The number of inventories in which each CWR species is present in the association and the fidelity to the association are shown. Inventories: Number of inventories in which the species is present. Fidelity (%): percentage of inventories of the association in which the species is present.

	Target Associations	Priority CWR Species	Inventories	Fidelity (%)
		<i>Poa pratensis</i> L.	70	57.4
		<i>Trifolium repens</i> L.	78	63.9
		<i>Trifolium arvense</i> L.	87	52.9
11	<i>Trifolium cherleri</i> - <i>Taeniatheretum caput-medusae</i>	<i>Trifolium angustifolium</i> L.	70	45.8
		<i>Trifolium campestre</i> Schreb.	89	58.2
		<i>Trifolium striatum</i> L.	56	36.6
12	<i>Trifolium fragiferi</i> - <i>Cynodontetum dactyli</i>	<i>Medicago scutellata</i> (L.) Mill.	1	0.3
		<i>Trifolium resupinatum</i> L.	4	7.4
13	<i>Trifolium subterranei</i> - <i>Poetum bulbosae</i>	<i>Poa bulbosa</i> L.	54	100.0
		<i>Trifolium subterraneum</i> L.	31	57.4
14	<i>Trifolium cherleri</i> - <i>Plantaginetum bellardii</i>	<i>Ornithopus compressus</i> L.	81	53.6
15	<i>Lino biennis</i> - <i>Cynosuretum cristati</i>	<i>Lolium multiflorum</i> Lam.	22	5.9

Ecogeographical Analyses

Creation of Ecogeographical Land Characterization Map and Representativeness Analysis

The clustering algorithm using the selected environmental variables resulted in an ELC map with 49 different ELC categories. The total number of inventories of the selected associations was 1283, distributed across 39 ELC categories. Inventories found to be in ELC categories '0' or 'NA' (no available information) were excluded from the analysis, which resulted in 1227 total inventories. The distribution of the inventories of the associations along the ELC categories depended on the frequency of the ELC categories in the territory and the sampling effort (Table S3). The associations that were found in a greater number of ELC categories were *Holoschoenetum vulgaris* and *Medicagini rigidulae-Aegilopetum geniculatae*, which also had the highest number of inventories. On the other hand, two other associations (*Lino biennis-Cynosuretum cristati* and *Festuco amplae-Agrostietum castellanae*) with a similar sampling effort had a low number of ELC categories, which relate to more specific-habitat requirements (Figure 1).

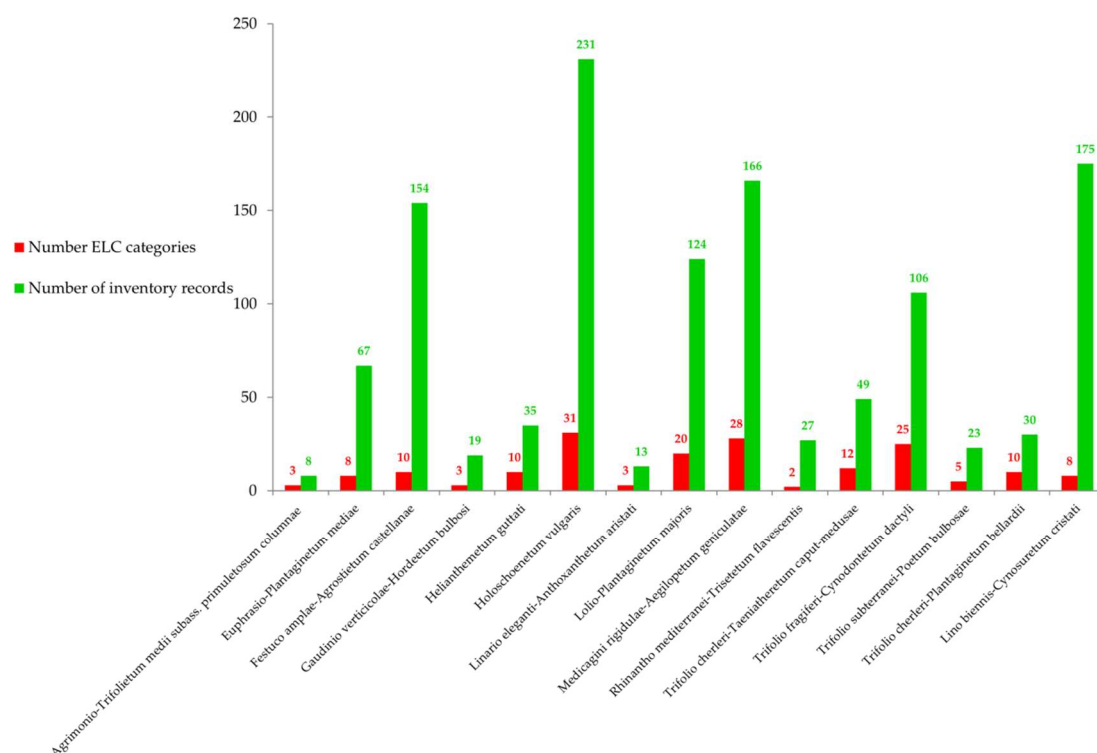


Figure 1. Number of inventory records of the target phytosociological associations containing prioritized species of forage and fodder crop wild relatives of Spain and the number of Ecogeographical Land Characterization categories represented in the inventories of each association.

Lastly, the combination of the 39 ELC categories with the occurrence data of the 15 phytosociological associations resulted in 165 unique Asso-EcoUs. Figure 2 shows a representation of the ELC map with the 165 Asso-EcoUs distribution.

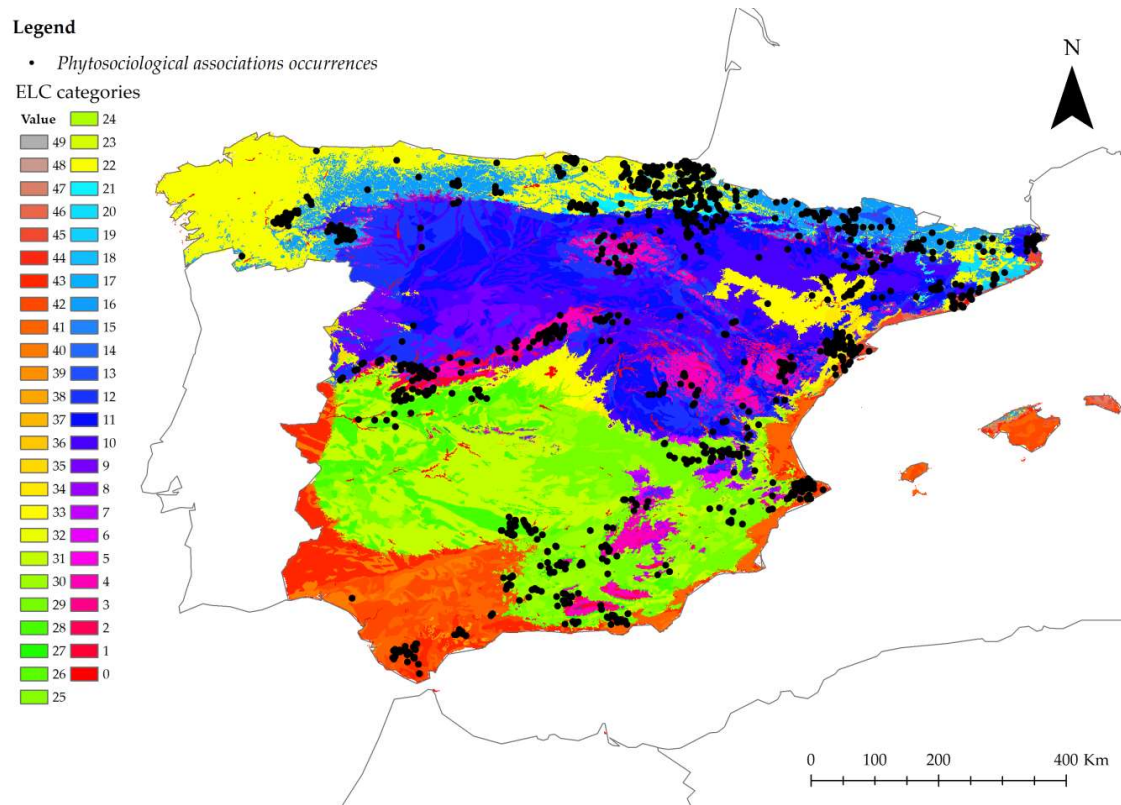


Figure 2. Ecogeographical Land Characterization (ELC) map of Spain composed of 49 ecogeographical categories. Black dots depict the occurrences of 1227 inventories representing 165 Asso-EcoUs (combination of associations with ELC categories) (15 phytosociological associations that contain 39 priority forage and fodder crop wild relative species).

Gap Analysis, Coverage, and Complementarity Analyses

The gap analysis showed that 447 of the 1227 inventories were found within the SCI areas of the Natura 2000 network, representing at least once, all 15 associations (100%) (Table S4) and 114 of the 165 targeted Asso-EcoUs (69%) (Table S5).

The Natura 2000 network of Spain contains 1449 SCI areas and the coverage analysis showed that 127 SCI areas will suffice to passively protect the 15 associations and the 114 Asso-EcoUs present in the network. These 127 areas represent 8.8% of the total areas composing the SCI network of Natura 2000 in Spain, which points to a low efficiency of the network in covering our target conservation units. Table S6 shows the 127 SCI areas covering the targeted conservation units. It is worthy to note that 37 areas have a greater number of Asso-EcoUs inventories than phytosociological associations inventories, which indicates that these associations are found under diverse environmental conditions in the same SCI area.

The complementarity analysis showed that seven SCI areas (0.48% of the network) would be needed to conserve all 15 associations. In addition, these seven areas would contain 22 different Asso-EcoUs (17.5% of Asso-EcoUs inside SCI areas) (Table 2). Same analysis performed with the 114 Asso-EcoUs showed that 52 SCI areas (3.59% of the network) is the minimum number of areas to represent Asso-EcoU at least once each (Table S7). Figure 3 shows the maps representing the SCI areas obtained from complementarity analysis targeting the associations or the Asso-EcoUs. Fourteen SCI areas add at least three new different Asso-EcoUs to the sites selected through complementarity analysis, which represents around 41% of total Asso-EcoUs (67 different Asso-EcoUs) and 14 of the 15 associations. To complete the coverage of the associations and include the missing association, (*Linario eleganti*-*Anthoxanthetum aristati*). The SCI area named Baixa Limia must be added since it is the only one including that association (see Table S4). These 15 areas constitute our proposal for establishing genetic reserves of phytosociological associations containing prioritized forage and fodder CWR of Spain (Figure 4). This proposal includes all the targeted associations and 41% of the targeted Asso-

EcoUs (68 different Asso-EcoUs). Both results using the associations or the Asso-EcoUs point at a high effectiveness of the network in covering our target conservation units.

Table 2. Areas belonging to the Sites of Community Importance of Spain that will cover the 15 selected associations under study obtained through complementarity analysis.

Name of SCI Area	Site Code	Autonomous Community	Number of New Associations Added	Number of Asso-EcoUs Present
Cuenca del río Lozoya y Sierra Norte	ES3110002	Comunidad de Madrid	5	9
Aiguamolls de l'Alt Empordà	ES0000019	Cataluña	3	3
Ordesa y Monte Perdido	ES0000016	Aragón	3	3
Los Alcornocales	ES0000049	Andalucía	1	4
Montaña Oriental	ES1300002	Cantabria	1	1
Baixa Limia	ES1130001	Galicia	1	1
Montes Aquilanos y Sierra de Teleno	ES4130117	Castilla y León	1	1

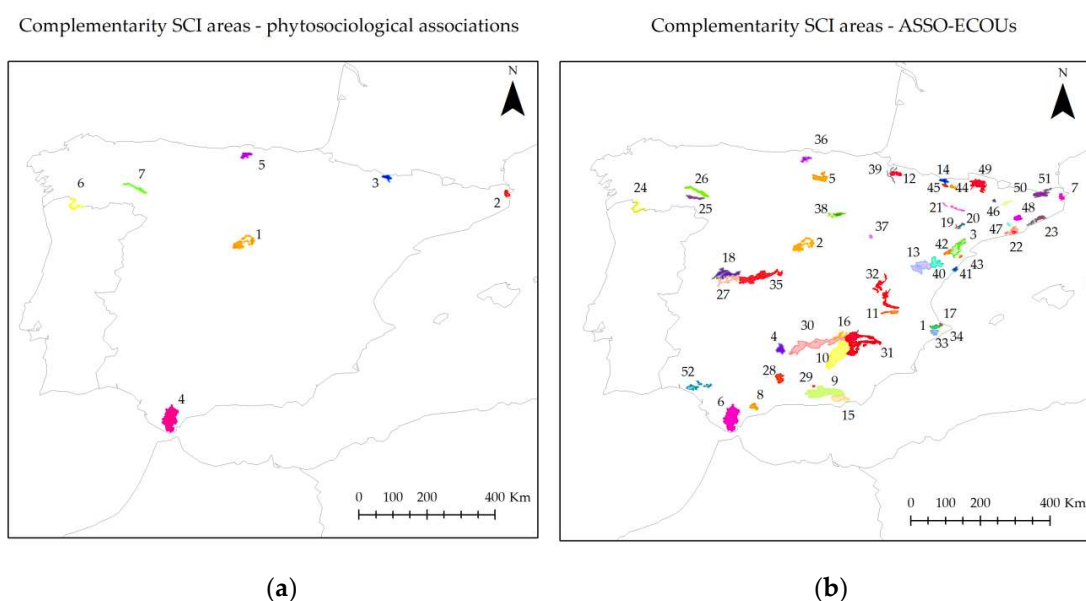


Figure 3. Graphic comparison between the seven Sites of Community Importance (SCI) areas needed to conserve the 15 phytosociological associations (a) vs. the 52 SCI areas needed to conserve the 114 Asso-EcoUs (combination of associations with ecogeographical categories) under study (b). The selection of areas was made through complementarity analysis and numbers by the SCI areas indicate the ranking in the selection process.

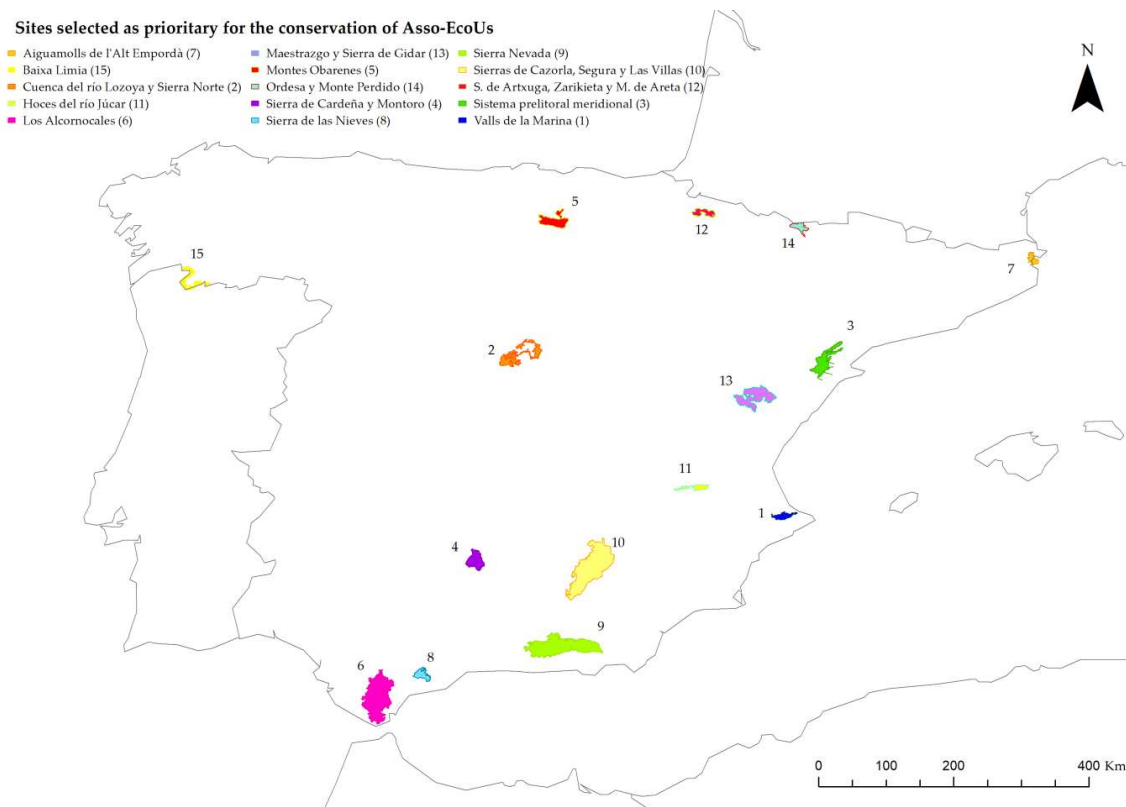


Figure 4. Proposal of 15 Sites of Community Importance (SCI) areas of the Natura 2000 network to establish genetic reserves for the conservation of prioritized forage and fodder crop wild relatives of Spain grouped in Asso-EcoUs. Selection of SCI areas was made through complementarity analysis, and the numbers by the SCI areas indicate the ranking in the selection process.

Discussion

Relevance of Genetic Diversity in the Conservation of Crop Wild Relatives

The preservation of target CWR species, which protects both their taxonomic diversity and genetic diversity, should be one of the objectives when planning CWR conservation strategies, especially at the national level [29]. Traditionally, the steps taken in the implementation of national CWR strategies include the creation of checklists listing all CWR species of a country followed by their prioritization, according to different criteria depending on the country's idiosyncrasy [14]. However, in several cases, the national CWR conservation strategies do not go much further and do not take practical steps for the conservation of a representative sample of the genetic diversity of adaptive value present in each CWR. As a result, the genetic diversity of the target species may not be conserved or it is, at most, conserved in an inefficient manner.

One of the most important characteristics of CWR is that their populations can provide useful genes to their domesticated relatives [4,30]. Thus, CWR are an important source to take into account in crop breeding [7,31] given the expected negative effects of climate change on crop yields [32] and the inability of crops to overcome extreme events due to their narrow genetic diversity [3]. Thus, conservation goals should not be focused on the conservation of the target CWR species, but on the conservation of several populations per species containing a representative sample of their genetic diversity of adaptive value.

To that aim, maps that capture the specific environmental attributes of an area (ELC maps are built considering climatic, edaphic, and geophysical variables) [15] can be used as a proxy to survey the genetic differentiation of adaptive value among CWR populations [16]. Consequently, they have already been used for this purpose in several cases, such as in the improvement of seed collections of germplasm banks [33], the search for tolerance to drought and salinity in *Aegilops* collections of Spain, by taking into consideration different adaptive contexts

[17] or the assessment of CWR diversity in Spain [19] and Norway [18]. When this approach is used, results must be validated by proper phenotyping and/or molecular characterization procedures. A further step forward in this line involves considering each potentially different adaptive environment of a CWR as a conservation target. However, one of the drawbacks of this approach is that the number of conservation targets may become too large to handle. Hence, new approaches to achieve the in situ conservation of CWR in an efficient manner are needed.

Phytosociological Associations as a Means for Multiple Species Conservation Management

Phytosociology, as a discipline with a long tradition in Europe, facilitates the management of various species at a time – a desirable characteristic for conservation actors – using the association as a reference entity. This can be especially advantageous if the objective is to conserve species and their genetic diversity while considering the ecogeographical information associated with them [34]. The link of ecological information with phytosociological associations has been previously approached from a landscape perspective by proposing a landscape unit named geosigmetum [35], which consists of an integrated set of vegetation series that is repeated in an area of land with the same edaphic, climatic, and biogeographic characteristics. On the contrary, in our case, we are interested in the different environmental conditions that can be found within the distribution range of an association and which can generate different evolutionary adaptations in their populations. Using the Asso-EcoU as target conservation units in CWRs can be helpful in establishing genetic reserves if there are several CWR species that form part of the same association. Fodder and forage CWR can be a good group to test the viability of this novel approach, since many of these species are known to grow together. Results in the selection of associations support this hypothesis since the associations contain from seven to 20 prioritized forage and fodder crop wild relatives, except for one that has three target species (see Table S2).

The viability of using the Asso-EcoU approach also depends on the quality and quantity of distribution data of the phytosociological associations. In this sense, we verified this premise by finding that most selected associations (15 of 21) had accurate georeferencing data at the 1x1 km grid cell scale. Considering that, even if the species is very frequently linked with the association, its presence in a citation of an association is not assured. It becomes necessary to use only inventory records of the association where the species has been cited. Alternatively, it is possible to cross the association distribution data with the target species distribution data obtained from an independent source (e.g., Global Biodiversity Information Facility (GBIF)) to verify the presence of the species at one location.

Ecogeographical Analyses

Creation of ELC Map and Representativeness Analysis

The generalist ELC map created for forage and fodder crop wild relatives of Spain and used in this study resulted in 49 ELC categories, which were assigned to the territory with a 1x1-km grid resolution. The distribution of inventories throughout the ELC categories agrees in general terms with their expected distribution, according to the frequency of ELC categories in the ELC map of Peninsular Spain and Balearic Islands (see Table S3). ELC categories with low frequency occurrence in the studied territory have a lower number of inventory records than ELC categories with higher frequencies. Thus, nine of the 10 ELC categories that do not have any inventory records correspond to ELC categories with low representation in the territory studied (<0.03). The exception is category 49, which has a mid-high distribution. The database of inventories of phytosociological associations of SIVIM provides a balanced sample of studies distributed across the different ELC categories. This screening also indicates that the set of phytosociological associations selected for this study cover almost all of the most frequent types of environmental conditions (ELC categories) found in Peninsular Spain and Balearic Islands.

Gap Analysis, Coverage, and Complementary Analyses

Gap analysis help answer the question of how our target conservation units – phytosociological associations or Asso-EcoUs – are passively protected by the Sites of Community Importance of the Natura 2000 network of Spain. The N2000 network was developed under the Habitats Directive seeking the protection of threatened habitats and species [36]. Results in this work point to a very high effectiveness (in terms of representation of

all targeted CWR diversity) of the network, since all phytosociological associations and almost 70% of the Asso-EcoUs have at least one inventory within the network. This is congruent with previous studies performed on the Natura 2000 network in Spain in which they found a high effectiveness in covering threatened species, since only 5% of threatened species under study had less than 10% of their distributions not covered by the Natura 2000 network [37]. Other works, however, report low effectiveness of the Natura 2000 network concerning other parts of Europe or other groups of species, such as, plant regional biodiversity in Crete [38], terrestrial vertebrates and fresh water fishes in Italy [39], Mediterranean lichen species in Spain [40], or insects in Italy [41]. As pointed out by many of these authors, the adequacy of the Natura 2000 network to fulfill biodiversity conservation goals is heavily dependent on the taxonomic group under study and the heterogeneous contributions to the network made by the different countries, dependent on economic and political issues.

The use of existing protected areas should be the first approach for planning CWR in situ conservation actions since it minimizes costs and takes advantage of existing conservation laws and management figures. Nevertheless, there are some Asso-EcoUs not protected by the network that should also be included in a future network of genetic reserves. The absence of these Asso-EcoUs in the SCI network could be explained by two different hypotheses. The first one is that the ELC categories of those Asso-EcoUs are simply not found inside the limits of the SCI network. From our point of view, this is unlikely to happen, as the Natura 2000 network covers 118 different habitats in Spain, representing about 37% of the territory of the country (https://www.miteco.gob.es/es/biodiversidad/temas/espacios-protegidos/red-natura-2000/rn_espan_a.aspx, last accessed 2/09/2019). In addition, the diversity of environments found within the limits of the SCI network is highlighted in previous works [18,19]. The second hypothesis is that the missing Asso-EcoUs are actually present in the SCI network but have not been captured by the current association inventories. We should exclude the distribution patterns of the associations as an explanation, as all associations are represented in the SCI network. In this sense, we recommend to prospect the missing associations in the SCI areas that contain the ELC categories corresponding to the targeted Asso-EcoUs.

If the missing targeted Asso-EcoUs cannot be found within the SCI network, the inclusion of new areas to formal networks of protected areas can be considered, which is a matter that has already been addressed [42]. In the case of in situ conservation of CWR, the existence of conservation targets outside protected areas is a common event and their management is an important matter that must be dealt with [43]. This intermediate solution would, thus, combine the network and other areas of interest, which could be designated micro-reserves or genetic reserves, as pointed out by Maxted et al. [16].

The coverage analysis denoted a low efficiency of the SCI network in terms of maximizing the use of resources of the network. With a low number of SCI areas (127 out of 1449), all targeted phytosociological associations or Asso-EcoUs inventories are passively protected. Again, this is not at all surprising since the Natura 2000 network was not designed to conserve forage and fodder CWR.

Before any genetic reserve is established, the risk of outcrossing events with related crop species in surrounding areas should be evaluated and properly addressed. Furthermore, an experimental assessment of the adaptive traits of the selected populations should be performed if the aim is to consider these genetic reserves as an access point for using wild plant genetic resources for plant breeders, farmers, or other end users. Lastly, these genetic reserves should meet a minimum set of quality standards [56] to mitigate the effects of climate change and ensure the long-term conservation of these resources.

Conclusions

Phytosociological associations are a highly useful concept to facilitate the in situ conservation assessment of forage and fodder CWR species and identify an efficient network of sites for establishing genetic reserves. The incorporation of ecogeographical information to the selected associations as a proxy to represent potential genetic diversity of adaptive value among CWR populations by creating the Asso-EcoU target conservation unit, provides an inexpensive and simple approach for the in situ conservation of genetic diversity of several CWR species together. This is shown by the effectiveness of the SCI network in Spain in conserving the Asso-

EcoUs and the possibility to establish an efficient network in a reduced number of sites obtained through complementarity analysis. The identification of suitable areas for implementing genetic reserves is more effective when using this new approach. In addition, the selection of areas through this method facilitates higher probability of species' persistence, since it is not a species, but the whole plant community that is managed for conservation. Notwithstanding these positive results, more research is still needed to assess the validity of this approach in other groups of CWRs and further efforts should be made to improve the coverage of association inventories throughout the territory.

References

1. Heywood, V.; Casas, A.; Ford-Lloyd, B.; Kell, S.; Maxted, N. Conservation and sustainable use of crop wild relatives. *Agric. Ecosyst. Environ.* **2007**, *121*, 245–255.
2. Lidder, P.; Sonnino, A. *Biotechnologies for the Management of Genetic Resources for Food and Agriculture*, 1st ed.; In: *Advances in Genetics*, Elsevier Inc.: Amsterdam, The Netherlands, 2012; Volume 78, pp. 1–167. ISBN 9780123943941.
3. Esquinas-Alcázar, J. Science and society: Protecting crop genetic diversity for food security: Political, ethical and technical challenges. *Nat. Rev. Genet.* **2005**, *6*, 946–953.
4. Hawtin, G.; Iwanaga, M.; Hodgkin, T. Genetic resources in breeding for adaptation. *Euphytica* **1996**, *92*, 255–266.
5. Brozynska, M.; Furtado, A.; Henry, R.J. Genomics of crop wild relatives: Expanding the gene pool for crop improvement. *Plant Biotechnol. J.* **2016**, *14*, 1070–1085.
6. Dwivedi, S.L.; Crouch, J.H.; Mackill, D.J.; Xu, Y.; Blair, M.W.; Ragot, M.; Upadhyaya, H.D.; Ortiz, R. The Molecularization of Public Sector Crop Breeding: Progress, Problems, and Prospects. *Adv. Agron.* **2007**, *95*, 163–318.
7. Hajjar, R.; Hodgkin, T. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica* **2007**, *156*, 1–13.
8. Maxted, N. Conserving the genetic resources of crop wild relatives in European protected areas. *Biol. Conserv.* **2003**, *113*, 411–417.
9. Ford-Lloyd, B.V.; Schmidt, M.; Armstrong, S.J.; Barazani, O.; Engels, J.; Hadas, R.; Hammer, K.; Kell, S.P.; Kang, D.; Khoshbakht, K.; et al. Crop Wild Relatives—Undervalued, Underutilized and under Threat? *Bioscience* **2011**, *61*, 559–565.
10. MAGRAMA. Ministerio de Agricultura Alimentación y Medio Ambiente Estrategia Española de Conservación Vegetal 2014–2020. Principios y Orientaciones para la Conservación de la Diversidad Vegetal Silvestre en España; Ministerio de Agricultura, Alimentación y Medio Ambiente: Madrid, Spain., **2014**; 77 pp.
11. Labokas, J.; Maxted, N.; Kell, S.; Brehm, J.M.; Iriondo, J.M. Development of national crop wild relative conservation strategies in European countries. *Genet. Resour. Crop Evol.* **2018**, *65*, 1385–1403.
12. Fielder, H.; Hopkins, J.; Smith, C.; Kell, S.; Ford-Lloyd, B.; Al, E. UK wild species to underpin food security: Species selection, genetic reserves and targeted collection. *Crop Wild Relat.* **2012**, *8*, 24–27.
13. Pinheiro de Carvalho, M.Â.; Nóbrega, H.; Freitas, G.; Fontinha, S.; Frese, L. Towards the establishment of a genetic reserve for Beta patula Aiton. In *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*; Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M., Al, E., Eds.; CAB International: Wallingford, UK, 2012; pp. 36–44.
14. Iriondo, J.M.; Fielder, H.; Fitzgerald, H.; Kell, S.P.; Labokas, J.; Negri, V.; Phillips, J.; Teso, M.L.R.; Sensen, S.; Taylor, N.; et al. National Strategies for the Conservation of Crop Wild Relatives. In *Enhancing Crop Gene Pool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement*; Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Eds.; CAB International: Oxfordshire, UK, 2016; pp. 161–171.
15. Parra-Quijano, M.; Iriondo, J.M.; Torres, E. Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genet. Resour. Crop Evol.* **2012**, *59*, 205–217.
16. Maxted, N.; Kell, S.; Ford-Lloyd, B.; Dulloo, E.; Toledo, Á. Toward the systematic conservation of global crop wild relative diversity. *Crop Sci.* **2012**, *52*, 774–785.
17. Garcia, R.M.; Parra-Quijano, M.; Iriondo, J.M. Identification of ecogeographical gaps in the Spanish Aegilops collections with potential tolerance to drought and salinity. *PeerJ* **2017**, *5*, e3494.
18. Phillips, J.; Asdal, Å.; Magos Brehm, J.; Rasmussen, M.; Maxted, N. In situ and ex situ diversity analysis of priority crop wild relatives in Norway. *Divers. Distrib.* **2016**, *22*, 1112–1126.

19. Rubio Teso, M.L.; Iriondo, J.M.; Parra-Quijano, M.; Torres Lamas, E. National Strategy for the Conservation of Crop Wild Relatives of Spain. 2013. Available online: https://pgrsecure.bham.ac.uk/sites/default/files/documents/public/National_CWR_Conservation_Strategy_Spain.pdf (accessed on 10/09/2019).
20. Taylor, N.G.; Kell, S.P.; Holubec, V.; Parra-Quijano, M.; Chobot, K.; Maxted, N. A systematic conservation strategy for crop wild relatives in the Czech Republic. *Divers. Distrib.* **2017**, *23*, 448–462.
21. Braun-Blanquet, J. *Fitosociología. Bases Para el Estudio de Comunidades Vegetales*; Lalucat Jo, J., de Bolos Capdevilla, J.O., Eds.; H. Blume Ediciones: Madrid, Spain, 1979; ISBN 9781604138795.
22. Caro, T. Reserve Site Selection. In *Conservation by Proxy: Indicator, Umbrella, Keystone, Flagship and Other Surrogate Species*; Island Press: Whashington, DC, USA, 2010; pp. 61–98.
23. Rubio Teso, M.L.; Torres, E.; Parra-Quijano, M.; de la Rosa, L.; Fajardo, J.; Iriondo, J.M. National inventory and prioritization of crop wild relatives in Spain. *Genet. Resour. Crop Evol.* **2018**, *65*, 1237–1253.
24. Harlan, J.R.; de Wet, J.M.J. Toward a rational classification of cultivated plants. *Taxon* **1971**, *20*, 506–517.
25. Parra-Quijano, M.; Torres Lamas, E.; Iriondo Alegría, J.M.; López, F. Herramientas CAPFITOGEN. V. 2.0. Manual. In *Herramientas Capfitogen*; FAO: Rome, Italy, 2016; 292 pp.
26. Ketchen, D.J.; Shook, C.L. The Application of Cluster Analysis in Management Strategic Research: An Analysis and Critique. *Strateg. Manag. J.* **1996**, *17*, 441–458.
27. Scott, J.M.; Davis, F.; Csuti, B.; Noss, R.; Butterfield, B.; Anderson, H.; Caicco, S.; Erchia, F.D.; Edwards, T.C.; Ulliman, J. Gap analysis: A Geographic approach to protection of Biological Diversity. *Wildl. Monogr.* **1993**, *123*, 3–41.
28. Rebelo, A.G. Iterative selection procedures: Centres of endemism and optimal placement of reserves. In *Botanical Diversity in Southern Africa*; Huntley, B.J., Ed.; National Botanical Institute: Pretoria, South Africa, 1994; pp. 231–257.
29. Maxted, N.; Brehm, J.M.; Kell, S. *Resource Book for the Preparation of National Plans for Conservation of Crop Wild Relatives and Landraces*; FAO: Rome, Italy, 2013.
30. Turner, N.J.; Luczaj, L.J.; Migliorini, P.; Pieroni, A.; Dreon, A.L.; Sacchetti, L.E.; Paoletti, M.G. Edible and Tended Wild Plants, Traditional Ecological Knowledge and Agroecology. *CRC. Crit. Rev. Plant Sci.* **2011**, *30*, 198–225.
31. Dempewolf, H.; Baute, G.; Anderson, J.; Kilian, B.; Smith, C.; Guarino, L. Past and future use of wild relatives in crop breeding. *Crop Sci.* **2017**, *57*, 1070–1082.
32. Tubiello, F.N.; Soussana, J.-F.; Howden, S.M. Crop and pasture response to climate change. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 19686–19690.
33. Parra-Quijano, M.; Iriondo, J.M.; Torres, E. Improving representativeness of genebank collections through species distribution models, gap analysis and ecogeographical maps. *Biodivers. Conserv.* **2012**, *21*, 79–96.
34. Rubio Teso, M.L.; Kinoshita Kinoshita, K.; Iriondo, J.M. Optimized Site Selection for the In Situ Conservation of Forage CWRs: A Combination of Community- and Genetic-level Perspectives. In *Enhancing Crop GenePool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement*; Maxted, N., Dooloo, M.E., Ford-Lloyd, B.V., Eds.; CAB International: Oxfordshire, UK, 2016; pp. 199–205.
35. Biondi, E. Phytosociology today: Methodological and conceptual evolution. *Plant Biosyst.* **2011**, *145*, 19–29.
36. European Commission Council Directive 92/43/ECC. *Off. J. Eur. Union* **1992**, *94*, 40–52.
37. Trochet, A.; Schmeller, D.S. Effectiveness of the Natura 2000 network to cover threatened species. *Nat. Conserv.* **2013**, *4*, 35–53.
38. Dimitrakopoulos, P.G.; Memtsas, D.; Troumbis, A.Y. Questioning the effectiveness of the Natura 2000 species areas for conservation strategy: The case of Crete. *Glob. Ecol. Biogeogr.* **2004**, *13*, 199–207.
39. Maiorano, L.; Falcucci, A.; Garton, E.O.; Boitani, L. Contribution of the Natura 2000 network to biodiversity conservation in Italy. *Conserv. Biol.* **2007**, *21*, 1433–1444.
40. Rubio-Salcedo, M.; Martínez, I.; Carreño, F.; Escudero, A. Poor effectiveness of the Natura 2000 network protecting Mediterranean lichen species. *J. Nat. Conserv.* **2013**, *21*, 1–9.
41. D’Amen, M.; Bombi, P.; Campanaro, A.; Zapponi, L.; Bologna, M.A.; Mason, F. Protected areas and insect conservation: Questioning the effectiveness of natura 2000 network for saproxylic beetles in Italy. *Anim. Conserv.* **2013**, *16*, 370–378.
42. Faith, D.P.; Carter, G.; Cassis, G.; Ferrier, S.; Wilkie, L. Complementarity, biodiversity viability analysis, and policy-based algorithms for conservation. *Environ. Sci. Policy* **2003**, *6*, 311–328.
43. Heywood, V. Conservation strategies for species/populations occurring outside protected areas. In *Crop Wild Relatives: A Manual of in Situ Conservation*; Hunter, D., Heywood, V., Eds.; Earthscan: London, UK.; Washington, DC, USA, 2011; pp. 253–294.

44. Traba, J.; García De La Morena, E.L.; Morales, M.B.; Suárez, F. Determining high value areas for steppe birds in Spain: Hot spots, complementarity and the efficiency of protected areas. *Biodivers. Conserv.* **2007**, *16*, 3255–3275.
45. Marignani, M.; Blasi, C. Looking for important plant areas: Selection based on criteria, complementarity, or both? *Biodivers. Conserv.* **2012**, *21*, 1853–1864.
46. Vane-Wright, R.I.; Humphries, C.J.; Williams, P.H. What to protect? Systematics and the agony of choice. *Biol. Conserv.* **1991**, *55*, 235–254.
47. Maxted, N.; Dulloo, E.; Ford-Lloyd, B.V.; Iriondo, J.M.; Jarvis, A. Gap analysis: A tool for complementary genetic conservation assessment. *Divers. Distrib.* **2008**, *14*, 1018–1030.
48. Fielder, H.; Brotherton, P.; Hosking, J.; Hopkins, J.J.; Ford-Lloyd, B.; Maxted, N. Enhancing the Conservation of Crop Wild Relatives in England. *PLoS ONE* **2015**, *10*, e0130804.
49. Fitzgerald, H. The National Crop Wild Relative Strategy Report for Finland MTT: AgriFood Research Finland: Helsinki, Finland, 2013.
50. Phillips, J.; Kyrtzsis, A.; Christoudoulou, C.; Kell, S.; Maxted, N. Development of a national crop wild relative conservation strategy for Cyprus. *Genet. Resour. Crop Evol.* **2014**, *61*, 817–827.
51. Reyers, B.; van Jaarsveld, A.S.; Krüger, M. Complementarity as a biodiversity indicator strategy. *Proc. R. Soc. B Biol. Sci.* **2000**, *267*, 505–513.
52. Kati, V.; Devillers, P.; Dufrêne, M.; Legakis, A.; Vokou, D.; Lebrun, P. Hotspots, complementarity or representativeness? Designing optimal small-scale reserves for biodiversity conservation. *Biol. Conserv.* **2004**, *120*, 471–480.
53. Araújo, M.B.; Williams, P.H. The bias of complementarity hotspots toward marginal populations. *Conserv. Biol.* **2001**, *15*, 1710–1720.
54. Faith, D.P.; Walker, P.A. How do indicator groups provide information about the relative biodiversity of different sets of areas? On hotspots, complementarity and pattern-based approaches. *Biodivers. Lett.* **1996**, *3*, 18–25.
55. Araújo, M.B.; Williams, P.H. Selecting areas for species persistence using occurrence data. *Biol. Conserv.* **2000**, *96*, 331–345.
56. Iriondo, J.M.; Maxted, N.; Kell, S.P.; Ford-Lloyd, B.V.; Lara-Romero, C.; Labokas, J.; Magos Brehm, J. Quality standards for genetic reserve conservation of crop wild relatives. In *Agrobiodiversity Conservation. Securing the Diversity of Crop Wild Relatives and Landraces*; Maxted, N., Dulloo, E., Ford-Lloyd, B.V., Frese, L., Iriondo, J., Pinheiro de Carvalho, M.A.A., Eds.; CAB International: Oxfordshire, UK, 2012; pp. 72–77.

Supplementary Materials

The following are available online at: <https://github.com/MI.RubioTeso/In-situ-conservation-assessment-of-forage-and-fodder-CWR-in-Spain-using-phytosociological-associations>

Table S1: Table_S1_RubioTeso&Iriondo_2019_List_phytosociol_asso_Sustainability

Table S2: Table_S2_RubioTeso&Iriondo_2019_Phytosociol_asso_sel_Sustainability

Table S3: Table_S3_RubioTeso&Iriondo_2019_Inventories_ELC_freq_Sustainability

Table S4: Table_S4_RubioTeso&Iriondo_2019_Gap_analysis_Aso_Sustainability

Table S5: Table_S5_RubioTeso&Iriondo_2019_Gap_analysis_Aso-EcoU_Sustainability

Table S6: Table_S6_RubioTeso&Iriondo_2019_Coverage_analysis_Sustainability

Table S7: Table_S7_RubioTeso&Iriondo_2019_Complementarity_Aso-EcoU_Sustainability

CAPÍTULO 4:

Searching for Abiotic Tolerant and Biotic Stress Resistant Wild Lentils for Introgression Breeding Through Predictive Characterization

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Abstract

Crop wild relatives are species related to cultivated plants, whose populations have evolved in natural conditions and confer them valuable adaptive genetic diversity, that can be used in introgression breeding programs. Targeting four wild lentil taxa in Europe, we applied the predictive characterization approach through the filtering method to identify populations potentially tolerant to drought, salinity, and waterlogging. In parallel, the calibration method was applied to select wild populations potentially resistant to lentil rust and broomrape, using, respectively, 351 and 204 accessions evaluated for these diseases. An ecogeographic land characterization map was used to incorporate potential genetic diversity of adaptive value. We identified 13, 1, 21, and 30 populations potentially tolerant to drought, soil salinity, waterlogging, or resistance to rust, respectively. The models targeting broomrape resistance did not adjust well and thus, we were not able to select any population regarding this trait. The systematic use of predictive characterization techniques may boost the efficiency of introgression breeding programs by increasing the chances of collecting the most appropriate populations for the desired traits. However, these populations must still be experimentally tested to confirm the predictions.

Keywords: crop wild relatives, predictive characterization, ecogeographic land characterization maps, drought tolerance, salinity tolerance, waterlogging tolerance, rust resistance, broomrape resistance

Introduction

In the last century, the advances in plant breeding in search of the most productive and nutritional cultivars have allowed feeding millions of people (Khush, 2001). However, food security is menaced by the decrease in the diversity of crop species and the genetically uniform crop cultivars resulting from the breeding for higher yields (Esquinas-Alcázar, 2005; Khoury et al., 2014, 2021). Climate change is affecting crop production and food security, with different impacts depending on the area of the world and the economic status of the country (Rosenzweig and Parry, 1994; Challinor et al., 2009, 2014; Wheeler and von Braun, 2013; Rosenzweig et al., 2014). The reduction of genetic diversity in crops has made modern crop cultivars more vulnerable to biotic and abiotic stresses which are further aggravated by climate change (Heal et al., 2004; Massawe et al., 2016). Additionally, the development of adapted cultivars in many crops is constrained by this reduced genetic variation (Dempewolf et al., 2017). The adaptation of agriculture to climate change is imperative (Howden et al., 2007), and acquiring the traits to make crops tolerant to biotic or abiotic stresses is fundamental for food sustainability (Newton et al., 2011).

Crop wild relatives are plant species closely related to crops that can, relatively easily, transfer genetic material to them (Heywood et al., 2007). Because of their evolution under natural conditions with non-directed selective pressures, crop wild relatives are adapted to current environmental conditions and are, thus, a valuable source of genetic diversity of adaptive value and traits (Hawtin et al., 1996; Brozynska et al., 2016). Crop wild relatives have gained relevance for breeding in the last decades (Hajjar and Hodgkin, 2007; Jump et al., 2009) and their application as donors of useful traits is widely documented (e.g., Warschefsky et al., 2014; Choudhary et al., 2017). Nevertheless, their incorporation in breeding programs has been limited for several reasons. These include the potential incorporation of undesired traits during the breeding trials (Walley and Moore, 2015; Prohens et al., 2017 and references therein), the difficulty of generating favorable combinations of genes during the hybridization (Sano, 1993), and the impossibility of genetically characterizing every single wild population in the search for traits, being necessary to carefully select which accessions should be screened (Baute et al., 2015). However, given the great value of crop wild relatives as adaptive trait donors, their utilization is now considered a relevant pathway to incorporate genetic diversity (Guarino and Lobell, 2011; Greene and Warburton, 2017; Montenegro de Wit, 2017; Egan et al., 2018; Coyne et al., 2020; Kilian et al., 2020) and there have been considerable advances to overcome the above-mentioned limitations and enhance their use (Ford-Lloyd et al., 2011; Zhang et al., 2017; Zhang and Batley, 2020). Some of them include the creation of new elite materials from crop wild relatives, ready to be used for breeding through the “introgressiomics” approach (Prohens et al., 2017), the use of biotechnology and genomic tools (Baute et al., 2015; Walley and Moore, 2015;

Pratap et al., 2021) that make available a rapid gene targeting, or the speed breeding approach that has been successfully applied in different crops (Watson et al., 2018).

The use of ecogeographical and climatic data, together with ecological modeling is also arising as a promising tool, helping in the selection of wild germplasm or populations, both for its use in breeding and for prioritizing their conservation (Castañeda-Álvarez et al., 2016). Similarly, the Focused Identification of Germplasm Strategy (FIGS) (Mackay and Street, 2004) and predictive characterization techniques (Thormann et al., 2014a, 2016) have been developed to select subsets of landraces and crop wild relative populations with higher probabilities of containing the desired trait than if randomly selected. These techniques can be applied following two approaches: the “environmental filtering” method and the “calibration” method (Thormann et al., 2014a). Considering that the operation of different selective pressures will result in diverging genetic diversity of adaptive value, the environmental filtering method presumes that populations inhabiting certain areas with particular environmental characteristics will probably be better adapted to those conditions than other populations (e.g., populations inhabiting drier areas would be more tolerant to drought). The environmental filtering method does not require the previous characterization of the populations, just information about the environmental conditions existing in each population site, which can be obtained through environmental data available in global and national databases. This method involves the generation of ecogeographic land characterization maps (ParraQuijano et al., 2012b) that are used as a proxy to maximize the genetic diversity of the subset. Once the populations are ecogeographically and environmentally characterized, guided by literature or expert advice, environmental thresholds that may determine the presence of the desired trait are set, filtering in this way the subset of populations most likely to possess the trait (Thormann et al., 2014a). On the other hand, the calibration method, based on the same premises, needs a set of populations previously evaluated for a given trait (e.g., a set of populations with known resistance or sensitivity to a particular disease). These are used to train a model that will predict, in a given set of non-evaluated populations, those which most likely have the desired attribute. A recent example proving the usefulness of the calibration method is the successful prediction of the acyanogenic status of *Trifolium repens* L. populations after a worldwide screening (García Sánchez et al., 2019).

Cultivated lentil (*Lens culinaris* ssp. *culinaris* Medik.) was probably originated in the Fertile Crescent Area (South-Western Asia and the Mediterranean) (Smartt, 1984; Cokkizgin and Shtaya, 2013), during the Neolithic period, having as probable ancestor *L. culinaris* ssp. *orientalis* (Boiss.) Ponert (Chahota et al., 2019). Its cultivation and domestication probably date back to the late 5th or early 4th millennia BC and it has been described as probably being the most ancient crop among grain legumes (Sandhu and Singh, 2007). Lentils are commonly used in different cropping systems (Rawal and Bausal, 2019) and are traditionally used as a rotational crop which can increase their value as grain legumes if their potential as precrop cultivation is leveraged (Preissel et al., 2015). According to the latest FAO reports (FAO, 2020), lentil is cultivated in 44 countries, Jordan is the country with the highest yield (3,400 kg/ha calculated for 2019), and Canada is the country with the largest harvested area (close to 1.5 million hectares in 2019). In 2019, the harvested area in Europe was 137,161 hectares, which resulted in a total of about 125 thousand tons and a calculated yield of 909.6 kg/ha (FAO, 2020). Erskine (2009) reported an average production of 42 thousand tons per year for Europe in the period 2002–2006, which accounted for just 1.1% of global lentil production. However, in the last decade, there has been a large increase in lentil production (an average of 72.9 thousand tons per year in the 2010–2014 period and 173.25 thousand tons in 2015–2019) (FAO, 2020), which highlights a growing trend and increasing interest in its cultivation in Europe.

The lentil yield is affected by a series of biotic (rust, anthracnose, powdery mildew, sclerotinia stem rot or broomrape, among others) (Chen et al., 2009) and abiotic (drought, cold, salinity, or waterlogging, among others) stresses (Andrews and McKenzie, 2007; Kumar et al., 2014; Smýkal et al., 2015; Lake and Sadras, 2019). Biotic and abiotic stresses are affected by climate change and are likely to interact, which might cause higher damages to plants (Challinor et al., 2009). Additionally, the cultivated lentil is reported to contain a narrow genetic base (Fratini et al., 2004; Rawal and Bausal, 2019) and in the short history of breeding this crop, cultivated varieties or landraces have been, precisely, the main source of genetic variation for lentil crop improvement (Materne and McNeil, 2007).

Wild relatives of lentil possess valuable genes conferring resistance and tolerance to biotic and abiotic stresses (Gupta et al., 2011) and recent studies have reported promising results in the use of lentil wild relatives for breeding purposes (i.e., lentil rust, powdery mildew, or fusarium wilt resistance and drought and cold tolerance) (Mohar et al., 2020; Asghar et al., 2021). Other works point to the identification of novel genes or alleles involved in overcoming salinity tolerance and incorporating them into commercial lentils, as the path to increase productivity (Dissanayake et al., 2021), so the exploitation of genetic resources of lentil wild relatives gains special interest. The crossability among the different species of lentils has been widely studied, placing *Lens culinaris* ssp. *culinaris*, *L. culinaris* ssp. *orientalis* and *L. culinaris* ssp. *odemensis* (Ladiz.) into the primary gene pool, *L. nigricans* (M. Bieb.) Godr. and *L. ervoides* (Brign.) Grande into the secondary gene pool and *L. lamottei* Czefr. into the tertiary gene pool although in the latter, its placement in the secondary gene pool is under discussion [see Kumar et al. (2014) and references therein]. The crossability with species that are not in the primary gene pool is more difficult, but possible with the help of embryo rescue and hormone treatment (Fratini et al., 2004; Cubero et al., 2009; Tullu et al., 2013; Rawal and Bausal, 2019).

The aim of our study was to screen the variability of natural populations of the crop wild relatives of lentil and to select small subsets of populations that are more likely to contain, under different ecological contexts and considering potential genetic diversity, adaptations to drought, high soil salinity, waterlogging and resistance to rust [*Uromyces vicia-fabae* (Pers.) Schröt] and broomrape [*Orobanche crenata* Forsk.], some of the most important abiotic and biotic factors affecting lentil production. These subsets could then be evaluated for the corresponding traits and used in trait introgression breeding. We posed that it is possible to select populations potentially tolerant or resistant to the targeted traits, based on the ecological range of their distribution. Furthermore, we also posed that the ecogeographic information associated with each population will serve as explanatory variables to train models that successfully project the resistance to lentil rust and broomrape in nonevaluated populations. Because this study was developed under the framework of the Farmer's Pride project, a European H2020 project focused on the establishment of a network of genetic reserves of crop wild relatives and landraces in Europe and Turkey, the geographic scope of the study was delimited to these areas.

Material and Methods

Study taxa

Lens Mill. genus belongs to the Fabaceae family and contains four species and four subspecies according to the more recent accepted taxonomy (Ferguson et al., 2000). All of them are annual herbs (Castroviejo and Pascual, 1999) and are naturally distributed in the Mediterranean European countries (Euro+Med Plant Database, 2006). The four species are self-pollinating and diploid ($2n = 14$), with similar karyotypes (Ladizinsky, 1979; Erskine et al., 2016; Nair, 2019) although with potentially higher karyotypic variation than expected (Cubero et al., 2009).

We considered in our study the three species and the two subspecies of *Lens* naturally occurring in Europe and Turkey. These are *L. ervoides*, *L. nigricans*, and *L. lamottei*, as well as *L. culinaris* ssp. *orientalis* and *L. culinaris* ssp. *odemensis*.

Distribution data

Lens taxa distribution data were extracted from a highquality georeferencing occurrence database of crop wild relative populations in Europe and Turkey generated for the Farmer's Pride project¹. This database contains more than 3 million records for 616 prioritized taxa in Europe and Turkey and is the largest database of crop wild relatives built up to date (Rubio Teso et al., 2020). The generation of such database involved the downloading of records of targeted taxa from GBIF (GBIF.org, 2021) and Genesys2 repositories, using the packages *rgbif* (Chamberlain and Boettiger, 2017) and *genesysr* (Obreza, 2019) in the R environment (R Core Team, 2020). The raw data downloaded from these sources were further cleaned and filtered eliminating geographically non-accurate records, those dated before 1950, removing duplicates and records falling in urban areas, water bodies, or permanent ice or snow according to the ESA CCI Land Cover Project (2017). Finally, records of the same taxa found within a 1 km radius were also removed, assuming they belonged to the same

population. Further information and details about the generation and characteristics of this database can be found in Rubio Teso et al. (2020).

Generation of an Ecogeographic Land Characterization Map

Ecogeographic land characterization maps (ELC maps) are useful tools to represent potential adaptive scenarios, built on the combination of different bioclimatic, edaphic, and geophysics variables characterizing a territory (Parra-Quijano et al., 2012a). In this study, we generated an ELC map for Europe and Turkey based on the occurrence of *Lens* sp. populations, as a proxy of the different adaptive scenarios to which *Lens* populations may be subjected. The different ecogeographic categories obtained with the ELC map were used as an additional criterion in the selection of populations potentially adapted to the abiotic stresses. By maximizing the number of different ecogeographic categories in the selected populations, the background genetic diversity of the subset is likely to be increased, as well as the probability of gathering cases of convergent selection of the same trait through different evolutionary pathways.

The selection of variables explaining lentil taxa distribution, and thus of importance for the generation of the ELC map, was carried out using a modified R script developed for the SelecVar tool of CAPFITOGEN3 (Parra-Quijano, 2020). The analysis included ecogeographic variables with available data classified in three components: 65 bioclimatic variables, 35 edaphic variables, and 18 geophysics variables, and also included latitude and longitude, all available in CAPFITOGEN3 local mode (Parra-Quijano, 2020) (Supplementary Material 1). Variables' data were extracted at 2.5 arc-min resolution (around 5 km × 5 km) for each population, according to their geographical coordinates. The R script extracts the ecogeographic variables from the occurrence sites and assesses the importance of each variable in explaining the distribution of the study populations (Parra-Quijano et al., 2020). It estimates variable importance according to the Random Forest Classification (RF) machine learning algorithm and detects redundant variables through bivariate correlation analysis. RF categorizes variable importance according to their higher mean decrease accuracy (MDA) values (Cutler et al., 2007). The top fifteen bioclimatic and edaphic and geophysics variables were ordered according to their MDA value and correlated variables within the same group were removed (Pearson correlation coefficient > | 0.5 | and $p < 0.05$) following Garcia et al. (2017).

The selected variables were then used for the generation of an ELC map following a modified R script of the ELCmapas tool of CAPFITOGEN3 (Parra-Quijano, 2020). The map resolution was 2.5 arc-min. The “elbow” method was chosen for the determination of the optimal number of the ecogeographic clustering, allowing a maximum of six clusters per group of variables. This method, which is recommended for large territories (Parra-Quijano et al., 2020), determines the cutoff points based on the decrease in the sum of the intragroup squares (Ketchen and Shook, 1996). It reaches the optimal number of groups when the decrease in the sum of squares in a range of n and $n + 1$ group is less than 50% (Parra-Quijano et al., 2020). Using the ELC map, ecogeographic categories were extracted for each population using a modified R script of the Representa tool of CAPFITOGEN3 (Parra-Quijano, 2020) and incorporated into the master table of population occurrences along with the bioclimatic, edaphic, and geophysics information. All the analyses regarding the generation of ELC maps and subsequent data management were performed using the R 3.6.3 version of the R environment (R Core Team, 2020) and scripts downloaded and adapted from CAPFITOGEN3 website (<https://drive.google.com/file/d/19ppFLclsF05tptsKjK8eILlJcXnlH0qc/view>).

Predictive Characterization

Targeted abiotic stresses were drought, salinity, and waterlogging. The search for their tolerance in wild relative populations of lentils was performed using the environmental filtering method (Thormann et al., 2014a,b). Targeted biotic stresses were lentil rust (*Uromyces vicia-fabae*) and broomrape (*Orobancha crenata*). Resistance to both diseases, potentially found in wild populations, was modeled using the calibration method (Thormann et al., 2014a,b).

Calculation of Aridity Indexes, Soil Textures, and Delimitation of Saline and Non-saline Soils

The De Martonne aridity index (De Martonne, 1926) was obtained for each record as a proxy to estimate the drought stress experienced by the targeted populations. De Martonne aridity index (*IarDM*) was calculated as follows:

$$IarDM = \frac{P}{T + 10}$$

where “P” is the Annual Mean Precipitation, “T” the Annual Mean Temperature, and 10 is a constant to avoid negative values. Drought stress during the flowering season is reported to severely affect plant development (Kazan and Lyons, 2016). Thus, a De Martonne Aridity Index adapted to the flowering season (*IarDMf*) was generated by calculating the mean of the monthly De Martonne Aridity Index (*IarDMm*) for the flowering period (March, April, May, and June). The monthly De Martonne Aridity Index (*IarDMm*) and the Flowering season De Martonne index (*IarDMf*) were calculated for each population with the following expressions:

$$rDMm = 12 \frac{P}{Tm + 10}$$

$$IarDMf = \frac{IarDMmarch + IarDMapril + IarDMmay + IarDMjune}{4}$$

Calculated aridity indexes were included in the master table (Table 1).

Superficial soil contents in clay, silt, and sand of population occurrence sites, obtained from the Harmonized World Soil Database (Fischer et al., 2008), through the CAPFITOGEN3 tools (Parra-Quijano, 2020), were used to categorize soil texture into Clay, Silty-Clay, Sandy-Clay, and Silty-Clay-Loam or Other (Table 1) using the Soil Texture Calculator (USDA, 2020). The resulting categories were then added to the master data table.

Regarding soil salinity, sites were classified according to soil conductivity, into five categories, from non-saline to very strongly saline soils (Abrol et al., 1988; Table 1).

Table 1: Classification of areas according to De Martonne Aridity index (De Martonne, 1926). Classification of soil textures according to their content in clay, silt and sand (USDA, 2020). Classification of soils according to the conductivity of the saturation extract (dS/m) and their effects of crops (Abrol et al., 1988)

De Martonne classification – Aridity	De Martonne Index Value	Classification
	$0 \leq \text{IarDM} < 5$	Deserts. Extremely arid
	$5 \leq \text{IarDM} < 10$	Semi-desert. Arid
	$10 \leq \text{IarDM} < 20$	Drought Mediterranean countries. Semi-arid
	$20 \leq \text{IarDM} < 30$	Sub-humid
	$30 \leq \text{IarDM} < 60$	Humid
	$\text{IarDM} \geq 60$	Per-humid
Soil texture classification	Superficial soil content combinations	Soil Texture
	If Clay $\geq 40\%$, Sand $\leq 45\%$ and Silt $< 40\%$	Clay
	If Clay $\geq 40\%$ and Silt $\geq 40\%$	Silty Clay
	If Clay $\geq 35\%$ and Sand $> 45\%$	Sandy Clay
	If Clay $\geq 27\%$ and $< 40\%$ and Sand ≤ 20	Silty Clay Loam
	Different combinations	Other
Soil salinity classification (based on soil conductivity)	Conductivity (dS/m)	Type of soil and effect on crop plants
	0 – 2	Non saline: Salinity effects negligible
	2 – 4	Slightly saline: Yields of sensitive crops may be restricted
	4 – 8	Moderately saline: Yields of many crops are restricted
	8 – 16	Strongly saline: Only tolerant crops yield satisfactorily
	> 16	Very strongly saline: Only a few very tolerant crops yield satisfactorily

Environmental Filtering Method—Abiotic Stress Analyses

Populations more likely to be tolerant to drought, soil salinity, and waterlogging were selected using an R script adapted from Van Etten et al. (2011) (Supplementary Material 2). Ecogeographic categories of the ELC map of Europe and Turkey were taken into account so they were proportionally represented in the selected subset. The purpose was to generate three subsets with a maximum of 30 populations per trait, to be proposed for seed collecting and subsequent evaluation for the target traits in characterization and evaluation trials.

Drought Tolerance

Populations with an Annual De Martonne Aridity (IarDM) below 15 (mid-low semi-arid areas) were selected as a first subset. This initial selection contained less than 30 populations. From this subset, those populations with Flowering De Martonne Aridity indices (IarDMf) below 15 were selected and ranked according to IarDMf, from most to least arid conditions during the flowering season.

Salinity Tolerance

Since most crops reduce their yields under saline conditions (soil conductivity above 4 dS/m) (Panta et al., 2014; Zörb et al., 2018), a first subset targeted moderately saline soils or higher (i.e., soil conductivity above 4 dS/m). However, none of the populations of our dataset occurred under conditions that matched this criterion. Thus, a second subset was generated that selected populations occurring in slightly saline soils (soil conductivity $> 2 \text{ dS/m} < 4 \text{ dS/M}$).

Waterlogging Resistance

A first subset was generated by selecting populations inhabiting areas where limited water drainage was expected according to their soil texture (Clay, Silty Clay, Sandy Clay, or Silty Clay Loam). Subsequently, from this subset, a second selection was performed, identifying populations occurring in mid-high subhumid areas or more humid, according to their Annual De Martonne Aridity Indices.

Calibration Method—Biotic Stress Analyses

To train the models a database with evaluation information on resistance to lentil rust and broomrape for *Lens* sp. accessions was used. The database contained 419 georeferenced populations distributed worldwide that were evaluated using the Disease Severity Rating (DSr), 351 of them assessed for resistance to lentil rust (mean of 4 years' field trials) (Supplementary Material 3) and 204 for resistance to broomrape (mean of 3 years' field trials) (Rubiales, unpublished results). A basic description of the contents of the database is shown in Supplementary Material 4.

The binarization of levels of expression of both traits from quantitative values in a continuous range (DSr, 0–100) into qualitative values (resistant, sensitive), was approached by classifying as resistant the accessions with the lowest DSr values, i.e., those located in the first decile of the distribution. This criterion was chosen to ensure maximum levels of resistance in the predicted subset. The binarized levels of expression (0 = susceptible; 1 = resistant) were used as the dependent variables. The same ecogeographical variables that were previously used for the generation of the ELC map were used as explanatory variables.

The calibration process was performed using a modified R script developed for the Modela tool of CAPFITOGEN3 (ParraQuijano, 2020), based on the Biomod2 package (Thuiller et al., 2020). In this case, the R 3.1.2 version of the R environment was used, as recommended by Capfitogen developers (Parra-Quijano pers. comm.). Accessions classified as resistant to lentil rust or broomrape were used as “presence” data and sensitive accessions as “absence” data. Presences and absences were given the same weight independently of their number, the original balance of presences/absences was kept and 100% of the presence data was used to obtain the models. The True Statistic Skill value (TSS) (Allouche et al., 2006) was used to test the performance of nine algorithms: GLM—Generalized Linear Models (Nelder and Wedderburn, 1972), GAM—Generalized Additive Models (Hastie and Tibshirani, 1986), GBM—Generalized Boosting Models (Friedman, 2001, 2002), CTA—Classification Tree Analysis (Breiman et al., 1984), Artificial Neural Networks—ANN (Ripley, 1996), Flexible Discriminant Analysis—FDA (Hastie et al., 1994), Multivariate Adaptive Regression Splines—MARS (Friedman, 1991), Random Forest—RF (Breiman, 2001) and Surface Range Envelopes—SRE [similar to Bioclim (Busby, 1991)]. TSS is currently one of the most widely used evaluators for model performance, as it is not affected by the prevalence of the data (the proportion of sites in which the species is recorded, in our case described as resistant) (Allouche et al., 2006).

One hundred runs (models) were performed per each algorithm, using 75% of the data as “training” data—used to calibrate the models—, and 25% of the data as “testing” data, that is to evaluate each model. Variable importance for the models for each algorithm was determined with 100 permutations and ranked on a 0–1 scale (0: no influence in the model; 1: total influence in the model). Subsequently, the mean values of TSS of the 100 runs for each algorithm were calculated and the three algorithms with the highest mean TSS values were compared. Based on the index provided by Thuiller et al. (2010), the performances of the models according to the TSS values were classified into five categories: Fail or null ($0 > \text{TSS} < 0.2$), Poor ($0.2 > \text{TSS} < 0.4$), Fair ($0.4 > \text{TSS} < 0.6$), Good ($0.6 > \text{TSS} < 0.8$) and Excellent or High ($0.8 > \text{TSS} < 1$). From the selected algorithm, the best model (i.e., the run with the highest TSS) was chosen as the predictor model and

projected on the non-evaluated populations. Targeted wild populations were then classified as resistant or sensitive according to the cutoff suitability value given by the selected model. Populations with suitability values (ranked from 0 to 1,000) lower than the cutoff value of the model were classified as sensitive and those with suitability values higher than the cutoff value as resistant. From the latter and aiming to provide a manageable set of populations for breeding purposes, the 30 populations with the highest suitability values were selected as the subset with the highest probabilities of being resistant to lentil rust or broomrape.

Results

Distribution data

The subset with *Lens* wild relatives extracted from the database generated in the Farmer's Pride project contained 624 populations (Supplementary Material 5). From these, 105 were obtained from the Genesys database, which means that additionally to its probable in situ occurrence, are also ex situ preserved in gene banks. These entries are identified by Farmer's Pride Identifier codes starting with "GE". Populations belong to four different taxa and are distributed in 14 countries covering almost all the European countries in the Mediterranean Basin (Figure 1). The taxon with the highest number of records is *L. nigricans* with 443 populations found in 12 countries, followed by *L. ervoides* which has 145 populations in nine countries. *L. lamottei* and *L. culinaris* ssp. *orientalis* have 29 and 7 populations, respectively, found in three countries each. The countries with the highest number of populations recorded are Greece (173), France (165), and Spain (128).

The subspecies *L. culinaris* ssp. *odemensis* was not included in the analysis due to a lack of high-quality occurrence data.

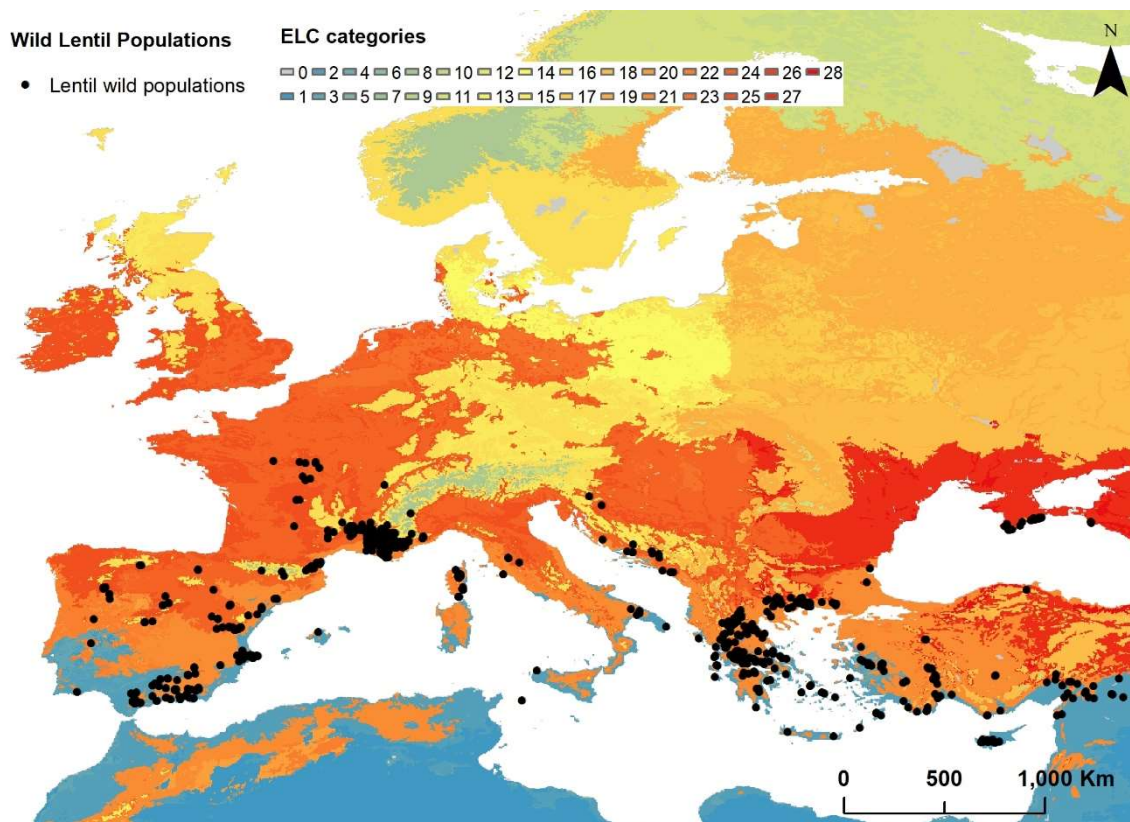


Figure 1: Ecogeographic land characterization map of the wild relatives of lentils in Europe and Turkey (2.5 arc-min resolution) and distribution of wild relatives of lentils in Europe

Ecogeographic analyses

Selection of Variables for the Ecogeographic Land Characterization Map

The 15 variables per component with the highest MDA values (Supplementary Material 6) were checked for high values of correlation between each other and, as a result, reduced to eight variables. Bivariate correlations for each component are shown in Supplementary Material 7. Hence, in the bioclimatic component, all variables were highly correlated to the one with the highest MDA values (annual mean temperature). In the edaphic component, four non-correlated variables were selected: bulk density (fine earth) of topsoil, topsoil available soil water capacity until a wilting point, topsoil total exchangeable bases, and topsoil sand fraction. Finally, in the geophysics component, three non-correlated variables were selected: annual solar radiation, December solar radiation and longitude.

Generation of Ecogeographic Land Characterization Map

The eight selected ecogeographic variables were used for the generation of the ELC map. ELC categories classified as “0” or “NA” were not considered for subsequent analyses, due to the lack of ecogeographic information. The resulting ELC map (Figure 1) has 28 different ecogeographic categories or units, grouped into four bioclimatic, three edaphic, and three geophysics clusters. Variable values per each ecogeographic category are shown in Supplementary Material 8.

Ecogeographic Characterization of the Targeted Populations

The extraction of bioclimatic, edaphic, and geophysics variables information assigned values to 619 of the 624 populations under analysis. *L. nigricans* had five populations classified into categories with no data (“0” or “NA”) due to the lack of ecogeographic data in their locations and thus were excluded from subsequent analyses. Populations of wild lentils were distributed in 13 of the 28 categories of the ELC map. *L. nigricans* was the species represented with the highest number of ELC categories (12), followed by *L. ervoides* (9). *L. lamottei* and *L. culinaris* ssp. *orientalis* populations were distributed across three ELC categories (Table 2).

Seventy-eight populations were classified as semi-arid according to the Annual De Martonne Aridity Index, but none were classified as arid or extremely arid. *L. culinaris* ssp. *orientalis* did not have any populations classified as semi-arid. According to the Flowering De Martonne Aridity Index, 82 populations were classified as semi-arid, and, again, no populations were found in drier areas. *L. culinaris* ssp. *orientalis* did not have any population in this category either. Concerning soil texture, 69 were classified in Clay soils, whereas no populations were classified with Silty Clay, Sandy Clay, or Silty Clay Loam textures (Table 2).

Table 2: Ecogeographic information of the targeted populations. Number of populations per taxon, number of different ecogeographic categories in which these populations are found, number of populations classified as semi-arid according to Annual (I_{arDM}) or Flowering (I_{arDM_f}) De Martonne Index values and number of populations in clayey soils. *Totals for No. ELC cat. are the different ELC categories in which the overall populations are found.

	No. pop.	No. ELC cat.	No. semi-arid pop (I_{arDM})	No. semi-arid pop. (I_{arDM_f})	No. pop. soil with clay texture
<i>L. culinaris</i> subsp. <i>orientalis</i>	7	3	0	0	4
<i>L. ervoides</i>	145	9	12	32	17
<i>L. lamottei</i>	29	3	12	10	1
<i>L. nigricans</i>	438	12	54	40	47
TOTALS	619	13*	78	82	69

Predictive characterization

We obtained three subsets of lentil wild relative populations that may contain adaptations to tolerate the targeted abiotic stresses and one subset potentially resistant to lentil rust. A complete database with all the ecogeographical information and the results on predictive characterization for each population is found in Supplementary Material 5.

Environmental Filtering Method—Abiotic Stress Analyses

Drought Tolerance

Thirteen populations belonging to three taxa (*L. nigricans*, *L. lamottei*, and *L. ervoides*) and distributed in Greece (3), Spain (9), and Turkey (1) were selected as potentially tolerant to drought (IarDM and IarDMf below 15). Only one ELC category (ELC category 2) is represented in this subset. Figure 2 shows the location of these populations and Supplementary Material 9 provides complete identification and location details of the populations along with the aridity indices values.

Salinity Tolerance

No populations were found to occur in moderately saline (4–8 dS/m soil conductivity) or more saline soils. One population (*L. lamottei*) is in slightly saline soil (soil conductivity above 2 and below 4 dS/m). This population is found in France (Figure 2) and is assigned to ELC category 21. Detailed information on the identification and location of the population can be found in Supplementary Material 9.

Waterlogging Tolerance

From the targeted soil texture categories (Clay, Silty Clay, Sandy Clay, or Silty Clay Loam), we only found wild lentil populations in clayey soils (69 populations). Subsequent criterion (IarDM > 25, mid-high subhumid areas or more humid) narrowed the selection to 21 populations. These populations belonged to the four taxa, are found in France (1), Greece (2), Spain (6), Turkey (4), and Ukraine (8) (Figure 2), and belong to four different ELC categories. Further details on the location of these populations, Annual De Martonne Aridity Indices, and ELC categories are shown in Supplementary Material 9.

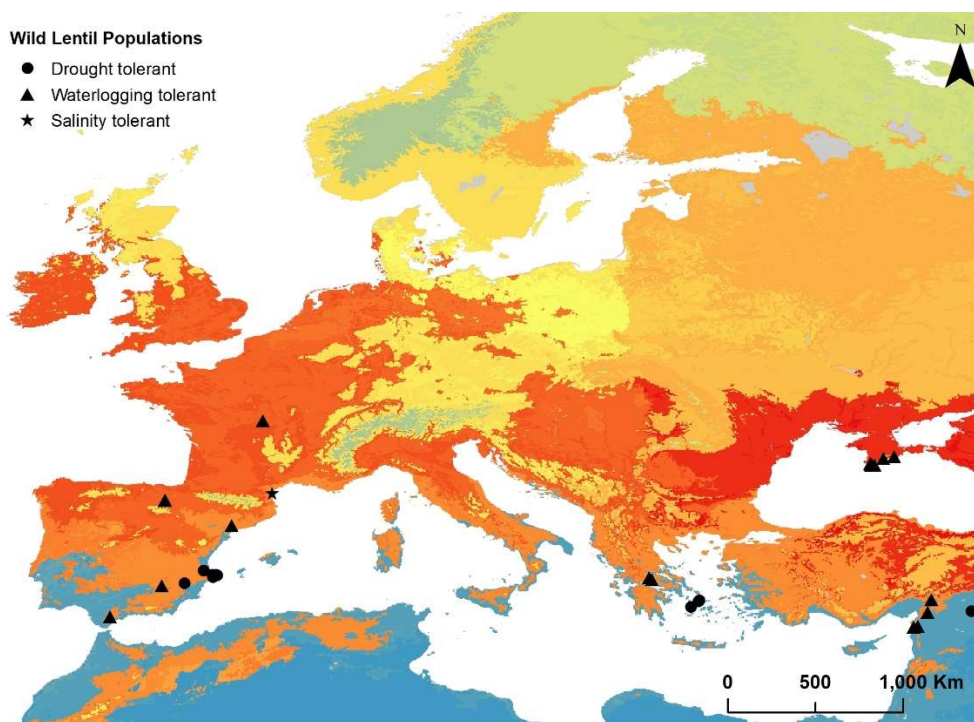


Figure 2: Location of populations of wild relatives of lentils in Europe and Turkey potentially tolerant to drought (black circles), to salinity (black star), and to waterlogging (black triangle). All populations were selected through the environmental filtering method of the predictive characterization technique. Locations are depicted on an ecogeographic land characterization map generated for these taxa to illustrate the potentially different adaptation landscapes.

The database with evaluation data for resistance to lentil rust contained 351 accessions whose DSr ranged from 0 (totally resistant) to 94.4 (almost totally sensitive). The binarization of data following the first decile criterion selected 33 accessions as resistant, with DSr values lower than 25. The rest of them were classified as sensitive (318).

Average performances of all models in the algorithms ranged from Null (SRE) to Fair (MARS, FDA, CTA, GLM, RF, and GBM) (Supplementary Material 10). From the algorithms classified as “fair”, the best three were GBM, RF, and GLM with very similar mean TSS values (0.588, 0.58, and 0.54, respectively). Thus, variables’ contribution to the model was assessed (Table 3). All the variables contributed to the best GBM algorithm (TSS value 0.828) giving the higher importance to longitude as an explanatory variable (0.168), followed by the topsoil water capacity until wilting point (0.09) and annual solar radiation (0.086). The random forest best model (TSS value 0.828) gave similar importance to the variables. Finally, the GLM best model (TSS value 0.879) only selected the longitude (0.836) and the topsoil water capacity (0.163) as contributors to the model. As a model-based essentially on longitude is not biologically meaningful from a mechanistic perspective, we excluded this option, and based on the similar variable contribution found in GBM and RF, these two approaches were considered. Subsequently, the highest TSS value criterion was used and the best model of the GBM algorithm was selected as a predictor and projected.

Table 3: Variable importance given by the best run of the selected algorithms modeling the resistance of wild lentils to rust. The algorithm selected for projection in bold.

Variable	Model		
	GBM	Random Forest	GLM
Longitude	0.168	0.152	0.836
Topsoil available soil water capacity until wilting point	0.09	0.073	0.163
Annual solar radiation	0.086	0.1	0
Annual Mean Temperature	0.037	0.042	0
Solar radiation December	0.03	0.049	0
Bulk density (fine earth) of topsoil	0.024	0.1	0
Topsoil total exchangeable bases	0.02	0.017	0
Topsoil sand fraction	0.011	0.018	0

A cutoff suitability value of 106 was obtained for the best model of the GBM algorithm. When this model was projected to the non-evaluated wild populations it identified 529 populations as potentially resistant to lentil rust (Figure 3). From these, the first 30 with the highest suitability values (248–295) were selected to constitute the subset for screening for lentil rust resistance.

These 30 populations belong to *L. ervoides* (21 populations) and *L. nigricans* (9 populations) and are distributed in six countries: Bosnia and Herzegovina (3) Croatia (4), Cyprus (6) Greece (6), Montenegro (1), and Turkey (10) (Figure 3 and Table 4). They represent five of the 13 ELC categories in which the taxa are distributed, although more than a half of them (17 populations) occur in ELC category 21. Detailed information on these populations can be found in Supplementary Material 11.

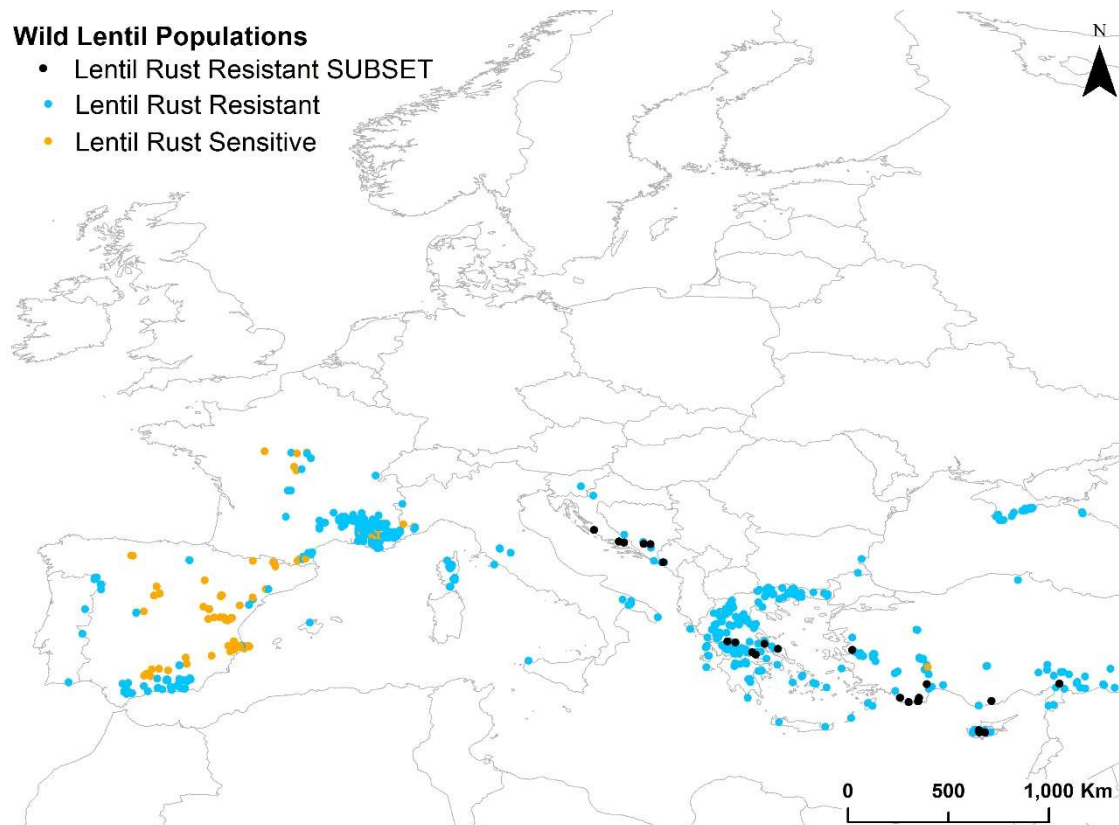


Figure 3: Location of projected lentil rust sensitive (orange dots) and resistant (blue dots) wild lentil populations. The subset of selected lentil rust-resistant (black dots) wild lentil populations

Table 4: Countries and taxa corresponding to the subset of 30 populations of wild relatives of lentils with higher probabilities of showing resistance to lentil rust. Number of populations per taxa and number of Ecogeographic Land Characterization (ELC) categories in which they are distributed.

Country	Taxon	No. populations	ELC categories
Bosnia and Herzegovina	<i>L. ervoides</i>	2	24, 25
	<i>L. nigricans</i>	1	25
Croatia	<i>L. ervoides</i>	2	21, 24
	<i>L. nigricans</i>	2	21, 24
Cyprus	<i>L. ervoides</i>	4	21
	<i>L. nigricans</i>	2	21
Greece	<i>L. ervoides</i>	3	21, 22
	<i>L. nigricans</i>	3	21, 22
Montenegro	<i>L. ervoides</i>	1	24
Turkey	<i>L. ervoides</i>	9	2, 21
	<i>L. nigricans</i>	1	21

All algorithms assayed to model broomrape resistance resulted in a very poor performance (All TSS values < 0.2). Cutoff values different from the ones initially set to define resistant individuals (first decile with the lowest DSr values) were also tested (first 17, 35, and 50% of the data with the lowest DSr values), but they did not improve the fit of the models. Consequently, no projections were made for broomrape resistance.

Targeted Trait Overlapping in Selected Subset Populations

None of the populations were simultaneously selected in the four subsets. However, there are some populations' coincidences between the biotic and abiotic selections. Four populations selected in the drought-tolerant subset were also classified as potentially resistant to lentil rust: three populations of *L. nigricans* in Greece (identifiers in the occurrence database generated for the Farmer's Pride project: ID_6954734, ID_6954730, and ID_6954729) and one population of *L. ervoides* in Turkey (ID_6953173). In addition, the population of *L. lamottei* selected in France as potentially tolerant to salinity was also classified as potentially resistant to lentil rust (ID_6953872). Finally, 19 populations belonging to the waterlogging tolerant subset were also classified as potentially resistant to lentil rust: two populations of *L. culinaris* ssp. *orientalis*, one in Ukraine (ID_6952604) and one in Greece (ID_6952438); nine populations of *L. ervoides*—one in Greece (ID_6953356), four in Ukraine (ID_6953026, ID_6953338, ID_6953339, ID_6953347) and four in Turkey (GE_222085, GE_222062, GE_222075, GE_222086); one population of *L. lamottei* in Spain (ID_6953903) and finally seven populations of *L. nigricans*, three of them in Spain (ID_6954642, ID_6954569, ID_6954618), one in France (ID_6954460) and three in Ukraine (ID_6954246, ID_6954619, ID_6954710). It is worth mentioning that the four *L. ervoides* populations in the Turkey subset as waterlogging tolerant and classified as potentially resistant to lentil rust, are not only potentially accessible to users in their *in situ* locations— according to our high-quality database—but also have stored accessions in gene banks.

Supplementary Material 5 provides the values obtained for each trait in each population and the target trait overlapping in the indicated populations.

Discussion

In this study we identified four subsets of populations of wild relatives of lentils, targeting different abiotic and biotic stresses affecting cultivated lentils. These subsets are more likely to be tolerant to drought, salinity, waterlogging, or resistant to lentil rust than randomly chosen populations. However, the modeling algorithms used were not able to satisfactorily identify a subset of populations more likely to be resistant to broomrape. We discuss below the benefits and limitations of our approach, providing arguments that support the use of predictive characterization.

Ecogeographic Analyses

The variables selected through the objective (non-directed) analyses (annual mean temperature, bulk density, water capacity until wilting point, exchangeable bases, topsoil sand fraction, annual solar radiation, December solar radiation and longitude) make sense when interpreted in the light of the ecology of *Lens* species and their origin. Lentil is reported to be sensitive to acid soils and waterlogging if compared to other grain legumes (Andrews and McKenzie, 2007) and also drought and heat are constraints to obtaining high lentil yields (Rawal and Bausal, 2019). Wild species usually prefer alkaline, stony, or sandy soils (Castroviejo and Pascual, 1999) and thus, the annual mean temperature, the presence of exchangeable bases, the bulk density, sand fraction agree as explanatory variables for their distribution. Furthermore, as in most cultivated plants, solar radiation is known to be a key element in lentil yield in association with other factors (e.g., sowing time, rainfall) (Andrews and McKenzie, 2007). Thus, the plant efficiency in the interception of solar radiation has been shown to be positively related to biomass and yield in lentils (Habib et al., 2021). Finally, the probable origin of lentils in the Fertile Crescent and the Mediterranean (Smartt, 1984; Cokkizgin and Shtaya, 2013) and the East-West distribution of the wild species in the Mediterranean (Ladizinsky, 1979; Ladizinsky et al., 1983, 1984 and references therein), support the selection of the longitude as an explanatory variable.

Ecogeographic approaches are considered a resource to select different populations with potentially useful traits (Hodgkin and Hajjar, 2008), based on the assumption that different environmental conditions will

differently shape the genetic diversity and will provide potential adaptations to populations inhabiting distinct environmental sites (Parra-Quijano et al., 2012b). The generated ELC map considered the most important variables for lentil wild relative distribution and covered the whole geographic scope of the project (Europe and Turkey). Given that wild lentil populations are not naturally distributed through the whole range of this territory, finding them in 13 of the 28 ecogeographical categories denotes a wide ecological range of these species. Such wide ELC representation across the wild lentil populations may entail a broad genetic diversity of adaptive value that can boost the potential benefits of using predictive characterization techniques. Other studies using ecogeographic approaches, such as the one by Mezghani et al. (2019) with wild relatives of carrots in Tunisia, also found a great diversification in the ecogeographic distribution of the populations within and across taxa, which they linked to traits of potential interest for breeding. In the same line, Kantar et al. (2015) also reported the presence of *Helianthus* wild species in diverse ecogeographic clusters, finding many of them in extreme environments that allow direct efforts in the selection of populations for abiotic stress tolerance breeding.

Environmental Filtering Method—Abiotic Stress Analyses

The relatively low number of populations found in semi-arid sites during the flowering period agrees with the literature that highlights that many legume species, including lentils, are severely affected by drought during this period (Andrews and McKenzie, 2007). The occurrence of lentil wild relative populations across a relatively wide range of water availability conditions (from semi-arid to almost sub-humid) suggests that populations living in the most arid conditions may have experienced adaptations to drought. The final subset of 13 populations, obtained by further filtering those simultaneously occurring in sites with De Martonne Annual and Flowering Indexes below 15, provides a more manageable fraction of populations to be handled by breeders, who could select from these those better fitting their particular pre-breeding programs.

Hamdi and Erskine (1996) consider that drought tolerance may not always be related to populations inhabiting arid sites and advocate field trials to confirm the tolerance. This implies that drought-tolerant lentils may be present in other environments and express this trait through phenotypic plasticity responses. These considerations may be sound but do not undermine the fact that drought tolerance is likely to be found, as a result of natural selection, in sites under high water limitation. Consequently, we think our results are trustworthy in the sense that the probability of finding drought tolerance in the given subset is likely to be significantly higher than if the same number of populations were randomly selected from the distribution area. The likelihood of identifying drought-tolerant populations through this approach is further supported by the fact that wild lentil species have shown a wider genetic diversity associated with drought tolerance than cultivated varieties, especially in *L. ervoides* (Singh et al., 2016) and *L. nigricans* (Coyne et al., 2020), and that experimental testing of drought tolerance of wild and cultivated peas found some wild pea accessions better adapted to drought than cultivated pea, as well as ecogeographical patterns associated to aridity gradients (Naim-feil et al., 2017). Additionally, lentils have other strategies to overcome drought, such as drought escape (advance or delay of the flowering time) (Shrestha et al., 2009), which can show a more intense response in semi-arid sites. Finally, other works with legume species have successfully selected drought-tolerant accessions of *Vicia* species through the calibration method of the FIGS approach (Khazaei et al., 2013; Bari et al., 2016). In any case, it is clear that the drought adaptation of the selected populations should be experimentally confirmed.

As for other traits, the identification of genetic variation for salinity tolerance is the first step prior to breeding for salinity tolerant genotypes in lentils (Maher et al., 2003). The predictive characterization method directs the selection of germplasm to be screened, aiming to increase the probabilities of finding the desired trait. Yet, there are few studies of predictive characterization targeting salinity tolerance. Thormann et al. (2016) and Garcia et al. (2017) generated subsets of populations through this method but did not experimentally evaluate them for this trait. In contrast to the number of populations selected for other traits, only one population (*L. lamottei* in slightly saline soil, located in France) was selected for salinity tolerance. Yet, it is a significant result since lentil is one of the most sensitive crops to soil salinity (Ashraf and Waheed, 1990; Yadav et al., 2009), not being able to grow even in slightly saline soils (conductivity between 2 and 4 dS/m) (Katerji et al., 2001). The low ratio of lentil wild relative populations found to inhabit saline soils (one out of 624 populations) suggests

that the sensitivity to soil salinity is widespread across the range of species comprising the genus *Lens*. Before the adaptation to soil salinity is tested in the selected population, it would be advisable to sample and assess the soil conductivity in that site to discard the possibility of an error in the digital cartography concerning this trait. Although the quality and accuracy of environmental information in digital cartography are continuously improving, we must be aware that the values assigned to geographical coordinates are the result of projections carried out through interpolation approaches and that the real values on site of the targeted variable may differ from the estimated ones. If the selected population is actually occurring in slightly saline soil, the transfer of the adaptive genes into cultivars will be hindered by the fact that *L. lamottei* is in the tertiary gene pool of cultivated lentils. Still, the potential benefits of accessing this wild species variability may be worth it (Van Der Maesen et al., 1988) and place the selected population as really valuable for pre-breeding purposes.

Finally, the environmental filtering method selected a relatively low number of wild lentil populations potentially tolerant to waterlogging (around 3.4% of total populations). Filtering criteria applied (bad drainage soils and humid sites according to the annual De Martonne aridity index) are in line with the main factors favoring waterlogging that affects lentil—type of soil and precipitation (Lake and Sadras, 2019). The low proportion of selected populations could agree with the high sensitivity of lentil to waterlogging in any of the vital stages, especially at the germination and vegetative stages (Materne et al., 2007; Nessa et al., 2007; Materne and Siddique, 2009; Malik et al., 2015), which would also be present in most of its wild relatives. A previous study on waterlogging tolerance carried out by Wiraguna et al. (2017) with cultivated accessions of lentils from major lentil-producing countries with different climates, found that only those from Bangladesh showed waterlogging tolerance at the germination stage. They argued that this could be due to its monsoonal climate (i.e., persistent rain depending on the season). The finding of this association supports our methodological approach in which we selected areas within the Mediterranean that relate to high precipitation and poor drainage.

Calibration Method—Biotic Stress Analyses

According to the selected model (GBM, TSS value 0.828) we obtained a high percentage of wild populations potentially resistant to lentil rust (84.8%). This agrees with the findings of Singh et al. (2014) who experimentally assessed 405 wild lentil accessions and reported that many of the accessions were resistant or moderately resistant to rust. They identified two accessions, one accession of *L. nigricans* and another of *L. ervoides* that were especially valuable. These two species are the species in the subset selected by the calibration method applied in this study (nine *L. nigricans* populations and 21 *L. ervoides* populations in the subset of 30). Furthermore, they suggested Turkey and the Aegean area as priority areas for collecting, which are also represented in the subset of 30 populations for lentil rust resistance (22 populations in Turkey, Cyprus, and Greece). The remaining eight populations are located in other close areas: Croatia, Montenegro and Bosnia, and Herzegovina. Although *L. nigricans* and *L. ervoides* both belong to the secondary gene pool of lentils, the progress in the application of biotechnological techniques to obtain viable hybrids with cultivated lentils supports their use. For example, *L. ervoides* is an important source of variability to breed both biotic and abiotic stresses (Tullu et al., 2011, 2013). In addition, crosses made by Ladizinsky (1979) supported the utilization of *L. nigricans* almost at the same level of *L. culinaris* ssp. *orientalis* during breeding processes, although it is worth mentioning that *L. nigricans* is classified into two groups, one from South Europe and the other from the Middle East, the first one being cross-incompatible with cultivated lentils (Ladizinsky et al., 1983). The nine populations of *L. nigricans* in the reduced subset (30 populations) are found in the eastern Mediterranean which may facilitate their use in pre-breeding programs.

Abiotic and biotic stresses are likely to be interrelated and the interpretation and selection of models should follow a “judicious choice” (Challinor et al., 2009). In this sense, and given the similar TSS mean values, we considered variable importance as an additional criterion prior to the selection of the model to be projected. Further exploring the subset of 30 populations selected for rust, we found that all of them inhabit humid or sub-humid sites according to the De Martonne aridity index (see Supplementary Material 5, ecogeographical information section), which provide suitable conditions for the development of rust.

The inability to find a suitable model for broomrape resistance may be related to several reasons. In the first place, it may simply be explained by a lack of association between the tested ecogeographic variables and the

pattern of resistance to broomrape. It may also be explained by the fact that the training set of the model was composed of lentil accessions that were mainly located in Spain and did not cover a wide enough environmental range. The incorporation of additional evaluation data on broomrape lentil resistance from wild populations in other countries could increase the quality of data and potentially improve predictive characterization results. We consider that the investment and enhancement in evaluation databases targeting broomrape resistance are of high interest and should be prioritized. Among others, resistance to broomrape is one of the traits lacking genetic variation in cultivated lentils (Sarker and Erskine, 2006) and the wider genetic diversity of wild lentil species could benefit its breeding. Another potential for improvement using the predictive characterization approach through the calibration method relies on the progress in the implementation of machine learning models. In this sense, the application of models that treat the response variable of biotic resistance on a quantitative basis may help improve the predictions.

Conclusion

The interest in the incorporation of genetic diversity of wild lentils in pre-breeding and breeding programs is endorsed by recent studies targeting these species and the reported genetic diversity of adaptive value they possess (Ferguson and Robertson, 1999; Coyne and McGee, 2013; Kumar et al., 2014; Singh et al., 2014; Coyne et al., 2020; Id et al., 2020), to both biotic and abiotic stresses. The application of the predictive characterization techniques has successfully been applied in the search for traits of interest in other species (Bhullar et al., 2010; Bari et al., 2012, 2016; Khazaei et al., 2013; García Sánchez et al., 2019), and thus we consider our results of interest for the lentil plant breeding community. Furthermore, the generation of subsets explicitly pointing to small subsets of wild populations may put the focus on wild populations with higher possibilities of containing the desired trait other than randomly screening wild populations. Abiotic stresses, such as drought, salinity, and waterlogging, are major constraints in the production of lentils worldwide. Although there is presently a better understanding of adaptations to those conditions, research advances in this area are more limited than those related to damages by biotic interactions (Materne et al., 2007). Our results may contribute to facilitating access to lentil wild genetic resources, directing the exploration of novel germplasm for abiotic breeding purposes. Therefore, we suggest that the 13, one and 21 wild lentil populations predicted to be tolerant to drought, salinity, and waterlogging respectively, should be prioritized in trials to confirm such tolerances. In the same vein, we strongly recommend to sample and test for lentil rust resistance in the 30 populations of the generated reduced subset.

The application of predictive characterization methodologies is currently constrained to their use in particular cases followed by subsequent experimental validation. Further advances in the wider implementation of these techniques rely on the development of basic research that could provide a soundproof of concept. This would involve the implementation of experiments with integrative approaches combining genomic, environmental and phenotypic data that would provide insight into the mechanistic causes underlying the environment-phenotype associations.

References

- Abrol, I. P., Yadav, J. S. P., and Massoud, F. I. (1988). *Salt-affected Soils and Their Management*. Rome: Food and Agriculture Organization of the United Nations. FAO Soils Bulletin 39.
- Allouche, O., Tsoar, A., and Kadmon, R. (2006). Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *J. Appl. Ecol.* 43, 1223–1232. doi: 10.1111/j.1365-2664.2006.01214.x
- Andrews, M., and McKenzie, B. A. (2007). “Adaptation and ecology,” in *Lentil: An Ancient Crop for Modern Times*, eds S. S. Yadav, D. McNeil, and P. C. Stevenson (Dordrecht: Springer), 23–32.
- Asghar, M. J., Hameed, A., Khan, M. R., Shahid, M., and Atif, R. M. (2021). “Lentil wild genetic resource: a potential source of genetic improvement for biotic and abiotic stress tolerance,” in *Wild Germplasm for Genetic Improvement in Crop Plants*, ed. Academic Press (London, UK: Academic Press).

- Ashraf, M., and Waheed, A. (1990). Screening of local/exotic accessions of lentil (*Lens culinaris* Medic.) for salt tolerance at two growth stages. *Plant Soil* 128, 167–176. doi: 10.1007/BF00011106
- Bari, A., Khazaee, H., Stoddard, F. L., Street, K., Sillanpää, M. J., Chaubey, Y. P., et al. (2016). In silico evaluation of plant genetic resources to search for traits for adaptation to climate change. *Clim. Change* 134, 667–680. doi: 10.1007/s10584-015-1541-9
- Bari, A., Street, K., Mackay, M., Endresen, D. T. F., de Pauw, E., and Amri, A. (2012). Focused identification of germplasm strategy (FIGS) detects wheat stem rust resistance linked to environmental variables. *Genet. Resour. Crop Evol.* 59, 1465–1481. doi: 10.1007/s10722-011-9775-9775
- Baute, G. J., Dempewolf, H., and Rieseberg, L. H. (2015). “Using genomic approaches to unlock the potential of CWR for crop adaptation to climate change,” in *Crop Wild Relatives and Climate Change*, eds R. Redden, S. S. Yadav, N. Maxted, M. E. Dooloo, L. Guarino, and P. Smith (Hoboken NJ: Wiley-Blackwell), 268–280. doi: 10.1002/9781118854396.ch15
- Bhullar, N. K., Zhang, Z., Wicker, T., and Keller, B. (2010). Wheat gene bank accessions as a source of new alleles of the powdery mildew resistance gene *Pm3*: a large scale allele mining project. *BMC Plant Biol.* 10:88. doi: 10.1186/1471-2229-10-88
- Breiman, L. (2001). Random forests. *Mach. Learn.* 45, 5–32. doi: 10.1201/9780367816377-11
- Breiman, L., Friedman, J. H., Olshen, R. A., and Stone, C. J. (1984). *Classification and Regression Trees*. London: Chapman and Hall.
- Brozynska, M., Furtado, A., and Henry, R. J. (2016). Genomics of crop wild relatives: expanding the gene pool for crop improvement. *Plant Biotechnol. J.* 14, 1070–1085. doi: 10.1111/pbi.12454
- Busby, J. R. (1991). “BIOCLIM - a bioclimate analysis and prediction system,” in *Nature Conservation: Cost Effective Biological Surveys and Data Analysis*, eds C. R. Margules and M. P. Austin (Canberra, ACT: CSIRO), 64–68.
- Castañeda-Álvarez, N. P., Khoury, C. K., Achicanoy, H. A., Bernau, V., Dempewolf, H., Eastwood, R. J., et al. (2016). Global conservation priorities for crop wild relatives. *Nat. Plants* 2:16022. doi: 10.1038/nplants.2016.22
- Castroviejo, S., and Pascual, H. (1999). “Lens Mill,” in *Flora Iberica*, eds S. Castroviejo, C. Aedo, M. Laínz, F. Muñoz Garmendia, G. Nieto Feliner, J. Paiva, et al. (Madrid: Real Jardín Botánico, CSIC), 417–423.
- Chahota, R. K., Sharma, T. R., and Sharma, S. K. (2019). *Conventional Genetic Manipulations*. Amsterdam: Elsevier Inc.
- Challinor, A. J., Ewert, F., Arnold, S., Simelton, E., and Fraser, E. (2009). Crops and climate change: progress, trends, and challenges in simulating impacts and informing adaptation. *J. Exp. Bot.* 60, 2775–2789. doi: 10.1093/jxb/erp062
- Challinor, A. J., Watson, J., Lobell, D. B., Howden, S. M., Smith, D. R., and Chhetri, N. (2014). A meta-analysis of crop yield under climate change and adaptation. *Nat. Clim. Chang.* 4, 287–291. doi: 10.1038/nclimate2153
- Chamberlain, S. A., and Boettiger, C. (2017). R Python, and ruby clients for GBIF species occurrence data. *PeerJ Prepr.* 5:e3304v1. doi: 10.7287/peerj.preprints.3304v1
- Chen, W., Basandrai, A. K., Basandrai, D., Banniza, S., Bayaa, B., Buchwaldt, L., et al. (2009). “Lentil diseases and their management,” in *The Lentil. Botany, Production and Uses*, eds W. Erskine, F. J. Muehlbauer, A. Sarker, and B. Sharma (Wallingford: CAB International), 262–281.
- Choudhary, M., Singh, V., Muthusamy, V., and Wani, S. H. (2017). Harnessing crop wild relatives for crop improvement. *LS Int. J. Life Sci.* 6:73. doi: 10.5958/2319-1198.2017.00009.4
- Cokkizgin, A., and Shtaya, M. J. Y. (2013). Lentil: origin, cultivation techniques and utilization and advances in transformation. *Agric. Sci.* 1, 55–62. doi: 10.12735/as.v1i1p55
- Coyne, C. J., Kumar, S., von Wettberg, E. J. B., Marques, E., Berger, J. D., Redden, R. J., et al. (2020). Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. *Legum. Sci.* 2:e36. doi: 10.1002/leg3.36
- Coyne, C., and McGee, R. (2013). “Lentil,” in *Genetic and Genomic Resources of Grain Legume Improvement*, eds M. Singh, H. D. Upadhyaya, and I. S. Bisht (Amsterdam: Elsevier Inc.), 157–180. doi: 10.1016/B978-0-12-397935-3.00007-4
- Cubero, J. I., Pérez, De La Vega, M., and Fratini, R. (2009). “Origin, phylogeny, domestication and spread,” in *The Lentil. Botany, Production and Uses*, eds W. Erskine, F. J. Muehlbauer, and B. Sharma (Wallingford: CAB International).

- Cutler, D. R., Edwards, T. C., Beard, K. H., Cutler, A., Hess, K. T., Gibson, J., et al. (2007). Random forests for classification in ecology. *Ecology* 88, 2783–2792. doi: 10.1890/07-0539.1
- De Martonne, E. (1926). Une nouvelle fonction climatologique: L'indice d'aridité. *La Meteorol.* 16, 449–458.
- Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., and Guarino, L. (2017). Past and future use of wild relatives in crop breeding. *Crop Sci.* 57, 1070–1082. doi: 10.2135/cropsci2016.10.0885
- Dissanayake, R., Cogan, N. O. I., Smith, K. F., and Kaur, S. (2021). Application of genomics to understand salt tolerance in lentil. *Genes (Basel)* 12:332. doi: 10.3390/genes12030332
- Egan, P. A., Muola, A., and Stenberg, J. A. (2018). Capturing genetic variation in crop wild relatives: an evolutionary approach. *Evol. Appl.* 11, 1293–1304. doi: 10.1111/eva.12626
- Erskine, W. (2009). “Global production, supply and demand,” in *The Lentil. Botany, Production and Uses*, eds W. Erskine, F. J. Muehlbauer, A. Sarker, and B. Sharma (Wallingford: CAB International), 4–12. doi: 10.1079/9781845934873.0004
- Erskine, W., Sarker, A., and Kumar, S. (2016). *Lentil Breeding*, 2nd Edn. Amsterdam: Elsevier Ltd, doi: 10.1016/b978-0-08-100596-5.00210-9
- ESA CCI Land Cover Project (2017). *Land Cover CCI Product User Guide Version 2*. Available online at: https://www.esa-landcover-cci.org/?q=webfm_send/84 (accessed January 21, 2022).
- Esquinas-Alcázar, J. (2005). Science and society: protecting crop genetic diversity for food security: political, ethical and technical challenges. *Nat. Rev. Genet.* 6, 946–953. doi: 10.1038/nrg1729
- Euro+Med Plant Database (2006). Euro+Med PlantBase - the Information Resource for Euro-Mediterranean Plant Diversity. Available online at: <http://ww2.bgbm.org/EuroPlusMed/> (accessed September 29, 2021)
- FAO (2020). Food and Agriculture Organization of the United Nations. Rome: FAO.
- Ferguson, M. E., Maxted, N., Slageren, M., Van, and Robertson, L. D. (1999). Morphological and phenological variation in the wild relatives of lentil. *Genet. Resour. Crop Evol.* 46, 3–12.
- Ferguson, M. E., Maxted, N., Slageren, M., Van, and Robertson, L. D. (2000). A re-assessment of the taxonomy of *Lens* Mill. (Leguminosae, Papilionoideae, Viciae). *Bot. J. Linn. Soc.* 133, 41–59. doi: 10.1006/boj.1999.0319
- Fischer, G., Nachtergaele, F. S., Prieler, H. T., van Velthuizen, L., Verelst, D., and Wiberg. (2008). *Global Agro-ecological Zones Assessment for Agriculture (GAEZ 2008)*. Rome: FAO.
- Ford-Lloyd, B. V., Schmidt, M., Armstrong, S. J., Barazani, O., Engels, J., Hadas, R., et al. (2011). Crop wild relatives—undervalued, underutilized and under threat? *Bioscience* 61, 559–565. doi: 10.1525/bio.2011.61.7.10
- Fratini, R., Ruiz, M. L., Pérez, and De La Vega, M. (2004). Intra-specific and inter-sub-specific crossing in lentil (*Lens culinaris* Medik.). *Can. J. Plant Sci.* 84, 981–986. doi: 10.4141/P03-201
- Friedman, J. H. (1991). Multivariate adaptive regression splines. *Ann. Stat.* 19, 1–67.
- Friedman, J. H. (2001). Greedy function approximation: a gradient boosting machine. *Ann. Stat.* 29, 1189–1232. doi: 10.1214/aos/1013203451
- Friedman, J. H. (2002). Stochastic gradient boosting. *Comput. Stat. Data Anal.* 38, 367–378. doi: 10.1016/S0167-9473(01)00065-2
- García Sánchez, R. M., Parra-Quijano, M., Greene, S., and Iriondo, J. M. (2019). Predictive characterisation identifies global sources of acyanogenic germplasm of a key forage species. *Crop Pasture Sci.* 70, 546–554. doi: 10.1071/cp18346
- García, R. M., Parra-Quijano, M., and Iriondo, J. M. (2017). Identification of ecogeographical gaps in the Spanish *Aegilops* collections with potential tolerance to drought and salinity. *PeerJ* 5:e3494. doi: 10.7717/peerj.3494
- GBIF.org (2021). Derived Dataset GBIF.org (10 December 2021) Filtered Export of GBIF Occurrence Data. Copenhagen: GBIF.org. Available online at: <https://doi.org/10.15468/dd.9yb585>
- Greene, S. L., and Warburton, M. L. (2017). Wading into the gene pool: progress and constraints using wild species. *Crop Sci.* 57, 1039–1041. doi: 10.2135/cropsci2017.04.0001in
- Guarino, L., and Lobell, D. B. (2011). A walk on the wild side. *Nat. Clim. Chang.* 1, 374–375. doi: 10.1038/nclimate1272
- Gupta, D., Ford, R., and Taylor, P. W. J. (2011). “Lens,” in *Wild Crop Relatives: Genomic and Breeding Resources. Legume Crops and Forages*, ed. C. Kole (Dordrecht: Springer), 127–139.

- Habib, M., Ahmad, I., Wang, D., Fahad, S., and Afzal, M. (2021). Influence of semi arid environment on radiation use efficiency and other growth attributes of lentil crop. *Environ. Sci. Pollut. Res.* 28, 13697–13711. doi: 10.1007/s11356-020-11376-w
- Hajjar, R., and Hodgkin, T. (2007). The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica* 156, 1–13. doi: 10.1007/s10681-007-9363-0
- Hamdi, A., and Erskine, W. (1996). Reaction of wild species of the genus *Lens* to drought. *Euphytica* 91, 173–179. doi: 10.1007/BF00021067
- Hastie, T., and Tibshirani, R. (1986). Generalized additive models. *Stat. Sci.* 1, 297–318.
- Hastie, T., Tibshirani, R., and Buja, A. (1994). Flexible discriminant analysis by optimal scoring. *J. Am. Stat. Assoc.* 89, 1255–1270. doi: 10.1080/01621459.1994.10476866
- Hawtin, G., Iwanaga, M., and Hodgkin, T. (1996). Genetic resources in breeding for adaptation. *Euphytica* 92, 255–266. doi: 10.1007/BF00022853
- Heal, G., Walker, B., Levin, S., Arrow, K., Dasgupta, P., Daily, G., et al. (2004). Genetic diversity and interdependent crop choices in agriculture. *Resour. Energy Econ.* 26, 175–184. doi: 10.1016/j.reseneeco.2003.11.006
- Heywood, V., Casas, A., Ford-Lloyd, B., Kell, S., and Maxted, N. (2007). Conservation and sustainable use of crop wild relatives. *Agric. Ecosyst. Environ.* 121, 245–255. doi: 10.1016/j.agee.2006.12.014
- Hodgkin, T., and Hajjar, R. (2008). “Using crop wild relatives for crop improvement: trends and perspectives,” in *Crop Wild Relative Conservation and Use*, eds N. Maxted, B. V. Ford-Lloyd, S. P. Kell, J. Iriondo, E. Dulloo, and J. Turok (Wallingford: CAB International), 535–548. doi: 10.1016/j.biotechadv.2019.107479
- Howden, S. M., Soussana, J.-F., Tubiello, F. N., Chhetri, N., Dunlop, M., and Meinke, H. (2007). Adapting agriculture to climate change. *Proc. Natl. Acad. Sci. U S A.* 104, 19691–19696. doi: 10.1073/pnas.0701890104
- Id, M. S., Kumar, S., Basandrai, A. K., Basandrai, D., Malhotra, N., Saxena, D. R., et al. (2020). Evaluation and identification of wild lentil accessions for enhancing genetic gains of cultivated varieties. *PLoS One* 15:e0229554.
- Jump, A. S., Marchant, R., and Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends Plant Sci.* 14, 51–58. doi: 10.1016/j.tplants.2008.10.002
- Kantar, M. B., Sosa, C. C., Khoury, C. K., Castañeda-Álvarez, N. P., Achicanoy, H. A., Bernau, V., et al. (2015). Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (*Helianthus annuus* L.). *Front. Plant Sci.* 6:841. doi: 10.3389/fpls.2015.00841
- Katerji, N., Van Hoorn, J. W., Hamdy, A., Mastrorilli, M., Oweis, T., and Erskine, W. (2001). Response of two varieties of lentil to soil salinity. *Agric. Water Manag.* 47, 179–190. doi: 10.1016/S0378-3774(00)00109-8
- Kazan, K., and Lyons, R. (2016). The link between flowering time and stress tolerance. *J. Exp. Bot.* 67, 47–60. doi: 10.1093/jxb/erv441
- Ketchen, D. J., and Shook, C. L. (1996). The application of cluster analysis in management strategic research: an analysis and critique. *Strateg. Manag. J.* 17, 441–458. doi: 10.1186/s13063-020-04982-z
- Khazaei, H., Street, K., Bari, A., Mackay, M., and Stoddard, F. L. (2013). The FIGS (focused identification of germplasm strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. *PLoS One* 8:e63107. doi: 10.1371/journal.pone.0063107
- Khoury, C. K., Bjorkman, A. D., Dempewolf, H., Ramirez-Villegas, J., Guarino, L., Jarvis, A., et al. (2014). Increasing homogeneity in global food supplies and the implications for food security. *PNAS* 111, 4001–4006. doi: 10.1073/pnas.1313490111
- Khoury, C. K., Brush, S., Costich, D. E., Curry, H. A., Haan, S., Engels, J. M. M., et al. (2021). Crop genetic erosion: understanding and responding to loss of crop diversity. *New Phytol.* 223, 84–118. doi: 10.1111/nph.17733
- Khush, G. S. (2001). Green revolution: the way forward. *Nat. Rev. Genet.* 2, 815–822. doi: 10.1038/35093585
- Kilian, B., Dempewolf, H., Guarino, L., Werner, P., Coyne, C., and Warburton, M. L. (2020). Crop Science special issue: adapting agriculture to climate change: a walk on the wild side. *Crop Sci.* 61, 32–36. doi: 10.1002/csc2.20418.
- Kumar, J., Srivastava, E., Singh, M., Mahto, D., Pratap, A., and Kumar, S. (2014). Lentil. *Alien Gene Transf. Crop Plants* 2, 191–205. doi: 10.1007/978-1-4614-9572-7
- Ladizinsky, G. (1979). The origin of lentil and its wild gene pool. *Euphytica* 28, 179–187.

- Ladizinsky, G., Braun, D., and Muehlbauer, F. J. (1983). Evidence for domestication of *Lens nigricans* (M. Bieb.) Godron in S Europe. *Bot. J. Linn. Soc.* 87, 169–176.
- Ladizinsky, G., Braun, D., Goshen, D., and Muehlbauer, F. J. (1984). The biological species of the genus *lens* L. *Bot. Gaz.* 145, 253–261. doi: 10.1086/337454
- Lake, L., and Sadras, V. O. (2019). “Screening lentil varieties for adaptation to waterlogging,” in *Cells to Satellites. Proceedings of the 2019 Agronomy Australia Conference*, ed. J. Pratley (Wagga, NSW).
- Mackay, M. C., and Street, K. (2004). “Focused identification of germplasm strategy - FIGS,” in *Proceedings of the 54th Australian Cereal Chemistry Conference and the 11th Wheat Breeders’ Assembly* (Melbourne: Royal Australian Chemical Institute), 138–141.
- Maher, L., Armstrong, R., and Connor, D. (2003). “Salt tolerant lentils - a possibility for the future?,” in *Proceedings of the 11th Australian Agronomy Conference*, (Geelong, Vic).
- Malik, A. I., Ailewe, T. I., and Erskine, W. (2015). Tolerance of three grain legume species to transient waterlogging. *AoB Plants* 7:lv040. doi: 10.1093/aobpla/plv040
- Massawe, F., Mayes, S., and Cheng, A. (2016). Crop diversity: an unexploited treasure trove for food security. *Trends Plant Sci.* 21, 365–368. doi: 10.1016/j.tplants.2016.02.006
- Materne, M., and McNeil, D. L. (2007). “Breeding methods and achievements,” in *Lentil: An Ancient Crop for Modern Times*, eds S. S. Yadav, D. McNeil, and P. C. Stevenson (Dordrecht: Springer), 241–254. doi: 10.1007/978-1-4020-6313-8_15
- Materne, M., and Siddique, K. H. M. (2009). “Agroecology and crop adaptation,” in *The Lentil. Botany, Production and Uses*, eds W. Erskine, F. J. Muehlbauer, A. Sarker, and B. Sharma (Wallingford: CAB International), 47–63.
- Materne, M., McNeil, D., Hobson, K., and Ford, R. (2007). “Abiotic stresses,” in *Lentil: An Ancient Crop for Modern Times*, eds S. S. Yadav, D. L. McNeil, and P. C. Stevenson (Dordrecht: Springer), 315–330.
- Mezghani, N., Khoury, C. K., Carver, D., Achicanoy, H. A., Simon, P., Flores, F. M., et al. (2019). Distributions and conservation status of carrot wild relatives in Tunisia: a case study in the Western Mediterranean Basin. *Crop Sci.* 59, 2317–2328. doi: 10.2135/cropsci2019.05.0333
- Mohar, S., Kumar, S., Basandrai, A. K., Basandrai, D., Malhotra, N., Saxena, D. R., et al. (2020). Evaluation and identification of wild lentil accessions for enhancing genetic gains of cultivated varieties. *PLoS One* 15:e0229554. doi: 10.1371/journal.pone.0229554
- Montenegro, and de Wit, M. (2017). Stealing into the wild: conservation science, plant breeding and the makings of new seed enclosures. *J. Peasant Stud.* 44, 169–212. doi: 10.1080/03066150.2016.1168405
- Naim-feil, E., Toren, M., Aubert, G., Rubinstein, M., Rosen, A., Eshed, R., et al. (2017). Drought response and genetic diversity in *pisum fulvum*, a wild relative of domesticated pea. *Crop Sci.* 57, 1145–1159. doi: 10.2135/cropsci2016.10.0880
- Nair, K. P. (2019). *Utilizing Crop Wild Relatives to Combat Global Warming*, 1st Edn. Amsterdam: Elsevier Inc, doi: 10.1016/bs.agron.2018.09.001
- Nelder, J. A., and Wedderburn, R. W. M. (1972). Generalized linear models. *J. R. Stat. Soc. Series A* 135, 370–384.
- Nessa, B., Islam, M., Haque, M., and Ahmed, J. (2007). Soil flooding tolerance in lentil at vegetative stage. *Progress. Agric.* 18, 17–24. doi: 10.3329/pa.v18i2.17460
- Newton, A. C., Johnson, S. N., and Gregory, P. J. (2011). Implications of climate change for diseases, crop yields and food security. *Euphytica* 179, 3–18. doi: 10.1007/s10681-011-0359-4
- Obreza, M. (2019). *genesysr: Genesys PGR Client. R Package Version 1.0.0*. Available online at: <https://cran.r-project.org/package=genesysr>
- Panta, S., Flowers, T., Lane, P., Doyle, R., Haros, G., and Shabala, S. (2014). Halophyte agriculture: success stories. *Environ. Exp. Bot.* 107, 71–83. doi: 10.1016/j.envexpbot.2014.05.006
- Parra-Quijano, M. (2020). CAPFITOGEN 3: A Toolbox for the Conservation and Promotion of the use of Agricultural Biodiversity. Bogotá, Colombia. Parra-Quijano, M., Iriondo, J. M., and Torres, E. (2012b). Review. applications of ecogeography and geographic information systems in conservation and utilization of plant genetic resources. *Spanish J. Agric. Res.* 10, 419–429. doi: 10.5424/sjar/2012102-303-11

- Parra-Quijano, M., Iriondo, J. M., and Torres, E. (2012a). Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genet. Resour. Crop Evol.* 59, 205–217. doi: 10.1007/s10722-011-9676-7
- Parra-Quijano, M., Iriondo, J. M., Torres, M. E., López, F., Maxted, N., and Kell, S. (2020). CAPFITOGEN3. A Toolbox for the Conservation and Promotion of the use of Agricultural Biodiversity. Bogotá, Colombia: Universidad Nacional de Colombia. Bogotá campus – Faculty of Agricultural Sciences. Available online at: <http://www.capfitogen.net/es/acceso/manuales/> (accessed November 17, 2021).
- Pratap, A., Das, A., Kumar, S., and Gupta, S. (2021). Current perspectives on introgression breeding in food legumes. *Front. Plant Sci.* 11:589189. doi: 10.3389/fpls.2020.589189
- Preissel, S., Reckling, M., Schläpke, N., and Zander, P. (2015). Field crops research magnitude and farm-economic value of grain legume pre-crop benefits in europe: a review. *F. Crop. Res.* 175, 64–79. doi: 10.1016/j.fcr.2015.01.012
- Prohens, J., Gramazio, P., Plazas, M., Dempewolf, H., Kilian, B., Díez, M. J., et al. (2017). Introgressomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. *Euphytica* 213, 1–19. doi: 10.1007/s10681-017-1938-9
- R Core Team (2020). R: A Language and Environment for Statistical Computing. Vienna: R Core Team.
- Rawal, V., and Bausal, V. (2019). “Lentil: emergence of large-scale, export oriented production,” in *The Global Economy of Pulses*, eds V.
- Rawal and D. K. Navarro (Rome: FAO. Food and Agriculture Organization of the United Nations), 71–85. Ripley, B. D. (1996). *Pattern Recognitions and Neural Networks*. Cambridge: Cambridge University Press, doi: 10.1017/CBO9781107415324.004
- Rosenzweig, C., and Parry, M. L. (1994). Potential impact of climatic change on world food supply. *Nature* 367, 133–138.
- Rosenzweig, C., Elliott, J., Deryng, D., Ruane, A. C., Müller, C., Arneth, A., et al. (2014). Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. *PNAS* 111, 3268–3273. doi: 10.1073/pnas.1222463110
- Rubio Teso, M. L., Álvarez-Muñiz, C., Gaisberger, H., Kell, S., Lara-Romero, C., Magos Brehm, J., et al. (2020). In Situ Plant Genetic Resources in Europe: Crop Wild Relatives. Montpellier: CGIAR Available at https://more.bham.ac.uk/farmerspride/wp-content/uploads/sites/19/2020/10/D1.2_In_situ_PGR_in_Europe_crop_wild_relativespdf
- Sandhu, J. S., and Singh, S. (2007). “History and origin,” in *Lentil: An Ancient Crop for Modern Times*, eds S. Yadav, D. McNeil, and P. Stevenson (Dordrecht: Springer). Sano, Y. (1993). “Constraints in using wild relatives in breeding: lack of basic knowledge on crop gene pools,” in *International Crop Science I*, eds D. R. Buxton, R. Shibles, R. A. Forsberg, B. L. Blad, K. H. Asay, G. M. Paulsen, et al. (Winsconsin, WI: Crop Science Society of America, Inc.), 437–443.
- Sarker, A., and Erskine, W. (2006). Recent progress in the ancient lentil. *J. Agric. Sci.* 144, 19–29. doi: 10.1017/S0021859605005800
- Shrestha, R., Siddique, K. H. M., Turner, D. W., and Turner, N. C. (2009). “Breeding and management to minimize the effects of drought and improve water use efficiency,” in *The Lentil. Botany, Production and Uses*, eds W. Erskine, F. J. Muehlbauer, A. Sarker, and B. Sharma (Wallingford: CAB International), 172–193.
- Singh, D., Singh, C. K., Singh Tomar, R. S., Taunk, J., Singh, R., Maurya, S., et al. (2016). Molecular assortment of lens species with different adaptations to drought conditions using SSR markers. *PLoS One* 11:e0147213. doi: 10.1371/journal.pone.0147213
- Singh, M., Bisht, I. S., Kumar, S., Dutta, M., Bansal, K. C., Karale, M., et al. (2014). Global wild annual lens collection: a potential resource for lentil genetic base broadening and yield enhancement. *PLoS One* 9:e107781. doi: 10.1371/journal.pone.0107781
- Smartt, J. (1984). Evolution of grain legumes. I. mediterranean pulses. *Exp. Agric.* 20, 275–296. doi: 10.1017/S0014479700017968
- Smykal, P., Coyne, C. J., Ambrose, M. J., Maxted, N., Schaefer, H., Blair, M. W., et al. (2015). Legume crops phylogeny and genetic diversity for science and breeding. *CRC. Crit. Rev. Plant Sci.* 34, 43–104. doi: 10.1080/07352689.2014.897904

- Thormann, I., Parra-Quijano, M., Endresen, D. T. F., Rubio-Teso, M. L., Iriondo, J. M., and Maxted, N. (2014a). Predictive Characterization of Crop Wild Relatives and Landraces. Rome: Bioversity International.
- Thormann, I., Parra-Quijano, M., Iriondo, J. M., Rubio-Teso, M. L., Endresen, D. T. F., Dias, S., et al. (2014b). “New predictive characterization methods for accessing and using CWR diversity,” in Proceedings of the International Conference on Enhanced Genepool Utilization: Capturing wild Relative and Landrace Diversity for Crop Improvement (Cambridge).
- Thormann, I., Parra-Quijano, M., Rubio Teso, M. L., Endresen, D. T. F., Dias, S., Iriondo, J. M., et al. (2016). “Predictive characterization methods for accessing and using CWR diversity,” in Enhancing Crop Genepool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement, eds N. Maxted, M. E. Dooloo, and B. Ford-Lloyd (Wallingford: CAB International), 64–77.
- Thuiller, W., Georges, D., Engler, R., and Breiner, F. (2020). Package “biomod2.”. Thuiller, W., Lafourcade, B., and Araujo, M. (2010). Presentation Manual for BIOMOD.
- Tullu, A., Bett, K., Banniza, S., Vail, S., and Vandenberg, A. (2013). Widening the genetic base of cultivated lentil through hybridization of *Lens culinaris* “Eston” and *L. ervoides* accession IG 72815. *Can. J. Plant Sci.* 93, 1037–1047. doi: 10.4141/CJPS2013-072
- Tullu, A., Diederichsen, A., Suvorova, G., and Vandenberg, A. (2011). Genetic and genomic resources of lentil: status, use and prospects. *Plant Genet. Resour. Characterisation Util.* 9, 19–29. doi: 10.1017/S1479262110000353
- USDA (2020). Soil Texture Calculator. Washington, D.C: United States Department of Agriculture.
- Van Der Maesen, L. J. G., Kaiser, W. J., Marx, G. A., and Worede, M. (1988). “Genetic basis for pulse crop improvement: collection, preservation and genetic variation in relation to needed traits,” in *World Crops: Cool Season Food Legumes*, ed. R. J. Summerfield (Netherlands: Kluwer Academic Publishers), 55–66. doi: 10.1007/978-94-009-2764-3_7
- Van Etten, J., Parra-Quijano, M., Thormann, I. S. D., Rubio Teso, M. L., and Iriondo, J. (2011). Basic Script to Execute a Slightly Modified Version of the Classical Focused Identification of Germplasm Strategy. <https://github.com/MLRubioTeso/Searching-for-abiotic-tolerant-and-biotic-stress-resistant-wild-lentils-for-introgression-breeding-t>: GitHub.
- Walley, P. G., and Moore, J. D. (2015). “Biotechnology and genomics: exploiting the potential of CWR,” in *Crop Wild Relatives and Climate Change*, eds R. Redden, S. S. Yadav, N. Maxted, M. E. Dooloo, L. Guarino, and P. Smith (Hoboken NJ: Wiley-Blackwell), 212–223. doi: 10.1002/9781118854396.ch12
- Warschefsky, E., Penmetsa, R. V., Cook, D. R., and von Wettberg, E. J. B. (2014). Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *Am. J. Bot.* 101, 1791–1800. doi: 10.3732/ajb.1400116
- Watson, A., Ghosh, S., Williams, M. J., Cuddy, W. S., Simmonds, J., Rey, M. D., et al. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nat. Plants* 4, 23–29. doi: 10.1038/s41477-017-0083-8
- Wheeler, T., and von Braun, J. (2013). Climate impacts on global food security. *Science* 341, 508–513. doi: 10.1126/science.1239402
- Wiraguna, E., Malik, A. I., and Erskine, W. (2017). Waterlogging tolerance in lentil (*Lens culinaris* Medik. subsp. *culinaris*) germplasm associated with geographic origin. *Genet. Resour. Crop Evol.* 64, 579–586. doi: 10.1007/s10722-016-0385-0
- Yadav, S. S., McNeil, D. L., Andrews, M., Chen, C., Brand, J., Singh, G., et al. (2009). “Soil nutrient management,” in *The Lentil. Botany, Production and Uses*, eds W. Erskine, F. J. Muehlbauer, and B. Sharma (Wallingford: CAB International), 194–212.
- Zhang, F., and Batley, J. (2020). Exploring the application of wild species for crop improvement in a changing climate. *Curr. Opin. Plant Biol.* 56, 218–222. doi: 10.1016/j.pbi.2019.12.013
- Zhang, H., Mittal, N., Leamy, L. J., Barazani, O., and Song, B. H. (2017). Back into the wild—apply untapped genetic diversity of wild relatives for crop improvement. *Evol. Appl.* 10, 5–24. doi: 10.1111/eva. 12434
- Zörb, C., Geilfus, C. M., and Dietz, K. J. (2018). Salinity and crop yield. *Plant Biol.* 21, 31–38. doi: 10.1111/plb.12884

Supplementary Material

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://github.com/MLRubioTeso/Searching-for-abiotic-tolerant-and-biotic-stress-resistant-wild-lentils-for-introgression-breeding-t>

For the reader service, Supplementary Material 9 and Supplementary Material 11 is shown below, providing details of the four generated subsets of wild lentils populations.

Supplementary Material 9:

SM 9: Populations of wild relatives of lentils in Europe selected through the Environmental Filtering Method of the Predictive Characterization technique and potentially tolerant to following abiotic stresses: Drought, soil salinity and waterlogging.										
		Taxa	Unique ID	IarDMf	IarDM	Latitude	Longitude	Country	Collecting site information	ELC category
Drought tolerant	1	<i>L. nigricans</i>	ID_6954734	10.72	13.94	37.133333	24.5	Greece	Serifos. 1-2 km S to SW of Livadion. Ep. Keas; Nom. Kikladon	2
	2	<i>L. nigricans</i>	ID_6954730	10.79	13.83	37.4	24.9	Greece	Siros. The mt N of Finikas. Ep. Sirou, Nom. Kikladon	2
	3	<i>L. nigricans</i>	ID_6954729	10.88	14.07	37.45	24.933333	Greece	Siros. Mt. Siringas. Ep. Sirou; Nom. Kikladon	2
	4	<i>L. lamottei</i>	GE_222367	11.43	12.01	38.7775	0.11889	Spain	Close to Petronor gas station and carob trees, Javea, Alicante province	2
	5	<i>L. lamottei</i>	ID_6953881	12.07	12.15	38.74	0.18	Spain	La Granadella, Xabia, Alicante province	2
	6	<i>L. lamottei</i>	GE_222365	12.07	12.15	38.75583	0.16	Spain	Tossal del Rebaldi, Javea, Alicante province	2
	7	<i>L. nigricans</i>	GE_222437	13.62	11.75	38.3333	-1.5	Spain	Hozel del Lino, Murcia	2
	8	<i>L. lamottei</i>	GE_222366	14.21	13.28	38.74028	-0.01694	Spain	Calvari, Jalon, Alicante province	2
	9	<i>L. lamottei</i>	GE_222343	14.42	13.63	38.983333	-0.516667	Spain	Castle hillside, Xativa, Valencia province	2
	10	<i>L. nigricans</i>	ID_6954675	14.42	13.63	38.983337	-0.516666	Spain	Jativa, Valencia province	2
	11	<i>L. nigricans</i>	ID_6954474	14.44	13.40	38.77	-0.07	Spain	Jalón; Calvari, Alicante province	2
	12	<i>L. ervoides</i>	ID_6953173	14.59	12.55	36.9	38.916667	Turkey	29 km South of Urfa on road to Akcakale, In pine woodland. Koruklu, Urfa	2
	13	<i>L. nigricans</i>	ID_6954230	14.74	13.36	38.66	-0.05	Spain	Jalon, Alicante province	2

		Taxa	Unique ID	Soil salinity (conductivity)	Latitude	Longitude	Country	Collecting site information	ELC category	
Salinity toler.	1	<i>L. lammoretei</i>	ID_6953872	2.099999905	42.96808	2.98863	France	La Palme [INSEE:11188], Aude	21	
		Taxa	Unique ID	Soil Texture	IarDM	Latitude	Longitude	Country	Collecting site information	ELC cat
Waterlogging tolerance	1	<i>L. culinaris</i> subsp. <i>orientalis</i>	ID_6952438	CLAY	40.11	38.523891	22.502781	Greece	Fokidos, Parnassidhos, NW Ag. Triada	21
	2	<i>L. ervoides</i>	ID_6953356	CLAY	35.77	38.633333	22.366667	Greece	The Amvlema-pass, N of Amphissia. Ep. Parnassidos. Nom. Fokidos	21
	3	<i>L. ervoides</i>	GE_222062	CLAY	34.38	36.1333	36.1667	Turkey	Harbiye edge of village on road leading south	2
	4	<i>L. ervoides</i>	GE_222085	CLAY	33.21	36.1	35.95	Turkey	2 km from Samandagi on road to Kaburluk	2
	5	<i>L. nigricans</i>	ID_6954642	CLAY	33.07	42.61	-2.5	Spain	Bernedo; Peña Alta. At the foot of a limestone rocky place. Rocky flat area, organic matter. Álava province	24
	6	<i>L. nigricans</i>	ID_6954569	CLAY	33.07	42.616005	-2.507488	Spain	Peña Alta, Bernedo, Alava province	24
	7	<i>L. nigricans</i>	ID_6954460	CLAY	32.85	46.6907	2.51218	France	La Groutte	24
	8	<i>L. nigricans</i>	ID_6954619	CLAY	31.97	44.433333	33.783333	Ukraine	near Orlinoe sett.	27
	9	<i>L. culinaris</i> subsp. <i>orientalis</i>	ID_6952604	CLAY	29.43	44.483333	33.716667	Ukraine	Shirokoye sett., Chernaya river valley	27
	10	<i>L. ervoides</i>	ID_6953026	CLAY	29.43	44.483333	33.716667	Ukraine	Sevastopol	27
	11	<i>L. nigricans</i>	ID_6954771	CLAY	29.37	41.31	0.91	Spain	Cornudella de Montsant; Albarca. Tarragona province	21
	12	<i>L. nigricans</i>	ID_6954710	CLAY	29.01	44.75	34.4	Ukraine	Demerdzhi mntn, road to top	27
	13	<i>L. ervoides</i>	ID_6953338	CLAY	28.98	44.4	33.766667	Ukraine	near Foros sett.	27
	14	<i>L. lamottei</i>	ID_6953903	CLAY	28.91	36.625	-5.3273	Spain	Cortes de la Frontera: Sierra de los Pinos. Málaga province	21

15	<i>Lens nigricans</i> (M. Bieb.) Godr.	ID_6954618	CLAY	28.91	36.632	-5.321	Spain	Cortes de la Frontera; Sierra de los Pinos. Elevation 700-1000 m. Malaga province	21
16	<i>Lens ervoides</i> (Brign.) Grande	ID_6953339	CLAY	28.01	44.416667	33.933333	Ukraine	near Opolznevoye sett.	27
17	<i>Lens nigricans</i> (M. Bieb.) Godr.	ID_6954246	CLAY	27.61	44.466667	33.761111	Ukraine	Sevastopol	27
18	<i>Lens nigricans</i> (M. Bieb.) Godr.	ID_6954658	CLAY	25.80	38.2337	-2.6699	Spain	Segura de la Sierra: Forest path to Yelmo. Jaén province	21
19	<i>Lens ervoides</i> (Brign.) Grande	GE_222075	CLAY	25.62	36.85	36.6667	Turkey	44 km W of Kilis on Kilis to Wkhiye	2
20	<i>Lens ervoides</i> (Brign.) Grande	ID_6953347	CLAY	25.34	44.857451	34.934721	Ukraine	gorodskoi okrug Sudak. Crimea	27
21	<i>Lens ervoides</i> (Brign.) Grande	GE_222086	CLAY	25.16	37.5	36.8667	Turkey	Heyelan 16 km from Kozan on road to Feke	2

Supplementary Material 11:

SM 11: Subset of populations of wild relatives of lentils in Europe selected through the Calibration Method of the Predictive Characterization technique and potentially resistant to lentil rust (*Uromyces vicia-fabae* (Pers.) Schröt)

Ord	Unique ID	Taxa	Country	Collecting site information	Latitude	Longitude	ELC Category
1	GE_222021	<i>L. ervoides</i>	Turkey	Antalya. Finike (km 18805)	36.2833	30.1	2
2	GE_222024	<i>L. ervoides</i>	Turkey	Icel. Gulnar (km 19471)	36.3	33.4	2
3	GE_222020	<i>L. ervoides</i>	Turkey	Antalya. Finike (km 18801)	36.3	30.1333	2
4	GE_222195	<i>L. ervoides</i>	Turkey	Kas to Antalya road 1 km after end sign of Kas on left in olive grove	36.25	29.6833	21
5	ID_6954659	<i>L. nigricans</i>	Croatia	Biograd, Zadar-Sibenik road	43.957236	15.606079	21
6	ID_6953331	<i>L. ervoides</i>	Cyprus	Lemesos, Pano Platres	34.886389	32.859444	21
7	GE_222073	<i>L. ervoides</i>	Turkey	Karatas 22 km from Osmaniye on road to Yarpuz	37.0667	36.4333	21
8	GE_222087	<i>L. ervoides</i>	Turkey	15 km from Osmaniye on road to Karatas	37.0833	36.4333	21
9	ID_6953062	<i>L. ervoides</i>	Croatia	Kroatien, ca. 22 km E Split, Tal der CetiN-exponierter Hang	43.44861	16.71222	21
10	GE_222196	<i>L. ervoides</i>	Turkey	19 km from Finike junction on Kas-Antalya road towards Elmali on left side	36.45	30.15	21
11	ID_6953057	<i>L. ervoides</i>	Greece	Évvoia, N Rovies	38.85722	23.237499	21
12	ID_6953329	<i>L. ervoides</i>	Croatia	4 km N of Baska Voda, Split-Makarska road	43.392945	16.950277	24
13	ID_6954661	<i>L. nigricans</i>	Croatia	4 km N of Baska Voda, Split-Makarska road	43.392945	16.950277	24
14	GE_222009	<i>L. ervoides</i>	Montenegro	Montenegro. Risan	42.5147	18.7	24
15	GE_222011	<i>L. ervoides</i>	Bosnia & Herzegovina	Bosnia-Herzegovina. Mostar	43.3333	17.8333	25
16	ID_6953042	<i>L. ervoides</i>	Greece	Evrytania, WNW Dytiki Frangista	38.965832	21.57139	21
17	GE_222433	<i>L. nigricans</i>	Bosnia & Herzegovina	Bosnia-Herzegovina. Mostar	43.3333	17.8333	25

SM 11: Subset of populations of wild relatives of lentils in Europe selected through the Calibration Method of the Predictive Characterization technique and potentially resistant to lentil rust (*Uromyces vicia-fabae* (Pers.) Schröt)

Ord	Unique ID	Taxa	Country	Collecting site information	Latitude	Longitude	ELC Category
18	GE_222522	<i>L. nigricans</i>	Turkey	Izmir-Karagol 1 km after junction Izmir-Menemen (forest road) Yamanlar Dag	38.5833	27.1667	21
19	ID_6953327	<i>L. ervoides</i>	Bosnia & Herzegovina	16 km E. of Dubrovnik	43.323891	18.129205	24
20	ID_6954149	<i>L. nigricans</i>	Greece	Voiotía, SW Davlia	38.478611	22.68528	22
21	GE_222194	<i>L. ervoides</i>	Turkey	Fethiye to Kas 1 km after Kuscukurincir on right in middle of hairpin bend. After Ese	36.45	29.3	2
22	GE_222326	<i>L. ervoides</i>	Cyprus	Nicosia District Prodromos. Prodromos	34.96417	32.8175	21
23	GE_222545	<i>L. nigricans</i>	Cyprus	Prodromos on the road side to the Forestry Collage 150 m from the parking area	34.9643	32.8174	21
24	GE_663332	<i>L. nigricans</i>	Cyprus	Nicosia District Kalopanagiotis. Kalopanagiotis	34.99333	32.83306	21
25	GE_222197	<i>L. ervoides</i>	Turkey	33 km after junction to Korkuteli on Antalya to Korkuteli road. Hill on R.	37.05	30.5	21
26	GE_222322	<i>L. ervoides</i>	Cyprus	Nicosia District Palaichori. Palaichori	34.90528	33.09111	21
27	GE_222237	<i>L. ervoides</i>	Cyprus	Profitas Elias up the track up the village after water tank on the road to Farmaka	34.8925	33.1125	21
28	ID_6953071	<i>L. ervoides</i>	Greece	Fthiótis, Mesea Kapsi	38.91917	21.929171	22
29	ID_6954148	<i>L. nigricans</i>	Greece	Voiotía, NO Elikon	38.3825	22.839439	21
30	ID_6954663	<i>L. nigricans</i>	Greece	Delphi	38.63333	23.833333	22

GENERAL DISCUSSION

Overview

The main objective posed in this thesis was to contribute to the conservation-access-utilization continuum of plant genetic resources at the national level, focusing on CWR as a source of novel genetic variation worth to be preserved in the interest of food security, given the limited genetic diversity held by current crops and the challenges brought by climate change. In this sense, not only the taxonomic diversity of CWR was taken into account as a conservation target, but also the genetic diversity and potential adaptations to biotic and abiotic stresses that their populations contain. To that aim, we have prioritized CWR of importance for Spain and applied ecogeographical approaches to estimate the among-population genetic diversity of CWR populations, to contribute to a proposal of sites for *in situ* conservation and *ex situ* collection, strengthening the access and, in this manner, the utilization of these valuable plant genetic resources. This discussion section is structured in three subsections relating to the i) identification and prioritization of CWR, ii) conservation of CWR and iii) access and utilization of CWR. The genetic variation of adaptive value component is transversally discussed over the three subsections, as the genetic diversity held by CWR must be the basis for the design of conservation plans that should pursue boosting the use of CWR as gene donors for plant breeding. Figure 1 shows a simplified schema of the processes involved and the products obtained in this thesis, which are the main subjects of this general discussion.

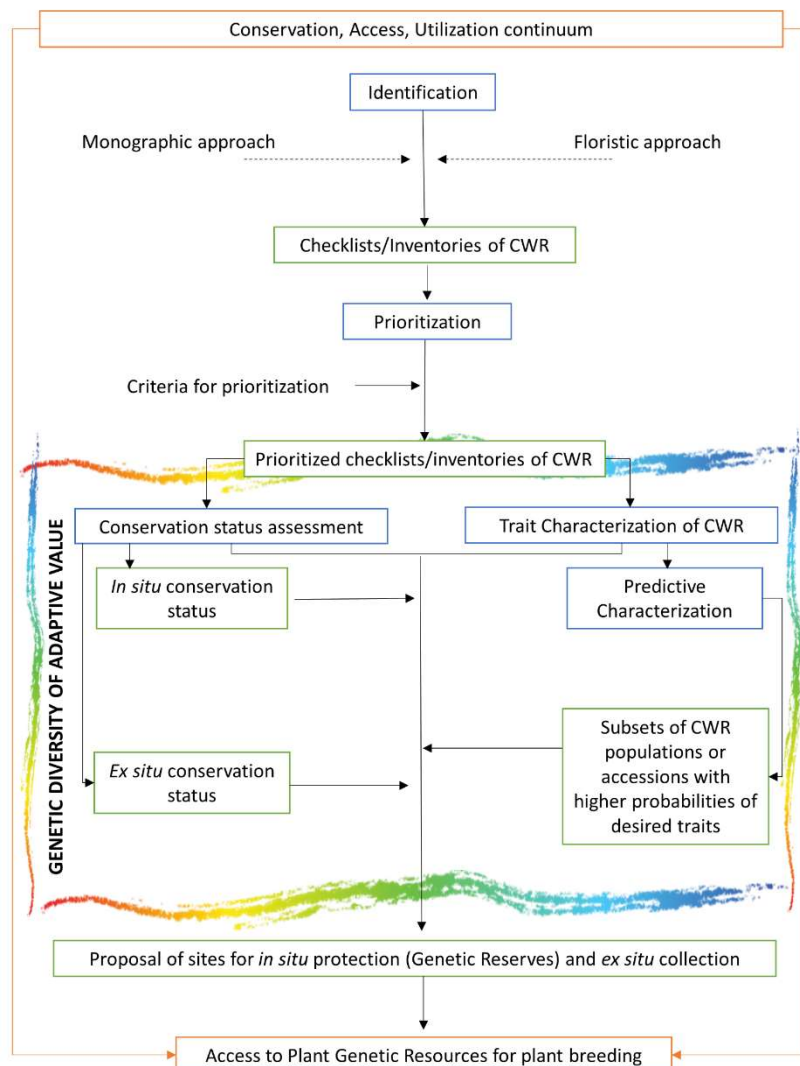


Figure 1: Processes (blue) and products (green) developed in the framework of this thesis.

Identification and prioritization of crop wild relatives

The broad definition of a crop wild relative (CWR) as a wild plant species closely related to a crop provides an ambiguous context for the identification of CWR, sometimes leading to consider as much as 80% of the national Floras as CWR species. This is, for instance, the case of Spain and other countries in the Euro-Mediterranean region (Kell et al., 2007). However, in other cases, a much lower proportion of the Flora of the countries is considered a CWR species (e.g. 14.8% in The Netherlands (van Treuren et al., 2017), around 10% in Benin (Idohou et al., 2013), 35% in England (Fielder et al., 2015a) or 43% in Scotland (Fielder et al., 2015b)). Accordingly, and accounting for the differences in plant species richness among countries, the number of species in national CWR checklists can be very different. For example, we find checklists with more than 24000, 6500 and 7000 species in China (Kell et al., 2015), Italy (Ciancaleoni et al., 2021) and Turkey (Tas et al., 2019), respectively, and other checklists with less than 500 species (e.g. Zambia (Mwila et al., 2019)), Benin and The Netherlands. Nevertheless, among the published checklists, the final number of species normally ranges between 1200 species (England and Scotland) and 2000 - 3000 species (e.g. Russia (Smekalova, 2008; Chukhina et al., 2020), United Kingdom (Maxted et al., 2007); Portugal (Magos Brehm et al., 2008), Czech Republic (Taylor et al., 2017), Finland (Fitzgerald, 2013), Cyprus (Phillips et al., 2014) or Norway (Phillips et al., 2016), which implies, in every case, larger checklists than the Spanish checklist proposed in this thesis (929 species). Overall, these are large numbers for implementing effective conservation strategies for CWR and all these checklists were subsequently prioritized.

Criteria for prioritization varies among countries, depending on their idiosyncrasy, but there are shared criteria that all countries applied. The two criteria applied in all cases are ease of crossability with crops (except for the United Kingdom) and economic value of related crops. The application of both criteria, whether the approach for the generation of checklists was monographic (selection of crops and then list related wild species) or floristic (list of all CWR in the area using floristic inventories) (Maxted et al., 2011; Magos Brehm et al., 2013), resulted in the definition of different groups of CWR depending on the use of the related crop. In this sense, in Spain we established four categories (Food, Fodder and Forage, Ornamental and Industrial and Other Uses) that were similarly established in all consulted national inventories, with the exception of Venezuela (Berlingeri and Crespo, 2012), Mexico (Contreras-Toledo et al., 2018) and Italy (Ciancaleoni et al., 2021), that only focused on CWR related to human food. Thus, the overall trend followed in the last decade, and opposite to the suggested existence of biases favoring wild food crop relatives against other groups such as ornamentals or forage species in the generation of checklists and inventories (Meilleur and Hodgkin, 2004), is the inclusion of categories not only important for food security, but also for the economic sustainability of the countries. In any case, the criteria applied for the prioritization of CWR in Spain was intentionally biased in favor of food and forage categories in the interest of food security. This preferential treatment is also implemented in other national inventories: for example, Khoury et al., (2013) only prioritized CWR in major agricultural crops for the USA, Fielder et al. (2015a and 2015b) only prioritized species in the human and animal food categories, and Cyprus and Turkey only included these two categories in their inventories (Phillips et al., 2014; Tas et al., 2019).

Following on the main criteria applied for prioritization of checklists, in Spain we considered as a priority those CWR classified in any of the IUCN threat categories or those endemic to the country (excluding Ornamental and Industrial and Other uses categories, as explained above). Regarding relative threat status, it is worth mentioning that it is a common criterion for prioritization of CWR as shown in the processes followed by 10 out of the 18 published national prioritized inventories (Venezuela, Finland, USA, China, Scotland, England, Turkey, The Netherlands, Russia and United Kingdom) and in the prioritization of plant genetic resources for *ex situ* collection in Israel (Barazani et al., 2008). Furthermore, it is also a widespread criterion for prioritization when following point scoring procedures (*i.e.*, assigning values according to agreed criteria, as first described for CWR prioritization in Portugal (Magos-Brehm et al., 2010) and subsequently applied in the prioritization of inventories of Benin (Idohou et al., 2013), Mexico (Contreras-Toledo et al., 2018) or Zambia (Mwila et al., 2019), increasing to 13 the number of countries the threat status criterion for prioritization. The endemic status is also a common criterion used to select those CWR of particular importance for the country. Except for China or USA, all countries apply the endemism criterion for prioritization or similar criteria that considers its rarity (e.g. Russia) or national distribution (Mexico, Zambia or Benin) of inventoried CWR.

Last, but not least, the native status of prioritized CWR should be addressed in this discussion, directly linking to genetic diversity of CWR. In this sense, we found different strategies among consulted inventories. Introduced and naturalized taxa are included in most of the inventories (*e.g.*, Portugal, Italy, Zambia, the Czech Republic, Norway, Scotland, England, the USA, Benin, Venezuela), claiming that these genetic resources occurring in the country and linked to important crops should be taken into account. For instance, Bossdorf et al. (2005) argue that the evolution in the country (although short) might have provided these CWR with useful genetic diversity for crop breeding. On the contrary, highly biodiverse countries such as Turkey or China, directly focused on native wild species, as done in the Spanish case. As discussed in Chapter 1, both approaches may entail benefits, however it is expected a higher genetic variation in native CWR, as they are closer to their centers of diversity (Corinto, 2014). In this sense, and despite the inclusion of introduced and naturalized CWR in the inventories, many of them restrict their presence in the prioritized inventories. That is the case for the selected CWR for *ex situ* collection in Israel or priority CWR in Benin; the case of Scotland and the Czech Republic in which only native or archeophytes were considered for prioritization; the Netherlands which only considered naturalized or introduced species if these naturalization events took place before 1900, or the USA, in which higher priority was given to native taxa.

Conservation Status Assessment

The assessments of CWR conservation status – both *in situ* and *ex situ* – are the starting point to evaluate the preservation of CWR in each country. Remarkably, the conservation status assessment has been traditionally performed independently, separating *in situ* and *ex situ* actions. This is partly due to the different responsibilities granted to institutions taking care of conservation actions, assuming that genebanks implement *ex situ* conservation actions and that protected areas managers take care of the *in situ* conservation. In most cases, although pursuing the same conservation objective, these different actions are uncoordinated, causing gaps in conservation actions. Furthermore, if particularly focusing on CWR, their *in situ* conservation is neglected in many cases due to a simplified view that assumes that protected area managers can easily adapt ongoing management plans to include CWR (Hunter et al., 2012). Hunter et al. (2012) also warned about the threats faced by gene banks for the *ex situ* conservation of CWR, based on the lack of coordinated systems and insecure funding, facts that might compromise the adequate conservation of the accessions. Thus, CWR preservation must involve multiple actors that are expected to coordinate actions to achieve conservation goals (Magos Brehm et al., 2013). The implementation of assessments for CWR conservation must seek the complementary conservation, developing integrated strategies (Maxted et al., 2015) and management plans (Iriondo et al., 2021). In this sense, in this thesis both approaches have been explored searching for complementary conservation: we have evaluated the distribution of priority CWR and their *ex situ* conservation status (Chapters 1 and 2) and performed gap and complementarity analyses using protected areas in the Natura 2000 network for the identification of suitable sites for the establishment of genetic reserves (Chapters 2 and 3). In line with this, most of the above-mentioned inventories of other countries have also been subject of *in situ* and *ex situ* conservation assessments. For instance, *in situ* conservation status was evaluated for priority CWR of Benin, Portugal, Venezuela, Finland, Cyprus, England, Scotland, Norway, the Czech Republic, Turkey, Zambia, United Kingdom and Russia, including also a regional global assessment (Vincent et al., 2019). The *ex situ* conservation status was assessed for Portugal, Venezuela, USA, England, Scotland and Norway, plus the global assessment performed by Castañeda-Álvarez et al. (2016). Furthermore, The Netherlands assessed the vulnerability of CWR populations to climate change, in an attempt to clearly direct conservation efforts in a pragmatic manner. The lack of evaluation of the vulnerability of species or sites to climate change in this thesis might be considered as a limitation, as it might compromise the long-term stability of genetic reserves. However, the work on conservation of CWR in Spain has been continued beyond the development of this thesis and these type of analyses are now included in the National Strategy for the Conservation and Use of CWR and wild harvested plants of Spain (Molina Pertíñez et al. in press). In this Strategy, the vulnerability of CWR to climate change is addressed, making this issue one of the objectives to accomplish within the Goal A of the Strategy.

The *in situ* conservation assessment by any of the methodologies used (*i.e.* richness analysis, gap analyses against protected areas networks or complementarity analyses using protected areas or cells) is evidently affected by

the amount and quality of the available chorological data. In this thesis, the distribution data for *in situ* evaluation was obtained from the Global Information Facility (GBIF, www.gbif.org) which is, up to date, the most used open access species distribution database. However, this database is also reported to contain biases in the available data due to different sampling efforts, national involvement and funding, and even because of societal and taxonomic preferences (Beck et al., 2014; Troudet et al., 2017; Ronquillo et al., 2020). These biases have been discussed in recent works addressing European CWR complementary conservation, acknowledging also a low participation of certain countries in the GBIF database or restriction on access to sensitive data (i.e., threatened species, narrow endemic taxa) (Rubio Teso et al., 2021). Taking into account these limitations and the overall representation of high priority CWR in protected areas in Spain obtained (74% of target species have at least one population in Natura 2000, Chapter 2), we consider that CWR in Spain are well represented in protected areas, as our findings can be considered as conservative results (i.e., we can expect more CWR species and populations to occur inside protected areas). However, the mere presence of CWR populations within the limits of protected areas does not guarantee their conservation and, much less, the conservation of their genetic diversity.

It is worth mentioning another novel approach used for the conservation assessment of CWR diversity. That is the case of the recent work by González-Orozco et al., (2021) who applied a phylogenetic approach in combination with species distribution modeling to assess the *in situ* and *ex situ* conservation status of Colombian CWR. They stress the importance of paying attention not only the taxonomic richness but also to the suitability of the preserved genetic diversity for crop breeding when planning conservation actions for CWR (i.e. take into account genetic compatibility with crops; issue that has been addressed in this thesis by the application of the gene pool and taxon group concept, Chapter 1).

Conservation of genetic diversity is a priority in conservation management and should aim to preserve the evolutionary potential of the species in the long term (Foster Huenneke, 1991). The concern in the conservation of genetic diversity and how to approach it has been largely discussed in the context of both threatened plant species and plant genetic resources conservation. For instance, Brown and Briggs, (1991), already 30 years ago, suggested the sampling of, at least, five populations to adequately cover and *ex situ* preserve the genetic diversity of endangered species. They argued that endangered species are not widely distributed and normally have few populations. These minimum was further extended to 50 sites by Brown and Marshall in 1995, adducing that distribution restrictions experienced by threatened species are not applicable to non-endangered taxa, and thus a higher number of populations was needed to cover the genetic diversity of these species. Steps forward in the estimation of the genetic diversity of the species, led to the proposal of sampling at least 35% of the known populations of a species, targeting in this way 70% of the genetic diversity – avoiding arbitrary numbers that might not suit each target species – for *in situ* conservation planning (Whitlock et al., 2016). Up to date, the minimum of five populations is the most common used indicator in the field of CWR to assess their *in situ* or *ex situ* conservation status. Both the *in situ* and *ex situ* assessments performed in England (Fielder et al., 2015a) and Scotland (Fielder et al., 2015b) proposed the threshold of five populations/accessions to evaluate the conservation status of the targeted CWR. Concerning the *ex situ* assessments, five accessions were also the minimum threshold settled for Finland (Fitzgerald, 2013), Norway (Phillips et al., 2016) or Zambia (Mwila et al., 2019) and slightly increased to a minimum of ten accessions in the global assessment of the *ex situ* conservation of CWR by Castañeda-Álvarez et al. (2016). In this thesis, both the minimum five populations/accessions and the 35% threshold of populations have been assessed (Chapter 1 and Chapter 2) as starting point for the evaluation of the conservation of genetic diversity.

Following on the importance of not only the genetic diversity of a species, but also in its potential adaptive value, Brown and Briggs (1991) suggested the interest on sampling diverse ecotypes as a source of potential adaptations, idea that directly links to the ecogeographic approach used in this thesis (Chapter 3 and Chapter 4). The use of Ecogeographic Land Characterization maps (ELC maps) is considered as a step forward in the estimation of genetic variation of adaptive value for plant genetic resources conservation (Parra-Quijano et al., 2020). Having in mind that the main objective for the establishment of a genetic reserve of CWR is the preservation of genetic diversity of the species and the evolutionary potential of their populations (Maxted et al., 2008; Iriando et al., 2012), the use of ELC maps is widely followed in the conservation assessments of CWR. For instance, Phillips et al. (2016) used the ELC categories for sampling design in Norway, trying to

cover the full ecogeographic range of the species' populations and assessed the ELC categories representation within protected areas. Similar approaches were followed for the conservation assessments in Sweden (Weibull and Phillips, 2020), Mexico (Contreras-Toledo et al., 2019), the Czech Republic (Taylor et al., 2017), in the global *in situ* assessment carried out by Vincent et al. (2019) and similar maps were used in the global *ex situ* analyses of Castañeda-Álvarez et al. (2016).

In this thesis, not only ELC maps have been used, but we have also proposed an innovative ELC application by linking populations to ELC categories in which they occur (Chapter 3). We propose a new conservation target unit that is built by joining the populations' occurrences of CWR to each of the ELC categories in which they occur, putting the focus on the adaptive value of each population of a species. To our knowledge, this is the first time that such combinations are used as conservation target units. Further to current approaches in which ELC representation of *in situ* CWR populations is assessed (Rubio Teso et al., 2013; Phillips et al., 2016; Taylor et al., 2017; Contreras-Toledo et al., 2019; Vincent et al., 2019; Weibull and Phillips, 2020) or *ex situ* collections planned or evaluated in their genetic representation according to ELC categories (Parra-Quijano et al., 2012a; García et al., 2017; García et al., 2017) this methodology proposes to conserve at least one population of each of the CWR-Eco combinations and cover in this way the overall potential genetic diversity of the targeted species.

Based on the hypothesis that different environments can exert divergent selective pressures on plant populations, and thus genetic differentiation in genes of adaptive value (Thormann et al., 2014), Parra-Quijano et al. (2012b) provided a conceptual framework supporting the applicability of ELC maps for the design of conservation plans for plant genetic resources. In this way, ELC maps have been reported to show a good performance for predicting phenotype differentiation for *Lupinus* species in the field according to ELC categories (Parra-Quijano et al., 2012a). Nonetheless, linking the genetic diversity to environmental patterns does not always find a close association. For instance, Foster Huenneke (1991) provided some examples of unsuccessful correlations, citing some works carried out in the decade of the 70's (mainly with isozymes). In a similar way, Thormann et al. (2016b) did not find a significant correlation among climatic differences and genetic structure in barley using microsatellites markers. However, it should be noted that these works do not estimate genetic diversity of adaptive value, but neutral genetic diversity. Neutral genetic diversity, by definition, does not affect fitness, it is not subjected to selective pressures and, therefore, does not allow to know to which extent the evolutionary and adaptive potential of a species could be affected by environmental conditions (González Campos, 2019).

More recently, and using data from sequenced regions that are neutral and also under selection, Reeves and Richards (2018) claimed that geographic and environmental distances are not good proxies for the estimation of functional genetic diversity, finding biases in haplotypic representation in *Populus* and *Sorghum* species. However, García Sánchez (2020) provided some limitations to the findings of Reeves and Richards (2018), pointing to the lack of edaphic variables in the populations characterization and also that they did not take into account environmental variables' contribution to the distribution of the species, contrary to what is done for the generation of ELC maps. In addition, Di Santo and Hamilton (2020) tested the neutral, adaptive and functional genetic diversity of 15 plant species using geographic and environmental distances as predictors for such diversity, providing strong arguments (close to 71% of adaptive variation and 45% of functional diversity captured) for the use of environmental distances in the design of *ex situ* collecting plans.

Most published works related to CWR conservation consider multi-species approaches to increase the efficiency of *in situ* conservation. For example, richness analyses are applied in the collecting strategy of 98 CWR to cereal crops in Spain (García et al., 2017), also in the proposal of 150 sites for global conservation of CWR (Vincent et al., 2019) or for the proposal of sites for the establishment of genetic reserves in the UK (Maxted et al., 2007). Complementarity analyses, iterative processes that maximize the number of species in the minimum number of places (Rebelo, 1994), are also a methodology that intends to protect multiple species at the minimum cost. Complementarity analyses have been applied in the conservation assessments of Norway, Cyprus, Zambia or Turkey, just to cite some. Both richness and complementarity analyses have been implemented in this thesis (Chapter 1, Chapter 2 and Chapter 3), and results show that they are an excellent approach to conserve CWR *in situ* and *ex situ* in Spain. However, the increment in the number of target

conservation units, resulting when the combination of CWR with ELC categories is used, may considerably increase the resources needed to implement *in situ* and *ex situ* conservation actions. Therefore, there is an urgency to implement more efficient approaches based on multiple-species conservation. The integration of phytosociological approaches may be helpful in this regard. Results obtained in Chapter 3 highlight the usefulness of phytosociological associations to combine multiple-species conservation along with the potential genetic diversity their populations might contain. Since this methodology has been implemented to a reduced group of CWR, its application to other groups of CWR must be tested. This approach might be useful to enhance the already established genetic reserves of specific CWR with allied CWR species. This could be the case for the established network of *Apium* genetic reserves in Germany (Bönisch et al., 2015) or the proposed genetic reserves of *Beta* relatives (Frese et al., 2017).

Access and utilization of crop wild relatives

The establishment of genetic reserves should contemplate the current and future utilization of the genetic diversity preserved, making it available for potential users (Maxted et al., 1997). Besides, one of the aims of the *ex situ* conservation is to make available the material both to reinforce natural populations or to be used in scientific essays, among others (Bacchetta et al., 2008). In this way, the access to genetic diversity to be used would be linking the conservation efforts to the sustainable use of these genetic resources, as suggested by Maxted et al. (2008). However, an enhanced access to plant material whether from the *in situ* genetic reserves or from the *ex situ* collections, needs a previous characterization of the contained diversity, both in the genotypic and the phenotypic component. In this way, potential users could orientate sampling and trials efforts; otherwise, the effective access to genetic variation of adaptive value would be impractical. The genotypic characterization of all populations that are part of a genetic reserve network or in a gene bank collection is a costly and time-consuming process (Dulloo et al., 2008). Thus, the implementation of methodologies that may help the characterization of genetic variation of adaptive value is a key element when designing conservation plans aimed also to facilitate the access to such variation.

Falconer (1952) already proposed the selection of populations under the same environment in which the improved breed is destined to live. That is the principle applied to the selection of CWR for crop breeding through the predictive characterization methodology (Thormann et al., 2014a, 2016a) and the Focused Identification of Germplasm Strategy (FIGS) (Mackay and Street, 2004). Heterogeneous environmental conditions, by generating different types of limitations to living organisms, are likely to promote divergent selection in the populations of a given species and lead to phenotypic differentiation through local adaptation. Predictive characterization aims to help in the selection of germplasm for breeding and pre-breeding purposes, identifying CWR populations with higher probabilities of containing trait adaptations than if randomly chosen. To reach this objective, the incorporation of indicators helping in the discrimination of genetic variation of adaptive value is required. In this regard, predictive characterization techniques involve the use of ecogeographic information, including bioclimatic, edaphic, geophytic and other environmental variables to characterize populations. Furthermore, implications of phenotypic plasticity (*i.e.*, the capacity of some genotypes to produce different phenotypic responses depending on the environmental pressures) are not tested in these works.

FIGS and predictive characterization techniques have been shown useful for the identification of germplasm with adaptive traits for crop breeding, by using climatic and environmental variables in the search of adaptive diversity. Regarding the FIGS approach (used with landraces and cultivated diversity), and just to cite some examples, Khazaei et al. (2013) found ecotypic differentiation due to drought pressures in faba bean, Bari et al. (2012) reported resistance to wheat stem rust associated to certain environments and Endresen et al. (2011) tested the subset selections for wheat stem rust and barley net blotch resistances, successfully finding correlations between resistance and certain climate variables. On the other hand, although predictive characterization is becoming popular in the search for adaptive traits in CWR (Thormann et al., 2014b; García et al., 2017; Rubio Teso et al., 2020), works validating the results of predictive characterization subsets are still scarce. García Sánchez et al., (2019) experimentally validated the results obtained through this methodology, finding a significant association between the ecogeographic variables used and the acyanogenic status of white

clover. Although further experimental studies are needed to validate the results of this approach, based on the successful results of the FIGS methodology with landraces, we expect similar or even higher performance of predictive characterization techniques with CWR. That is because, contrary to landraces, CWR have only evolved under natural selective pressures and thus, one would expect to find stronger correlations between adaptive traits and the type of environment. Nonetheless, experimental essays and quantitative genetic studies to assess the wide applicability and appropriate scale of these methodologies are unavoidable steps to adequately assess the benefits of predictive characterization.

In conclusion, the link between conservation and access to the genetic diversity of adaptive value of CWR is approached in this thesis through the identification of priority CWR of interest for Spain (Chapter 1), the identification of high-priority CWR that are in urgent need of conservation (Chapter 2), the proposal of establishment of multispecies genetic reserves considering adaptive genetic variation – through the use of ELC maps – (Chapter 3) and the proposal of populations for collecting germplasm of wild lentil as candidates in their tolerance to drought, soil salinity and waterlogging and their resistance to lentil rust (Chapter 4), taking into account environmental variables for the selection of subsets.

References

- Bacchetta, G., Bueno Sánchez, A., Fenu, G., Jiménez-Alfaro, B., Mattana, E., Piotto, B., et al. eds. (2008). *Conservación ex situ de plantas silvestres*. Principado de Asturias / La Caixa.
- Barazani, O., Perevolotsky, A., and Hadas, R. (2008). A problem of the rich: Prioritizing local plant genetic resources for ex situ conservation in Israel. *Biol. Conserv.* doi:10.1016/j.biocon.2007.10.014.
- Bari, A., Street, K., Mackay, M., Endresen, D. T. F., de Pauw, E., and Amri, A. (2012). Focused identification of germplasm strategy (FIGS) detects wheat stem rust resistance linked to environmental variables. *Genet. Resour. Crop Evol.* 59, 1465–1481. doi:10.1007/s10722-011-9775-5.
- Beck, J., Böller, M., Erhardt, A., and Schwanghart, W. (2014). Spatial bias in the GBIF database and its effect on modeling species' geographic distributions. *Ecol. Inform.* 19, 10–15. doi:10.1016/j.ecoinf.2013.11.002.
- Berlinger, C., and Crespo, M. B. (2012). Inventory of related wild species of priority crops in Venezuela. *Genet. Resour. Crop Evol.* doi:10.1007/s10722-011-9709-2.
- Bönisch, M., Herden, T., Nachtigall, M., Friesen, N., Zander, M., and Frese, L. (2015). Establishment of Genetic Reserves for Crop Wild Relatives. poster. doi:10.13140/RG.2.2.15771.67363.
- Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., and Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144, 1–11. doi:10.1007/s00442-005-0070-z.
- Brown, A. H. D., and Briggs, J. D. (1991). "Sampling strategies for genetic variation in ex situ collectoins of endangered plant species," in *Genetics and conservation of rare plants*, eds. D. A. I. Falk and K. E. Holsinger (New York; Oxford: Oxford University Press), 99–119.
- Brown, A. H. D., and Marshall, D. R. (1995). "A basic sampling strategy: theory and practice," in *Collecting plant genetic diversity: Technical guidelines*, eds. L. Guarino, R. V Ramanantha, and R. Reid (Wallingford, UK: CAB International), 75–91.
- Castañeda-Álvarez, N. P., Khoury, C. K., Achicanoy, H. A., Bernau, V., Dempewolf, H., Eastwood, R. J., et al. (2016). Global conservation priorities for crop wild relatives. *Nat. Plants* 2, 1–6. doi:10.1038/nplants.2016.22.
- Chukhina, I., Shipilina, L., Bagmet, L., Talovina, G., and Smekalova, T. (2020). Results of studying wild relatives of the cultivated plants of Russia. *Biol. Commun.* 65, 41–52. doi:10.21638/spbu03.2020.104.
- Ciancaleoni, S., Raggi, L., Barone, G., Donnini, D., Gigante, D., Domina, G., et al. (2021). A new list and prioritization of wild plants of socioeconomic interest in Italy: toward a conservation strategy. *Agroecol. Sustain. Food Syst.* doi:10.1080/21683565.2021.1917469.
- Contreras-Toledo, A. R., Cortés-Cruz, M. A., Costich, D., Rico-Arce, M. L., Magos Brehm, J., and Maxted, N. (2018). A crop wild relative inventory for Mexico. *Crop Sci.* 58, 1292–1305. doi:10.2135/cropsci2017.07.0452.
- Contreras-Toledo, A. R., Cortés-Cruz, M., Costich, D. E., Rico-Arce, M. D. L., Magos Brehm, J., and Maxted, N. (2019). Diversity and conservation priorities of crop wild relatives in Mexico. *Plant Genet. Resour. Characterisation Util.* 17, 140–150. doi:10.1017/S1479262118000540.
- Corinto, G. L. (2014). Nikolai Vavilov 's Centers of Origin of Cultivated Plants With a View to Conserving Agricultural Biodiversity. *Hum. Evol.* 29, 1–17.

- Di Santo, L. N., and Hamilton, J. A. (2020). Using environmental and geographic data to optimize ex situ collections and preserve evolutionary potential. *Conserv. Biol.* 35, 733–744. doi:10.1111/cobi.13568.
- Dulloo, M. E., Labokas, J., Iriondo, J. M., Maxted, N., Lane, E., Laguna, E., et al. (2008). “Genetic Reserve Location and Design,” in *Conserving Plant Genetic Diversity in Protected Areas. Population Management of Crop Wild Relatives*, eds. J. M. Iriondo, N. Maxted, and M. E. Dulloo (Oxfordshire, UK), 23–64.
- Endresen, D. T. F., Street, K., Mackay, M., Bari, A., and de Pauw, E. (2011). Predictive association between biotic stress traits and eco-geographic data for wheat and barley landraces. *Crop Sci.* 51, 2036–2055. doi:10.2135/cropsci2010.12.0717.
- Falconer, D. S. (1952). The problem of environment and selection. *Am. Nat.* LXXXVI, 293–298.
- Fielder, H., Brotherton, P., Hosking, J., Hopkins, J. J., Ford-Lloyd, B., and Maxted, N. (2015a). Enhancing the Conservation of Crop Wild Relatives in England. *PLoS One* 10, e0130804. doi:10.1371/journal.pone.0130804.
- Fielder, H., Smith, C., Ford-Lloyd, B., and Maxted, N. (2015b). Enhancing the conservation of crop wild relatives in Scotland. *J. Nat. Conserv.* 29, 51–61. doi:10.1016/j.jnc.2015.11.002.
- Fitzgerald, H. (2013). The National Crop Wild Relative Strategy Report for Finland.
- Foster Huenneke, L. (1991). “Ecological Implications of Genetic Variation in Plant Populations,” in *Genetic and Conservation of Rare Plants*, eds. D. A. I. Falk and K. E. Holsinger (New York; Oxford: Oxford University Press), 31–44.
- Frese, L., Bülow, L., Castro, S., Duarte, M. C., Iriondo, J. M., Lohwasser, U., et al. (2017). ECPGR Activity Grant Scheme – First Call, 2014 Final Activity Report: Genetic diversity of Patellifolia species (GeDiPa).
- García, R. M., Parra-Quijano, M., and Iriondo, J. M. (2017). Identification of ecogeographical gaps in the Spanish Aegilops collections with potential tolerance to drought and salinity. *PeerJ* 2017. doi:10.7717/peerj.3494.
- García, R. M., Parra-Quijano, M., and Iriondo, J. M. (2017). A multispecies collecting strategy for crop wild relatives based on complementary areas with a high density of ecogeographical gaps. *Crop Sci.* 57, 1059–1069. doi:10.2135/cropsci2016.10.0860.
- García Sánchez, R. M. (2020). Conservation of plant genetic resources: a spatial and ecogeographical approach. PhD thesis.
- García Sánchez, R. M., Parra-Quijano, M., Greene, S., and Iriondo, J. M. (2019). Predictive characterisation identifies global sources of acyanogenic germplasm of a key forage species. *Crop Pasture Sci.* 70, 546–554.
- González-Orozco, C. E., Sosa, C. C., Thornhill, A. H., and Laffan, S. W. (2021). Phylogenetic diversity and conservation of crop wild relatives in Colombia. *Evol. Appl.* 14, 2603–2617. doi:10.1111/eva.13295.
- González Campos, J. M. (2019). Diversidad genética neutral y adaptativa, una simple explicación. *Cienc. Investig. For.* 25, 81–98. doi:10.52904/0718-4646.2019.512.
- Hunter, D., Guarino, L., Khoury, C. K., and Dempewolf, H. (2012). “A community divided: Lessons from the conservation of crop wild relatives around the world,” in *Agrobiodiversity Conservation: Securing the diversity of crop wild relatives and landraces*, eds. N. Maxted, M. E. Dulloo, B. V. Ford-Lloyd, L. Frese, J. M. Iriondo, and M. A. A. Pinheiro de Carvalho (Wallingford, Oxfordshire, UK: CAB International), 298–304.
- Idohou, R., Assogbadjo, A. E., Fandohan, B., Gouwakinnou, G. N., Glele Kakai, R. L., Sinsin, B., et al. (2013). National inventory and prioritization of crop wild relatives: Case study for Benin. *Genet. Resour. Crop Evol.* 60, 1337–1352. doi:10.1007/s10722-012-9923-6.
- Iriondo, J. M., Magos-Brehm, J., Dulloo, M. E., and Maxted, N. eds. (2021). *Crop Wild Relative Population Management Guidelines*. Farmer’s Pride: Networking, partnerships and tools to enhance in situ conservation of European plant genetic resources. Available at: https://more.bham.ac.uk/farmerspride/wp-content/uploads/sites/19/2021/07/Crop_Wild_Relative_Population_Management_Guideli.
- Iriondo, J. M., Maxted, N., Kell, S. P., Ford-Lloyd, B. V., Lara-Romero, C., Labokas, J., et al. (2012). “Quality standards for genetic reserve conservation of crop wild relatives,” in *Agrobiodiversity Conservation: Securing the diversity of crop wild relatives and landraces*, eds. N. Maxted, M. E. Dulloo, B. V. Ford-Lloyd, L. Frese, J. M. Iriondo, and M. A. A. Pinheiro de Carvalho (Oxfordshire, UK: CAB International), 72–77.
- Kell, S. P., Knupffer, H., Jury, S. L., Ford-Lloyd, B. V., and Maxted, N. (2007). “Crops and wild relatives of the Euro-Mediterranean region: making and using a conservation catalogue,” in *Crop Wild Relative Conservation and Use*, eds. N. Maxted, B. V. Ford-Lloyd, S. P. Kell, J. M. Iriondo, M. E. Dulloo, and J. Turok (CAB International), 69–109. Available at: <http://centaur.reading.ac.uk/10030/>.
- Kell, S., Qin, H., Chen, B., Ford-Lloyd, B., Wei, W., Kang, D., et al. (2015). China’s crop wild relatives: Diversity for agriculture and food security. *Agric. Ecosyst. Environ.* 209, 138–154. doi:10.1016/j.agee.2015.02.012.
- Khazaei, H., Street, K., Bari, A., Mackay, M., and Stoddard, F. L. (2013). The FIGS (focused identification of germplasm

- strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. *PLoS One* 8, e63107. doi:10.1371/journal.pone.0063107.
- Khoury, C. K., Greene, S., Wiersema, J., Maxted, N., Jarvis, A., and Struik, P. C. (2013). An inventory of crop wild relatives of the United States. *Crop Sci.* 53, 1496–1508. doi:10.2135/cropsci2012.10.0585.
- Mackay, M. C., and Street, K. (2004). Focused identification of germplasm strategy – FIGS. in *Proceedings of the 54th Australian Cereal Chemistry Conference and the 11th Wheat Breeders' Assembly*. (Melbourne: Royal Australian Chemical Institute), 138–141.
- Magos-Brehm, J., Maxted, N., Martins-Loução, M. A., and Ford-Lloyd, B. V. (2010). New approaches for establishing conservation priorities for socio-economically important plant species. *Biodivers. Conserv.* 19, 2715–2740. doi:10.1007/s10531-010-9871-4.
- Magos Brehm, J., Kell, S., Thormann, I., Gaisberger, H., Dulloo, E., and Maxted, N. (2013). INTERACTIVE TOOLKIT FOR CROP WILD RELATIVE CONSERVATION PLANNING version 1.0. Resource Book for the Preparation of National Plans of Crop Wild Relatives and Landraces. Food and Agriculture Organization of the In Situ Conservation and Use of Crop Wild Rela.
- Magos Brehm, J., Maxted, N., Ford-Lloyd, B. V., and Martins-Loução, M. A. (2008). National inventories of crop wild relatives and wild harvested plants: Case-study for Portugal. *Genet. Resour. Crop Evol.* 55, 779–796. doi:10.1007/s10722-007-9283-9.
- Maxted, N., Avagyan, A., Frese, L., Iriondo, J., Brehm, J. M., Singer, A., et al. (2015). ECPGR Concept for in situ conservation of crop wild relatives in Europe. Wild Species Conservation in Genetic Reserves Working Group. European Cooperative Programme for Plant Genetic Resources. Rome, Italy. Available at: www.ecpgr.cgiar.org/fileadmin/temp.21. Available at: www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/WG_UPLOADS_PHASE_IX/WILD_SPECIES/%0AConcept_for_in__situ_conservation_of_CWR_in_Europe.pdf%0A.
- Maxted, N., Hawkes, J. G., Ford-Lloyd, B. V., and Williams, J. T. (1997). “A practical model for in situ genetic conservation,” in *Plant Genetic Conservation: The In Situ Approach*, eds. N. Maxted, B. V. Ford-Lloyd, and J. G. Hawkes (Dordrecht, The Netherlands: Kluwer Academic Publishers), 339–367.
- Maxted, N., Iriondo, J. M., de Hond, L., Dulloo, M. E., Lefèvre, F., Asdal, A., et al. (2008). Genetic reserve management. *Conserv. plant Genet. Divers. Prot. areas*, 65–78.
- Maxted, N., Kell, S., and Brehm, J. M. (2011). Options to Promote Food Security: On-Farm Management and In Situ Conservation of Plant Genetic Resources for Food and Agriculture. *Commision Genet. Resour. Food Agric.*, 25. Available at: <http://www.fao.org/docrep/meeting/022/am489e.pdf>.
- Maxted, N., Scholten, M., Codd, R., and Ford-Lloyd, B. (2007). Creation and use of a national inventory of crop wild relatives. *Biol. Conserv.* doi:10.1016/j.biocon.2007.08.006.
- Meilleur, B. a., and Hodgkin, T. (2004). In situ conservation of crop wild relatives: Status and trends. *Biodivers. Conserv.* 13, 663–684. doi:10.1023/B:BIOC.0000011719.03230.17.
- Molina Pertúñez, A., Torres Lamas, E., Rubio Teso, M. L., Álvarez-Muñiz, C., de la Rosa, L., Tardío Pato, J., et al. Estrategia Nacional de Conservación y Utilización de Parientes Silvestres de los Cultivos (PSC) y Plantas Silvestres de Uso Alimentario (PSUA). 114 pp.
- Mwila, G., Gaisberger, H., International, B., Brehm, J. M., and Kell, S. (2019). Spatial analyses of occurrence data of crop wild relatives (CWR) taxa as tools for selection of sites for conservation of priority CWR in Zambia. *Plant Genet. Resour.*, 1–12. doi:10.1017/S1479262118000497.
- Parra-Quijano, M., Iriondo, J. M., and Torres, E. (2012a). Improving representativeness of genebank collections through species distribution models, gap analysis and ecogeographical maps. *Biodivers. Conserv.* 21, 79–96. doi:10.1007/s10531-011-0167-0.
- Parra-Quijano, M., Iriondo, J. M., and Torres, E. (2012b). Review. Applications of ecogeography and geographic information systems in conservation and utilization of plant genetic resources. *Spanish J. Agric. Res.* 10, 419–429. doi:10.5424/sjar/2012102-303-11.
- Parra-Quijano, M., Iriondo, J. M., Torres, M. E., López, F., Maxted, N., and Kell, S. (2020). CAPFITOGEN 3. Una caja de herramientas para la conservación y promoción del uso de la biodiversidad agrícola. Manual de usuario. 1–5. Available at: <http://www.etitudela.com/entrenadorcomunicaciones/downloads/gsmmanualsmsconfiguratorl.pdf>.
- Phillips, J., Asdal, Å., Magos Brehm, J., Rasmussen, M., and Maxted, N. (2016). In situ and ex situ diversity analysis of priority crop wild relatives in Norway. *Divers. Distrib.* 22, 1112–1126. doi:10.1111/ddi.12470.
- Phillips, J., Kyrtzisz, A., Christoudoulou, C., Kell, S., and Maxted, N. (2014). Development of a national crop wild relative conservation strategy for Cyprus. *Genet. Resour. Crop Evol.* 61, 817–827. doi:10.1007/s10722-013-0076-z.

- Rebello, A. G. (1994). "Iterative selection procedures: Centres of endemism and optimal placement of reserves," in *Botanical Diversity in Southern Africa*, ed. B. J. Huntley (Pretoria: National Botanical Institute), 231–257.
- Reeves, P. A., and Richards, C. M. (2018). Biases induced by using geography and environment to guide ex situ conservation. *Conserv. Genet.* 19, 1281–1293. doi:10.1007/s10592-018-1098-z.
- Ronquillo, C., Alves-Martins, F., Mazimpaka, V., Sobral-Souza, T., Vilela-Silva, B., Medina, N. G., et al. (2020). Assessing spatial and temporal biases and gaps in the publicly available distributional information of Iberian mosses. *Biodivers. Data J.* 8. doi:10.3897/BDJ.8.E53474.
- Rubio Teso, M. L., Álvarez-Muñiz, C., Gaisberger, H., Kell, S. P., Lara-Romero, C., Magos Brehm, J., et al. (2021). European crop wild relative diversity: towards the development of a complementary conservation strategy. 100.
- Rubio Teso, M. L., Álvarez-Muñiz, C., Raggi, L., Negri, V., García, R., Parra-Quijano, M., et al. (2020). Farmer's Pride: Identifying in situ areas with useful adaptive traits.
- Rubio Teso, M. L., Iriondo, J. M., Parra-Quijano, M., and Torres, E. (2013). Report for National Strategy for the Conservation of Crop Wild Relatives of Spain.
- Smekalova, T. N. (2008). "National Crop Wild Relative In Situ conservation Strategy for Russia," in *Crop Wild Relative Conservation and Use*, eds. N. Maxted, B. V. Ford-Lloyd, S. P. Kell, J. M. Iriondo, M. E. Dulloo, and J. Turok (Oxfordshire, UK: CAB International), 143–151.
- Tas, N., West, G., Kircalioglu, G., Topaloglu, S. B., Phillips, J., Kell, S., et al. (2019). Conservation gap analysis of crop wild relatives in Turkey. *Plant Genet. Resour. Characterisation Util.* 17, 164–173. doi:10.1017/S1479262118000564.
- Taylor, N. G., Kell, S. P., Holubec, V., Parra-Quijano, M., Chobot, K., and Maxted, N. (2017). A systematic conservation strategy for crop wild relatives in the Czech Republic. *Divers. Distrib.* 1–15. doi:10.1111/ddi.12539.
- Thormann, I., Parra-Quijano, M., Endresen, D. T. F., Rubio-Teso, M. L., Iriondo, J. M., and Maxted, N. (2014a). Predictive characterization of crop wild relatives and landraces. *Biodiversity Int.* 40.
- Thormann, I., Parra-Quijano, M., Iriondo, J. M., Rubio-Teso, M. L., Endresen, D. T. F., Dias, S., et al. (2014b). New predictive characterization methods for accessing and using CWR diversity. in *International Conference on ENHANCED GENEPOOL UTILIZATION - Capturing wild relative and landrace diversity for crop improvement, Cambridge, United Kingdom, 16-20 June 2014. Book of Abstracts*.
- Thormann, I., Parra-Quijano, M., Rubio Teso, M. L., Endresen, D. T. F., Dias, S., Iriondo, J. M., et al. (2016a). "Predictive Characterization Methods for Accessing and Using CWR Diversity," in *Enhancing Crop GenePool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement*, eds. N. Maxted, M. E. Dulloo, and B. Ford-Loyd (CAB International), 64–77.
- Thormann, I., Reeves, P., Reilley, A., Engels, J. M. M., Lohwasser, U., B??rner, A., et al. (2016b). Geography of genetic structure in barley wild relative *Hordeum vulgare* subsp. *Spontaneum* in Jordan. *PLoS One* 11, 1–19. doi:10.1371/journal.pone.0160745.
- Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., and Legendre, F. (2017). Taxonomic bias in biodiversity data and societal preferences. *Sci. Rep.* 7, 1–14. doi:10.1038/s41598-017-09084-6.
- van Treuren, R., Hoekstra, R., and van Hintum, T. J. L. (2017). Inventory and prioritization for the conservation of crop wild relatives in The Netherlands under climate change. *Biol. Conserv.* 216, 123–139. doi:10.1016/j.biocon.2017.10.003.
- Vincent, H., Amri, A., Castañeda-Álvarez, N. P., Dempewolf, H., Dulloo, E., Guarino, L., et al. (2019). Modeling of crop wild relative species identifies areas globally for in situ conservation. *Commun. Biol.* 2, 136. doi:10.1038/s42003-019-0372-z.
- Weibull, J., and Phillips, J. (2020). Swedish Crop Wild Relatives : towards a national strategy for in situ conservation of CWR. *Genet. Resour.* 1, 17–23. doi:10.46265/genresj.2020.1.17-23.
- Whitlock, R., Hipperson, H., Thompson, D. B. A., Butlin, R. K., and Burke, T. (2016). Consequences of in-situ strategies for the conservation of plant genetic diversity. *Biol. Conserv.* 203, 134–142. doi:10.1016/j.biocon.2016.08.006.

CONCLUSIONES

1. La enorme diversidad taxonómica de parientes silvestres en España hace necesaria una priorización de especies para focalizar los esfuerzos de conservación. Aplicando criterios de facilidad de cruzamiento con los cultivos, grado de amenaza y endemidad, la mayoría de las 578 especies de PSC priorizadas en este estudio poseen potencialidad de cruzamiento con sus parientes cultivadas, cerca de la mitad son endémicas de España y aproximadamente un cuarto de ellas tienen algún grado de amenaza.
2. El número de especies de PSC priorizadas en cada categoría de uso es homogéneo, destacando ligeramente las categorías de Pastos y Forraje y Ornamental. Asimismo, las comunidades autónomas muestran una riqueza en especies priorizadas de PSC homogénea, si bien Andalucía, Castilla y León, y Castilla La Mancha destacan sobre las demás.
3. La conservación *ex situ* de PSC priorizados es satisfactoria en cuanto que mantiene representadas casi tres cuartas partes de las especies en bancos de germoplasma. No obstante, es necesaria una recolección adicional de muestras de semillas que complete las colecciones, tanto a nivel de representatividad de especies como en número de accesiones, que permitan una adecuada conservación de la diversidad genética de las especies seleccionadas. En esta tesis se establecen cuatro categorías de prioridad de recolección que tienen en cuenta la importancia de los PSC en términos de facilidad de cruzamiento con los cultivos, grado de amenaza y endemidad, así como la representación de dichas especies en bancos de semillas.
4. El Catálogo Español de Especies Amenazadas protege a menos de la cuarta parte de las especies PSC amenazadas y seleccionadas para la toma de medidas urgentes de conservación. Sin embargo, la mayoría de especies identificadas sí se encuentran protegidas en los catálogos autonómicos de especies amenazadas.
5. La mayoría de especies de PSC prioritarios que requieren medidas de conservación urgentes están representadas en bancos de germoplasma y en áreas protegidas de la Red Natura 2000. En este último caso, algo más de la tercera parte contarían con un número suficiente de poblaciones que, presumiblemente, permitiría conservar de manera adecuada la diversidad genética de la especie.
6. La aproximación multiespecie utilizando asociaciones fitosociológicas demuestra gran efectividad en la cobertura de PSC forrajeras, encontrándose asociaciones que las contienen concentradas en gran número. Además, la combinación de las poblaciones (inventarios georreferenciados) de asociaciones fitosociológicas con las categorías ecogeográficas (Aso-Eco) en las que se encuentran presentes recoge una gran diversidad genética de valor adaptativo entre las asociaciones.
7. Los espacios de Natura 2000 cubren de manera muy satisfactoria tanto la diversidad sintaxonómica, como la diversidad de combinaciones Aso-Eco, permitiendo proponer un reducido número de áreas protegidas (15) para el establecimiento de reservas genéticas de PSC forrajeros. El establecimiento de estas reservas genéticas protegería todas las asociaciones focales y aproximadamente el 40% de la diversidad genética de valor adaptativo estimada, siguiendo una aproximación multiespecie, que reduciría los costes de gestión, así como facilitaría el acceso a recursos genéticos para la mejora genética de cultivos forrajeros.
8. El método de filtrado ambiental de las técnicas de caracterización predictiva identificó tres subconjuntos de poblaciones de PSC de lentejas con mayor probabilidad de contener tolerancia a la sequía (21 poblaciones), la salinidad del suelo (una población) y el encharcamiento (13 poblaciones), que facilita la selección de germoplasma para ser utilizado en mejora vegetal de la lenteja, disminuyendo en gran medida el número de poblaciones candidatas para ensayos de evaluación de tolerancia a estos estreses abióticos.
9. El método de calibración de las técnicas de caracterización predictiva identificó un amplio porcentaje de poblaciones silvestres de PSC de lentejas potencialmente resistentes a la roya (cerca del 85.5%) y permite seleccionar de entre estas, un subconjunto con mayor probabilidad de resistencia (30 poblaciones), candidatas para su evaluación en este rasgo y ser utilizadas en procesos de pre-mejora y mejora genética de la lenteja cultivada. Sin embargo, con el conjunto de datos de evaluación para el modelo de entrenamiento, el método de calibración no se ha mostrado eficaz para la selección de poblaciones de PSC de lentejas potencialmente resistentes al jopo.

LISTA DE PUBLICACIONES

La investigación derivada de esta tesis doctoral ha permitido desarrollar varios trabajos que han derivado en las siguientes publicaciones:

Peer reviewed articles (JRC indexed)

- 1.- Frese, L; Lorenz Bülow, L; Nachtigall, M; **Rubio Teso, ML**; Duarte, MC; Rey, E; Iriondo, JM (2017). Genetic diversity of *Patellifolia patellaris* from the Iberian Peninsula, a crop wild relative of cultivated beets. *Euphytica* 213: 187. doi: 10.1007/s10681-017-2003-4
- 2.- **Rubio Teso, ML**; Torres Llamas, E; Parra-Quijano, M; de la Rosa, L; Fajardo, J; Iriondo, JM (2018). National inventory and prioritization of crop wild relatives in Spain. *Genetic Resources and Crop Evolution* 65: 1237–1253. doi: 10.1007/s10722-018-0610-0 (CAPÍTULO 1)
- 3.- **Rubio Teso, ML**; Torres Lamas, E; Parra-Quijano, M; Iriondo, JM (2018). Identification and assessment of the crop wild relatives of Spain that require most urgent conservation actions. *Mediterranean Botany* 39: 67-75. doi: 10.5209/MBOT.60074 (CAPÍTULO 2)
- 4.- Frese, L; Nachtigall, M; Iriondo, JM; **Rubio Teso, ML**; Duarte, MC; Pinheiro de Carvalho, MA (2018). Genetic diversity and differentiation in *Patellifolia* (Amaranthaceae) in the Macaronesian archipelagos and the Iberian Peninsula and implications for genetic conservation programmes. *Genetic Resources and Crop Evolution* 66:225-241. doi:10.1007/s10722-018-0708-4
- 5.- **Rubio Teso, ML** & Iriondo, JM (2019). In situ conservation assessment of forage and fodder CWR in Spain using phytosociological associations. *Sustainability* 11, 5882. doi:10.3390/su11215882 (CAPÍTULO 3)
- 6.- Matesanz, S; Ramos Muñoz, M; Moncalvillo, B; **Rubio Teso, ML**; López García de Dioniso, S; Romero, J & Iriondo, JM (2020). Plasticity to drought and ecotypic differentiation in populations of a crop wild relative. *Annals of Botany - Special Issue: The Ecology and Genetics of Population Differentiation in Plants*. 10.1093/aobpla/plaa006
- 7.- Raggi, L; Paccico, LC; Caprioni, L; Álvarez-Muñiz, C; Annamaa, K; Barata, AM; Batir-Rusu, D; Díez, MJ; Heinonen, M; Holubec, V; Kell, S; Kutnjak, H; Maierhofer, H; Poulsen, G; Prohens, J; Ralli, P; Rocha, F; **Rubio Teso ML**; Sandru, D; Santamaria, P; Sensen, S; Shoemark, O; Soler, S; Strajeru, S; Thormann, I; Weibull, J; Maxted, N & Negri, V (2022). Analysis of landrace cultivation in Europe: A means to support in situ conservation of crop diversity. *Biological conservation* 10.1016/j.biocon.2022.109460
- 8.- **Rubio Teso, ML**; Lara-Romero, C; Rubiales, D; Parra-Quijano, M & Iriondo, JM (2022). Searching for abiotic tolerant and biotic stress resistant wild lentils for introgression breeding through predictive characterization. *Frontiers in Plant Science. Plant Breeding Section. Introgression Breeding in Cultivated Plants, Volume II*, 10.3389/fpls.2022.817849 (CAPÍTULO 4).

Book chapters:

- 1.- Iriondo, JM; Fielder, H; Fitzgerald, H; Kell, SP; Labokas, J; Magos-Brehm, J; Negri, V; Phillips, J; **Rubio Teso, ML**; Sensen, S; Taylor, N; Maxted, N (2016). National strategies for the conservation of crop wild relatives. In: Maxted, N; Dulloo, ME; Ford-Lloyd, E (eds.) *Enhancing crop genepool use: Capturing wild relative and landrace diversity for crop improvement*: 161-171. CAB International, Oxfordshire.
- 2.- **Rubio Teso, ML**; Kinoshita, K; Iriondo Alegría, JM (2016). Optimized site selection for the in situ conservation of forage CWRs: a combination of community and genetic level perspectives. In: Maxted, N; Dulloo, ME; Ford-Lloyd, E (eds.) *Enhancing crop genepool use: Capturing wild relative and landrace diversity for crop improvement*: 199- 205. CAB International, Oxfordshire
- 3.- Thormann, I; Parra-Quijano, M; **Rubio Teso, ML**; Endresen, DT; Dias, S; Iriondo, JM; Maxted, N (2016). Predictive characterization methods for accessing and using CWR diversity. In: Maxted, N; Dulloo, ME; Ford-

Lloyd, E (eds.) Enhancing crop genepool use: Capturing wild relative and landrace diversity for crop improvement: 64- 77. CAB International, Oxfordshire.

Other scientific articles (non-indexed JCR):

1.- Álvarez, C; **Rubio Teso, ML**; Torres, E & Iriondo, JM (2018). Primeros pasos del proyecto europeo «Farmer's Pride». *Conservación Vegetal* 22: 19-20.

2.- Álvarez-Muñiz, C; **Rubio Teso, ML**; Torres, E; Molina A & Iriondo, JM (2019). Integrated in situ and ex situ conservation of crop wild relatives in Spain. *Crop wild relative newsletter* 12: 19-21.

3.- Álvarez-Muñiz, C; **Rubio Teso, ML**; Magos Brehm, J; Ralli, P; Palmé, A; Dulloo, ME; Maxted, N; Negri, V; Löwenhardt, HMR; Aykas, L; Kell, SP & Iriondo, JM (2021). Crop wild relative network showcases. *Crop wild relative newsletter* 13, issue October, 16-18.

4.- **Rubio Teso, ML**; Álvarez -Muñiz, C; Gaisberger, H; Kell, S; Lara, C; Magos Brehm, J; Maxted, N & Iriondo JM (2021). CWR in Natura 2000 network. *Crop wild relative newsletter* 13, issue October, 19-22.

Technical and Scientific Reports:

1.- **Rubio Teso, ML**; Álvarez-Muñiz, C; Gaisberger, H; Kell, S; Lara-Romero, C; Magos Brehm, J; Maxted N and Iriondo JM (2020). Crop wild relatives in the Natura 2000 network. Deliverable report of the Farmer's Pride Project SFS-04 (GA 774271): MS19. Farmer's Pride: Conserving plant diversity for future generations. Project funded by the Horizon 2020 Framework Programme of the European Union

2.- **Rubio Teso, ML**; Álvarez-Muñiz, C; Gaisberger, H; Kell, S; Lara-Romero, C; Magos Brehm, J; Maxted, N and Iriondo, JM. (2020). *In situ plant* genetic resources in Europe: crop wild relatives. Deliverable of the Farmer's Pride Project: D1.2, 'Knowledge of in situ resources/sites'. Farmer's Pride: Conserving plant diversity for future generations. Project funded by the Horizon 2020 Framework Programme of the European Union.

3.- Álvarez-Muñiz, C; **Rubio Teso, ML**; Magos Brehm, J.; Ralli, P; Palmé, A.; Dulloo, E; Negri, V; Kell, S; Maxted N and Iriondo, JM (2020). Crop wild relative in situ conservation case studies. Deliverable report of the Farmer's Pride Project SFS-04 (GA 774271): Farmer's Pride: Conserving plant diversity for future generations. Project funded by the Horizon 2020 Framework Programme of the European Union.

4.- **Rubio Teso, ML**; Álvarez-Muñiz, C; Raggi, L; Negri, V; García, R; Parra-Quijano, M., Green, S; Kell, S; Maxted, N; Brandehof, J; Civic, K; Janz, C; Weibull, J.; Tas, N. and Iriondo, JM. (2020). Identifying in situ areas with useful adaptive traits. Deliverable report "3.2" of the Farmer's Pride Project SFS-04 (GA 774271): Farmer's Pride: Conserving plant diversity for future generations. Project funded by the Horizon 2020 Framework Programme of the European Union.

5.- Álvarez-Muñiz, C; Magos Brehm, J; Ralli, P; Palmé, A; Dulloo, E; Maxted, N; Negri, V; Löwenhardt, H; Aykas, L; Kell, S.; **Rubio Teso, ML** and Iriondo JM (2021). Crop Wild Relative Network Showcases – Analysis and Best Practices. Deliverable report "1.5" of the Farmer's Pride Project SFS-04 (GA 774271): Farmer's Pride: Conserving plant diversity for future generations. Project funded by the Horizon 2020 Framework Programme of the European Union