EIDA3 - Escuela Internacional de Doctorado en Agroalimentación
Programa de doctorado en Biociencias y Ciencias Agroalimentarias
Departamento de Ingeniería Forestal

# TESIS DOCTORAL

# Aplicaciones SIG a la gestión de los conocimientos tradicionales asociados a la flora andaluza

*GIS applications for the management of traditional knowledge associated with the Andalusian flora*

Memoria presentada para optar al título de doctora por la Universidad de Córdoba

**Doctoranda**

Ing. Yalbeiry Claret Labarca Rojas

**Dirección de la tesis**

Dr. J. Esteban Hernández Bermejo
Dr. José L. Quero Pérez

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TITULO: *APLICACIONES SIG A LA GESTIÓN DE LOS CONOCIMIENTOS TRADICIONALES ASOCIADOS A LA FLORA ANDALUZA.*

AUTOR: *Yalbeiry Claret Labarca Rojas*

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

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Autora: Yalbeiry Claret Labarca Rojas

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Ctra. Nacional IV. Km. 396

14071, Córdoba

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## Aplicaciones SIG a la gestión de los conocimientos tradicionales asociados a la flora andaluza

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## INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS

El trabajo, o mejor dicho trabajos, de investigación desarrollados en el marco de esta tesis se desenvuelven en el espacio interdisciplinar generado por la búsqueda de soluciones de adaptación al cambio climático para los sistemas agrícolas y forestales de la península ibérica (con especial énfasis en el territorio andaluz bajo clima mediterráneo), el manejo de sistemas de información geográfica, los métodos cuantitativos en ecología para el estudio de los ecosistemas mediterráneos y la perspectiva de recuperación de especies marginados o infrautilizadas (NUS) a través del patrimonio que representan los conocimientos y sistemas de gestión tradicionales asociados a esas especies.

En esa compleja interacción la tesis se concreta en tres estudios de caso. Dos de ellos tienen como protagonista el argán (*Argania spinosa*) especie de Sapotácea endémica de Marruecos y Argelia y presente en la península ibérica desde hace siglos, que forma parte de un fascinante ecosistema que hoy en día sigue siendo objeto de una explotación estrictamente tradicional y que además de ver progresivamente reducida su área natural de distribución, presenta enormes expectativas económicas y agroecológicas como oleaginosa alternativa del olivar con usos cosméticos, alimentarios y medicinales. El segundo protagonista es el boj de Baleares (*Buxus balearica*), arbusto endémico del Mediterráneo occidental, con localidades en Andalucía e Islas Baleares que forma paisajes culturales antiguamente explotados por su madera con usos artesanales, carboneo, alimento de una megafauna mediterránea ya extinguida y con una cualidad común con el argán por ser ambas especies microcondensadoras, condensadoras de nieblas de advección, orográficas o atlánticas, según qué casos, generadoras de importantes aportes de agua en la recarga de los acuíferos mediterráneos y atlántico-norteafricanos.

Esta tesis ha utilizado metodologías innovadoras por el uso sinérgico de aproximaciones procedentes tanto de la ecología numérica como de los SIGs y modelos predictivos de idoneidad, evaluando la diversidad genética de las poblaciones y cultivos residuales, potenciando su uso futuro y conservación *ex situ* y en algún caso contrastando los resultados de la diversidad de hábitat calculada con la genética a nivel morfológico y molecular.

Ha sido un largo trabajo en el que fueron posibles diversas actividades, medidas, comprobaciones y experiencias de campo dentro de Andalucía, pero no así en Marruecos, donde la pandemia impidió en su momento visitas previstas que fueron sustituidas por un intenso trabajo *on line* entre la doctoranda y sus directores. Trabajo y experiencia que demostraron las grandes cualidades como investigadora de la Lcda. Labarca Rojas. Un camino recorrido en equipo, en el que los directores pudieron comprobar esas cualidades y disfrutar de la eficiencia, entusiasmo y riguroso trabajo de la doctoranda para la que tenemos simple y rotundamente dos palabras: profunda admiración.

Por todo ello se autoriza la presentación y defensa de la tesis doctoral de la Licenciada Yalbeiry Labarca Rojas

Fdo.:

Prof. Dr. J. Esteban Hernández Bermejo        Prof. Dr. José Luis Quero Pérez

Córdoba, a 06 febrero 2024

*Dedico esta tesis a mi madre, Yamira y a mi hermana, Yami, por siempre creer en mí, por apoyarme y por sostener los cuidados que muchas veces hasta yo misma olvidaba, y al resto de mi familia, quienes desde la distancia siempre me han acompañado a cumplir este sueño.*

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# Lista de abreviaturas

PC: Principal Component

PCA: Principal Component Analysis

PLS-DA: Partial Least-Squares Discriminant Analysis

VIP: Variable Importance Plots

AUC: Area under the curve

PV: Proportion of Variance

CP: Cumulative Proportion

GIS: Geographic Information System

SIG: Sistemas de Información Geográfica

ISSR: Interspread Single Sequence Repeats (secuencias intercaladas entre microsatelites)

SSR: Single Sequence Repeats (secuencias simples repetitivas o microsatélites)

CT: Conocimientos tradicionales

TK: Traditional knowledge

TM: término municipal

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# INFORME SOBRE APORTACIONES DERIVADAS DE LA TESIS DOCTORAL

## Publicaciones en revistas científicas (capítulos de la tesis)

**Labarca-Rojas, Y.**, Hernández-Bermejo, J. E., Quero, J. L., & Herrera-Molina, F. (2022). Bioclimatic habitat limitations for argan trees (*Argania spinosa* (L.) Skeels) in Northern Africa and Spain. *Regional Environmental Change*, 22(1), 14.

Factor de impacto: posición de la revista en relación con su categoría específica (Environmental Sciences) 4.2; Segundo cuartil (Q2)

**Labarca-Rojas, Y.**, Hernández-Bermejo, J. E., Herrera-Molina, F., Hernández-Clemente, M., & Quero, J. L. (2023). Assessing argan tree (*Argania spinosa* (L.) skeels) *ex situ* collections as a complementary tool to *in situ* conservation and crop introduction in the Mediterranean basin. *Trees-Structure and Function*, 37(2), 567-581.

Factor de impacto: posición de la revista en relación con su categoría específica (Forestry) 2.3; Segundo cuartil (Q2)

Hernández-Bermejo, J.E.; **Labarca-Rojas, Y.**; Herrera-Molina, F.; Quero, J.L.; Hernández-Clemente, R. (2023). Recovery of Neglected Species with Cloud Water Micro Condense Capacity as a Response to Climate Change: The Case of Sclerophyllous Boxwoods of *Buxus balearica* Lam. in the Southern Spanish Mediterranean. *Diversity*, 15, 1184.

Factor de impacto: posición de la revista en relación con su categoría específica (Biodiversity Conservation) 2.4; Segundo cuartil (Q2)

## Comunicaciones presentadas en congresos

**Labarca-Rojas, Y.**, Hernández-Bermejo, J. E., & Quero, J. L. (2022). Evaluación de las colecciones *ex situ* de argán (*Argania spinosa* (L.) Skeels) como herramienta complementaria a la conservación *in situ* en la cuenca mediterránea. In El arte de investigar: Córdoba (España), del 3 al 6 de mayo de 2022 (pp. 267-271). UCOPress.

**Labarca-Rojas, Y.**, Hernández-Bermejo, J. E., Herrera-Molina F., & Quero, J. L. (2021). Modelos de idoneidad basados en la heterogeneidad de hábitat para la conservación de *Sideroxylon spinosum* L. In Primer congreso español de botánica (SEBOT): Toledo (España), del 8 al 10 de septiembre de 2021 (pp. 115). Libro de actas.

**Labarca-Rojas, Y.**, Hernández-Bermejo, J. E., Herrera-Molina F., & Pochettino M.L. (2021). La Asociación Red CultIVA y el rescate de cultivos infravalorados como herramienta de innovación agroalimentaria. Caso de estudio: *Argania spinosa* en el norte de áfrica y España. In Memorias del primer congreso internacional "Transitando hacia una agroindustria sustentable": Cojedes (Venezuela), 30 de noviembre de 2021 (pp. 9-16). Fundación Editorial de la Universidad Nacional Experimental de los Llanos Occidentales.

## Otros trabajos en los que ha colaborado

Hernández-Bermejo, J.E.; Pochettino, M.L.; Herrera-Molina, F.; **Labarca-Rojas, Y.**; Tarifa-García, F. Eds. (2019). I Newsletter Red CultIVA (Elenco de especies marginadas e infrautilizadas (NUS). CYTED. 91 pp. ISBN: 978-84-15413-31-8.

## Proyecto de investigación y financiación

GOPG-AL-20-0005 ARGAN: El argán como alternativa a los cultivos leñosos en Andalucía. Ayudas dirigidas al funcionamiento de Grupos Operativos de la Asociación Europea de Innovación (AEI) en materia de productividad y sostenibilidad agrícolas para la realización de proyectos piloto y el desarrollo de nuevos productos, prácticas, procesos y tecnologías en los sectores agrícola, alimentario y forestal, en el marco del Programa de Desarrollo Rural de Andalucía 2014-2020.

Beca de especialización de la Organizzazione internazionale italo-latino americana (IILA) en el sector Ambiental - Ministerio de Asuntos Exteriores y de la Cooperación Internacional / Dirección General para la Cooperación al Desarrollo. Año 2019.

## **Divulgación científica**

Ganadora del 2do premio en Concurso de monólogos científicos "Cuéntame tu tesis" de la Universidad de Córdoba (2021).

Entrevista divulgativa en Canal Sur. El arca de las semillas en el Jardín Botánico de Córdoba. Fuente: https://www.canalsur.es/television/programas/espacio-protegido/noticia/1811910.html

## **Estancia de investigación**

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*“In the end, we will conserve only what we love,*
*we will love only what we understand, and we will*
*understand only what we are taught”.*
Baba Dioum

# RESUMEN

Los conocimientos tradicionales (CT) son los saberes y costumbres populares, generalmente delimitados a comunidades locales y asociados a los recursos naturales disponibles en cada territorio. Conociendo la importancia de esta sabiduría para la preservación de la biodiversidad global, en esta tesis doctoral se proponen metodologías basadas en ecología y análisis espacial para recuperar especies marginadas e infrautilizadas de la flora andaluza, que tienen potencial para paliar los efectos del calentamiento global a través de su manejo y conservación.

Hemos tenido en cuenta que el manejo tradicional de ciertos sistemas agroforestales en la cuenca mediterránea ha permitido la adaptación de estas especies a las condiciones climáticas de la región. Tal es el caso de las formaciones tanto de *Argania spinosa* (L.) Skeels como de *Buxus baleárica* Lam. que forman parte del elenco de especies espontáneas o agrícolas de la flora y cultivos presente en la comunidad Autónoma de Andalucía (España).

El caso específico de *A. spinosa*, árbol de la familia Sapotáceas, endémico de Marruecos y Argelia y en ciertos periodos de la historia también presente en la Península ibérica, forma parte de un fascinante ecosistema (*Arganeraie)*, que hoy día sigue siendo estrictamente tradicional en su manejo y uso humano. El aprovechamiento del *Arganeraie*, está muy arraigado a la cultura y las tradiciones locales de los pueblos bereberes del noroeste africano y proporciona ingresos y recursos alimentarios a las poblaciones locales. Es por ello por lo que el ecosistema alrededor de la explotación tradicional del argán en Marruecos es un ejemplo de la interconexión entre la biodiversidad, las prácticas culturales y la sostenibilidad económica.

Este ecosistema está sometido a políticas de conservación y reconocimiento internacional para su conservación debido a la fuerte reducción de su área de distribución en los últimos años. Por esta razón, la presencia ancestral del argán en la península ibérica supone una oportunidad para recuperar la diversidad genética del ecosistema, que está en peligro junto con todos sus conocimientos tradicionales de manejo y conservación.

Por su parte, *B. balearica*, una especie arbustiva de la familia Buxáceas, endémica del Mediterráneo occidental, con localidades en Andalucía e Islas Baleares donde forma parte de paisajes culturales que han sido desde hace siglos utilizados como fuente de madera de usos artesanales, leña y carboneo. Ciertos mamíferos extinguidos de área tirrénica parece ser, utilizaron esta planta como alimento (*Myotragus balearicus*). El llamado boj de Baleares forma parte de arbustedas que contribuyen al ingreso de agua en el ecosistema por la condensación de las nieblas orográficas y de advección, frecuentes en las montañas mediterráneas litorales.

Las medidas de protección de la flora andaluza reconocen la vulnerabilidad de esta especie y sus formaciones en el Decreto 104/1994, de 10 de mayo, por el que se establece el Catálogo Andaluz de Especies de la Flora Silvestre Amenazada.

El objetivo principal de esta tesis es proponer una metodología innovadora para determinar zonas idóneas en la recuperación y conservación de especies NUS de interés estratégico agrícola o forestal. A través del estudio de su amplitud ecológica se intenta reconocer áreas potenciales para su cultivo o reforestación.

**Así, en el capítulo I** se explica el papel de las especies infrautilizadas y marginadas como alternativa estratégica y de interés para la mitigación del cambio climático, considerándose una herramienta valiosa de innovación donde además de preservar la biodiversidad mundial, se promueve el desarrollo y la puesta en marcha de nuevas alternativas mejor adaptadas al cambio.

**Por otro lado, en el capítulo II** se ha estimado la distribución potencial de *Argania spinosa* L. Skeels en zonas de interés estratégico para su puesta en cultivo. Las localidades existentes en Marruecos, Argelia, Túnez y España fueron utilizadas para conocer la heterogeneidad de su hábitat y las condiciones ambientales que esta especie requiere para, de esta manera, calcular un modelo predictivo que simule las mismas condiciones en otros territorios para que esta especie prospere. Este modelo predictivo ha permitido obtener una aproximación de las zonas con mayor probabilidad de éxito para establecer programas de cultivo y conservación con poca intervención humana. Principalmente en el norte de África (costa mediterránea occidental de Argelia y las regiones costeras llanas del este de Túnez). Además, se ha observado un alto nivel de idoneidad en el sureste de la Península ibérica e Islas Canarias. Con la aplicación de esta metodología de análisis multivariable surge la oportunidad de encontrar territorios alternativos para expandir el cultivo del argán, lo que representa una ventana para mejorar su diversidad genética, promover programas de conservación y favorecer el desarrollo económico de los países donde se encuentra.

**Posteriormente, en el capítulo III** se ha evaluado la diversidad genética de una colección *ex situ* de más de 600 ejemplares de *Argania spinosa* (L.) Skeels en el sur de la Península ibérica. Esta colección, con 10 años de antigüedad, ha sido comparada con la diversidad genética y morfológica de especímenes procedentes de lugares tanto silvestres como cultivados. Además, en este estudio se han incorporado escenarios de cambio climático para evaluar la distribución del argán en diferentes condiciones.

Los resultados de la colección ibérica reflejan la presencia de un alto porcentaje de la diversidad genética respecto al observado en las poblaciones silvestres, por lo que se puede considerar como una colección núcleo punto de partida para fomentar el cultivo de argán y su domesticación. Estas áreas son de gran importancia estratégica debido a la reducción considerable de la distribución natural del argán para 2050 y 2080, de acuerdo con los resultados de nuestros modelos. Una vez más, se resalta el potencial económico del argán en la alimentación humana y la cosmética, así como a su adaptabilidad a las condiciones áridas, lo que reduce su susceptibilidad al cambio climático en comparación con otros cultivos.

**Por último, en el capítulo IV** se ha evaluado la heterogeneidad del hábitat de *Buxus balearica* Lam. para comprender sus requerimientos y evaluar el nivel de presión que sufre a causa del cambio climático. Al integrar el diagnóstico de los perfiles ecológicos, el análisis multivariante, la agrupación de las poblaciones y los modelos de distribución geográfica en función de ciertas variables ambientales, queda de manifiesto que la distribución de *B. balearica* en el sur de la Península ibérica está significativamente influenciada por las variaciones térmicas, el régimen de humedad y la microtopografía, lo que le hace altamente vulnerable a los escenarios de cambio climático actuales y futuros.

El estudio refuerza la necesidad de mejorar el estado actual de la especie y los paisajes asociados, haciendo hincapié en la importancia de establecer un plan de gestión que permitan reforzar las poblaciones y proteger su hábitat natural. Además, identifica una colección base de conservación y establece propuestas para los planes de gestión y recuperación de la especie.

En resumen, esta tesis, al hacer uso de una metodología integradora que además de considerar los requisitos ambientales toma en cuenta la distribución geográfica de las especies NUS estudiadas, que son de uso tradicional constitutivas de paisajes culturales y en todo caso infrautilizadas, permite; a) evaluar el nivel de afectación y vulnerabilidad debido especialmente al cambio climático, b) adelantar el diagnóstico de posibles cambios en la distribución y presencia y c) proponer nuevas directrices para los planes de conservación y recuperación de estas especies, asegurando no solo su conservación, sino reconociéndolas como herramientas al servicio de un desarrollo sostenible en las regiones de la cuenca mediterránea con énfasis especial en la península ibérica y Andalucía.

# ABSTRACT

Traditional knowledge (TK) involves popular customs, generally confined to local communities and associated with the natural resources available in each territory. Knowing the importance of this knowledge for the preservation of global biodiversity, this doctoral thesis proposes methodologies based on ecology and spatial analysis to recover marginalised and underutilised species of the Andalusian flora, which have the potential to mitigate the effects of global warming through their management and conservation.

We have considered that the traditional management of certain agroforestry systems in the Mediterranean basin has allowed these species to adapt to the climatic conditions of the region. Such is the case of the formations of both *Argania spinosa* (L.) Skeels and *Buxus balearica* Lam., which form part of the list of spontaneous or agricultural species of flora and crops present in the Autonomous Community of Andalusia (Spain).

The specific case of *A. spinosa*, a tree of the Sapotaceae family, is endemic to Morocco and Algeria. It has also been present in the Iberian Peninsula in certain periods of history and is part of a fascinating ecosystem (*Arganeraie*), which today remains strictly traditional in its management and human use. The use of the *Arganeraie* is deeply rooted in the culture and local traditions of the Berber people of northwest Africa and provides income and food resources for the local populations. This is why the ecosystem around the traditional exploitation of the argan tree in Morocco is an example of the interconnection between biodiversity, cultural practices, and economic sustainability.

This ecosystem is subject to conservation policies and international recognition for its conservation due to the sharp reduction in its distribution area in recent years. For this reason, the ancestral presence of the argan tree in the Iberian Peninsula represents an opportunity to recover the genetic diversity of the ecosystem, which is endangered along with all its traditional management and conservation knowledge.

*B. balearica*, a shrub species of the Buxaceae family, is endemic to the western Mediterranean, with localities in Andalusia and the Balearic Islands where it forms part of cultural landscapes that have been used for centuries as a source of wood for craft uses, firewood and charcoal. Certain extinct mammals from the Tyrrhenian area seem to have used this plant as food (Myotragus balearicus). The Balearic boxwood forms part of the shrubs that contribute to the entry of water into the ecosystem through the condensation of orographic and advection fogs, which are frequent in the coastal Mediterranean mountains.

Protection measures for the Andalusian flora recognise the vulnerability of this species and its formations in Decree 104/1994, of 10 May, establishing the Andalusian Catalogue of Threatened Wild Flora Species.

The main objective of this thesis is to propose an innovative methodology to determine suitable areas for the recovery and conservation of NUS species of strategic agricultural or forestry interest. The aim is to identify potential areas for their cultivation or reforestation, through the study of their ecological amplitude.

Thus, **chapter I** explains the role of underutilised and marginalised species as a strategic and relevant alternative for climate change mitigation, being considered a valuable tool for innovation where, in addition to preserving global biodiversity, it promotes the development and implementation of new alternatives that are better adapted to change.

On the other hand, **chapter II** estimated the potential distribution of *Argania spinosa* L. Skeels in areas of strategic interest for its cultivation. The existing localities in Morocco, Algeria, Tunisia, and Spain were used to determine the heterogeneity of its habitat and the environmental conditions that this species requires. This information allows us to calculate a predictive model that simulates the same conditions in other territories for this species to thrive. This predictive model has made it possible to obtain an approximation of the areas with the greatest probability of success for establishing cultivation and conservation programmes with little human intervention. Mainly in North Africa (western Mediterranean coast of Algeria and the flat coastal regions of eastern Tunisia). In addition, a high level of suitability has been observed in the southeast of the Iberian Peninsula and the Canary Islands. With the application of this multivariate analysis methodology, the opportunity arises to find alternative territories to expand the cultivation of the argan tree, which represents a window to improve its genetic diversity, promote conservation programmes and favour the economic development of the countries where it is found.

Subsequently, the genetic diversity of an *ex situ* collection of more than 600 specimens of *Argania spinosa* (L.) Skeels in the south of the Iberian Peninsula has been evaluated in **chapter III**. This 10-year-old collection has been compared with the genetic and morphological diversity of specimens from both wild and cultivated sites. In addition, climate change scenarios have been incorporated in this study to assess the distribution of argan under different conditions.

The results of the Iberian collection reflect the presence of a high percentage of genetic diversity with respect to that observed in the wild populations. Thus, it can be considered as a starting core collection to promote argan cultivation and domestication. These areas hold great strategic importance due to the considerable reduction of the natural distribution of the argan tree by 2050 and 2080, according to the results of our models. Once again, the economic potential of argan in human food and cosmetics is highlighted, as well as its adaptability to arid conditions, which reduces its susceptibility to climate change compared to other crops.

Finally, in **chapter IV** the habitat heterogeneity of *Buxus balearica* Lam. has been assessed to understand its requirements and to evaluate the level of pressure it faces due to climate change. By integrating the study of ecological profiles, multivariate analysis, population clustering and geographic distribution models applying certain environmental variables. The distribution of *B. balearica* in the southern Iberian Peninsula is significantly influenced by thermal variations, humidity regime and microtopography, which makes it highly vulnerable to current and future climate change scenarios.

The study reinforces the need to improve the current status of the species and associated landscapes, emphasising the importance of establishing a management plan to strengthen populations and protect their natural habitat. It also identifies a baseline conservation collection and sets out proposals for management and recovery plans for the species.

In summary, by making use of an integrative methodology that considers environmental requirements and the geographic distribution of the NUS species that are traditionally used, constitutive of cultural landscapes, and in any case are underutilised, this thesis allows to; a) assess the level of affectation and vulnerability due, especially to climate change; b) advance the diagnosis of possible changes in distribution and presence; and c) propose new guidelines for conservation and recovery plans for these species. This ensures not only their conservation but also recognises them as tools at the service of sustainable development in the regions of the Mediterranean basin, with special emphasis on the Iberian Peninsula and Andalusia.

# Capítulo I

## Introducción general

## Perspectiva política y de adaptación

El estado actual de la flora agrícola y forestal en Europa refleja una crisis acelerada como consecuencia del cambio climático. El aumento de las temperaturas, la alteración de los regímenes de precipitaciones y el incremento de los fenómenos meteorológicos extremos plantean importantes retos a los sistemas agrícolas y forestales tradicionales. El sistema que actualmente depende de un número reducido de cultivos, principalmente cereales (trigo, arroz y maíz), deja abandonada una gran cantidad de recursos genéticos con características potencialmente beneficiosas [1,2]. En respuesta, cada vez se reconoce más la necesidad imperiosa de rectificar estas prácticas para garantizar la seguridad alimentaria y preservar la biodiversidad. En este contexto, las especies olvidadas e infrautilizadas (NUS o NUCS) se perfilan como una vía prometedora para esta adaptación, ya que representan una riqueza de recursos genéticos con un gran potencial para contribuir a una agricultura sostenible, al tiempo que recupera una gran reserva de biodiversidad tanto de especies como de ecosistemas [3,4].

> *"Las especies olvidadas e infrautilizadas son aquellas a las que se presta poca atención o que son completamente ignoradas por los investigadores agrícolas, los fitogenetistas y los formuladores de políticas. Son variedades silvestres o semidomesticadas y especies forestales no maderables que normalmente no se comercializan como productos básicos"* [5]

Las NUS se han dejado de cultivar principalmente por la selección de otras con mayor rendimiento, sin embargo, estas especies han sobrevivido y son una muestra de resiliencia y adaptación. Por esta razón y por el alto valor nutricional, cosmético o de otro tipo que éstas presentan, se genera cada vez más expectación a nivel global en su recuperación, sobre todo para luchar contra la pobreza y contribuir a mitigar los efectos del cambio climático [6].

Una estrategia de adaptación innovadora gira en torno a un grupo de estas especies que integran mecanismos que capturan el vapor de agua atmosférica, lo que supone una oportunidad para mitigar los devastadores efectos de las sequías, de la disminución de la disponibilidad de agua y aumento de la evapotranspiración en todo el mundo.

Estas especies se caracterizan por tener hojas mirtoides o buxoides, (que podríamos llamar microlauroides) adaptadas y capaces de condensar la humedad ambiental por ser coriáceas, glabras, enteras e incluso con una filotaxia, *id est*, colocación de las hojas en el tallo, que permiten una buena eficacia condensadora equilibrada con un área foliar no demasiado grande para evitar una evaporación o exposición solar excesiva. Este tipo de mecanismo de supervivencia ha sido ampliamente estudiado en ambientes desérticos como el Desierto de Atacama en Chile, Lomas de Atiquipa en Perú, Desierto de Namibia, y la costa oeste del sur de África [7,8,9,10].

Explorando el papel de las NUS en dicho contexto, este trabajo de investigación profundiza en dos especies con dicha capacidad de microcondensación de agua, *Argania spinosa* (L.) Skeels y *Buxus baleárica* Lam.

Mediante el estudio de caso de estas especies, exploramos su eficacia en la supervivencia en zonas con regímenes hídricos alterados, como es el caso de la cuenca mediterránea. A través de un prisma de adaptación, desarrollo y conservación, se pretende contribuir al discurso sobre el aprovechamiento de especies marginadas e infrautilizadas como catalizadoras de la adaptación sostenible ante un clima cambiante.

# Perspectiva metodológica

Los estudios de hábitat son fundamentales para preservar la biodiversidad, gestionar los ecosistemas de manera sostenible, abordar los impactos del cambio climático y comprender el funcionamiento de los entornos naturales. En este sentido, caracterizar las condiciones de vida de cualquier especie proporciona una base para la toma de decisiones informadas en conservación, gestión de tierras e incluso sobre las políticas medioambientales asociadas.

Debido a la complejidad de los hábitats y sistemas de las especies NUS, se propone una metodología holística que integra cuatro métodos diferentes basados en la ecología numérica, para comprender las características cuantitativas específicas asociadas a cada especie [11,12]. Estos métodos son: (a) estadística descriptiva, (b) análisis multivariante, (c) modelado matemático y (d) ecología espacial.

(a) La estadística descriptiva permite describir las variables medioambientales utilizando medidas de tendencia central, dispersión y frecuencias, representadas con el uso de gráficas; estos son los llamados perfiles ecológicos.

(b) El análisis multivariante analiza conjuntos de datos complejos que involucran múltiples variables. Específicamente, se combina el análisis de componentes principales con el análisis de agrupamiento jerárquico.

(c) El modelo matemático integrado en esta metodología es el algoritmo de Máxima Entropía (MaxEnt). Se aplica para modelar la distribución de las especies con el objetivo de encontrar la distribución de probabilidad que sea más uniforme (máxima entropía) dadas las restricciones o variables medioambientales que se imponen a través de la información disponible.

(d) La ecología espacial y los SIG (Sistemas de Información Geográfica) añaden una compone espacial para analizar patrones de distribución geográfica, conectividad entre hábitats y efectos de la fragmentación del paisaje en las poblaciones y/o comunidades.

## Estudios de caso de especies NUS

Las especies microcondensadoras suelen mostrar una alta tolerancia a condiciones climáticas adversas, como sequías y bajas precipitaciones. Su capacidad de utilizar la humedad ambiental para su crecimiento les otorga resistencia ante desafíos climáticos cada vez más frecuentes. Esto es de suma importancia en un mundo en el que la sequía y la escasez de agua son preocupaciones crecientes, y donde la resiliencia de los sistemas agrícolas y forestales es esencial para la seguridad alimentaria y sostenibilidad a largo plazo. Su introducción en estos sistemas diversifica la gama de cultivos y especies relacionadas, que se traduce en fundamental en un contexto donde los patrones climáticos pueden volverse menos predecibles. Esta diversificación brinda opciones para enfrentar problemas climáticos específicos y promover la resiliencia en los sistemas agrícolas y forestales, ayudando a garantizar que las necesidades alimentarias puedan ser satisfechas de manera más confiable.

Además, estas especies también desempeñan un papel en la mitigación del cambio climático al contribuir a la restauración y conservación de suelos y ecosistemas. Mejoran la captura y retención de humedad en el suelo, reduciendo la erosión y manteniendo la salud del suelo, lo que, a su vez, almacena carbono y contribuye a la reducción de las emisiones de gases de efecto invernadero.

### *Argania spinosa* (L.) Skeels

*Argania spinosa*, el árbol del argán es un árbol que se presenta como un caso paradigmático de especie marginada y/o infrautilizada (NUS). A pesar de sus numerosas ventajas, el argán se encuentra en estas categorías. Varios factores contribuyen a su infrautilización, pero su resistencia a la sequía y baja demanda hídrica añaden nuevos valores de oportunidad en la lucha contra el cambio climático.

La limitada distribución geográfica del argán, lo sitúa principalmente en Marruecos y Argelia. Sin embargo, existe un interés creciente en su introducción y puesta en cultivo en otras regiones del mundo. Lo que agrega una dimensión adicional de importancia a su capacidad para prosperar en condiciones de escasa disponibilidad de agua.

El argán es un ejemplo elocuente de una especie marginada e infrautilizada que posee un gran potencial nutricional y cosmético. Su promoción, investigación en diversas aplicaciones y apoyo a las comunidades locales involucradas en su producción son esenciales para desbloquear su utilización y sostenibilidad en el contexto de las NUS.

### *Buxus baleárica* Lam.

*Buxus balearica*, conocido como el boj balear, es una planta endémica que se enfrenta a un peligro real de extinción. Este arbusto, nativo de las Islas Baleares (España) es uno de los muchos elementos de la flora tirrénica de origen terciario, común a las Islas y montañas litorales del occidente mediterráneo. Ha sido duramente afectado por una serie de amenazas y desafíos que han llevado a su clasificación como especie vulnerable (V) por la Lista Roja de la Flora Vascular de Andalucía [13]. Sin embargo, existen poblaciones de boj balear en las provincias de Málaga, Granada y Almería en las que se ha observado una capacidad micro condensadora que le permite aprovechar la humedad ambiental proveniente de las nieblas costeras originarias del Mar de Alborán, lo que añade una dimensión especial a su importancia.

Estas nieblas costeras, conocidas localmente como *Taró*, se forman por la diferencia de temperatura cuando una masa de aire cálido entra en contacto con temperaturas más bajas del Mar de Alborán (<25 °C), lo que evapora la humedad del aire y produce las nieblas que penetra en el continente.

La protección del hábitat natural del boj y la promoción de su cultivo sostenible pueden ayudar a preservar esta especie y el ecosistema en el que se encuentra, aprovechando su capacidad de condensar la humedad, como una especie arbustiva con interés forestal.

# Hipótesis

Basados en este marco de trabajo, la hipótesis general de esta Tesis Doctoral es que las especies NUS son una herramienta de desarrollo sostenible y su recuperación refuerza los sistema agrícolas y forestales actualmente afectados por el cambio climático acelerado.

# Objetivo general

Diseñar una metodología que integre diferentes métodos de la ecología numérica con análisis espacial para estudiar la ecología de especies NUS.

# Objetivos específicos

- Estudiar la heterogeneidad del hábitat actual de *Argania spinosa* y su hábitat potencial en el norte de África y España (Capítulo II).
- Evaluar la diversidad genética de una colección *ex situ* de *Argania spinosa* como herramienta de conservación y puesta en cultivo en la cuenca mediterránea (Capítulo III).
- Determinar la vulnerabilidad de *Buxus balearica* frente al cambio climático, analizando su heterogeneidad de hábitat y su diversidad genética en el sur del mediterráneo español (Capítulo IV).

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# Capítulo II

## Bioclimatic habitat limitations for argan trees (*Argania spinosa* (L.) Skeels) in Northern Africa and Spain

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## Abstract

*Argania spinosa* (L.) Skeels is an Algerian-Moroccan endemic tree. This species is part of various plant communities consisting of Mediterranean, Macaronesian and Saharan floristic elements. It has been introduced and perhaps sometimes naturalized in various regions of the Mediterranean basin.

Due to its role in combating desertification, high socio-economic value, and traditional use as fodder and food, the southwestern Moroccan argan grove (Arganeraie) was declared Biosphere Reserve. It had already been subject to conservation, and reforestation programs a century earlier. Its cultivation for oil production could be, besides an economic objective, an effective method to conserve its genetic diversity. Therefore, this study aims to estimate its potential distribution and establish efficient breeding programs by determining its ecological requirements, identifying its different habitats, and predicting habitat suitability models for Morocco, Algeria, Tunisia, and Spain.

Using 53 occurrence points, wind speed and direction data, and 29 bioclimatic variables, multivariate methods were applied to describe the ecological profiles and characterize the heterogeneity of its habitat to subsequently, train a Maxent model that establishes, besides Morocco and Algeria, suitable cultivation areas in Tunisia and Spain. The North African potential area is limited to the western Mediterranean coast of Algeria and flat and coastal areas of eastern Tunisia. The increased likelihood of suitability remains in the southeast Iberian Peninsula. A high probability of argan cultivation is also evident in the Canary Islands. These results provide possibilities for future expansion of argan crop and a window of opportunity to improve its genetic diversity and conservation.

**Key words**: Conservation, bioclimatic variables, habitat heterogeneity, geographic suitability, multivariate Analysis, MaxENT

# Introduction

*Argania spinosa* (L.) Skeels (hereafter argan) is an Algerian-Moroccan endemic tree. It has been introduced and locally naturalized mainly in Tunisia, Israel, and Spain [1–5]. The species is well known for the high socio-economic impact that its traditional exploitation has in large areas of Morocco by the extraction and use of oil from its seeds, wood, and other purposes [6]. These knowledge, techniques and practices related to argan were recognized as Intangible Cultural Heritage of Humanity on 27 November 2014 by UNESCO. In some parts of Morocco, due to its ethnobotanical uses, argan takes the role of the olive tree as a multipurpose specie, since it is used as fodder, wood, and even human food. For example, argan is the fuel of the Berber society, especially near Essaouira and, when food is scarce, goats climb the tree to forage. In 1998, UNESCO declared an area of 2 568 780 ha of the argan grove (Arganeraie) as a Biosphere Reserve situated in the Souss-Massa National Park, in southwestern Morocco near Agadir [7].

This native tree is more widely spread in southwestern Morocco (Figure 1), from the Saharan littoral plain to the valleys formed by *oueds* (wadis) draining the Great Atlas and the Anti-Atlas and disjoints with small populations growing more than 700 km away from it in northern Morocco and Algeria [8,9]. This dispersion pattern can be interpreted because of a habitat reduction or losing process. There are also ideas based on the molecular polymorphism of the species, explained by the human action that would have dispersed the species for its economic and ethnobotanical interest [10]. Despite the open debate about the possible fragmentation of its natural distribution area, different sources agree on the subspontaneous nature of these populations [11–13]. Some localities may even respond to ancient cultivation attempts. In fact, there is evidence of this dispersion of argan in the Iberian Peninsula where it was cultivated during the historical period of Al-Andalus (VII-XV centuries) [14]. There are other extensive regions in Argelia (northwest NW of the Tindouf region) where the argan grove is found in a spontaneous state [15].

The analysis of the plant communities in which arganappears evidence firstly its paleo-relict character from the late Tertiary vegetation, currently very fragmented and preserved in the Macaronesian flora, and secondly, its paleo-tropical character like other species sheltered in the Mediterranean littoral of Tyrrhenian area[16–18].

On the other hand, arganwas probably part of the woodlands that condensed the oceanic fog as nowadays the Laurel Forest does, and it still preserves that fog-harvesting ability, like other species that appear in argan formations such as *Pistacia lentiscus*, *Olea maroccana*, *Whitania frutescens* or *Buxus balearica* according to Ritter [19] and Navarro-Cerrillo [20]. Fog and dew regimes have been studied as a potential source of water in areas where argan is distributed, demonstrating the importance of the contributions of humidity through dew and fog as a complement to the scarce rainfall [21–23]. This happens especially in the southwestern arid and semiarid Morocco, where these two humidity sources have a significant role in different periods.

Since the end of the 1990s, numerous studies have attempted to explain the *A. spinosa* biological and ecological aspects to improve the conservation of its genetic diversity. The allogamous nature of argan with a small percentage of self-compatibility justifies a geographical model of distribution of genetic diversity that shows greater values of intra-population than inter-population variability and some independence from environmental or physiographic factors such as distance between populations or proximity to the coastline [7,10,24–28]. At the same time, this variability level is also a complex problem when establishing propagation, cultivation, and forest management mechanisms using sexual propagation [29].

The argan tree grows in areas of varied orography, however very little attention has been paid to its ecological range and the heterogeneity of its habitat [7,30]. This lack of information on the bioclimatic and physiographic factors that could determine argan presence throughout its natural range is extended to the suitability assessments aimed to determine the optimal areas to either afforestation or crop introduction. This is an intention already pointed out by López-Sáez and Alba-Sánchez [12], who demanded the development of habitat suitability models. They also highlight the need to better understand the ecophysiological behaviour of this plant (but see Díaz-Barradas [31]), especially when assessing its mechanisms against drought. Some of them suggested by Peltier [32] and Msanda [11] when referring to argan as an arid manifestation of "fog forests". Potential growing areas have been attempted to investigate in Argentina managing environment variables but have not dealt with the application of predictive suitability models [33].

A key aspect in Conservation Biology programs is the habitat heterogeneity studies that help to answer how variable the habitat of a species is, the diversity of its elements and the complexity of its relationships [34]. During decades and before the possibility of studying the genetic diversity of plant species at molecular level, forest science has used the analysis of the habitat diversity as an estimating parameter of the species genetic diversity. Nowadays, the habitat heterogeneity is incorporated to Geographical Information Systems (GIS), when establishing forest management models aimed to conservation, economic exploitation or sustainable use of species and landscapes applying ecogeographic variables. These models are very useful when choosing climatic refuges and establishing the areas and localities where its cultivation or introduction can be more efficient or carried out with better guarantees of success [20,35,36]. Thus, the main purpose of this study is to analyse the habitat diversity of argan to estimate its potential distribution and establish efficiency levels of potential breeding programs, pursuing three specific aims: 1) to determine its ecological requirements, 2) to assess habitat heterogeneity, and finally 3) to predict its habitat suitability by using the species distribution model (SDM) algorithm MaxEnt for its native countries (i.e., Morocco and Algeria), plus Tunisia and Spain, where cultivation for farming purposes may expand its genetic diversity and improve its conservation status.

## Materials and Methods

### *Considered area*

The area considered in this study extends over Morocco, Algeria, Tunisia, and Spain, between 15°N and 45°N of latitude and 20°W and 15°E of longitude (Figure 1). There are arid and semi-arid ecosystems with Atlantic influence between 29° and 32° N in southwestern Morocco. Inland, the larger populations occur along the Oued Souss on the S-side of the High Atlas, and on the N and S of the Anti-Atlas where it reaches 1300 to 1500 m. While the disjoint populations are influenced by the Mediterranean climate, in western Argelia it sometimes appears in microhabitats of certain humidity (usually at the bottom of the oueds).

Considering this evidence and to establish the potential area of argan cultivation, the occurrence points in its native area were used to explore the ecological requirements and habitat heterogeneity. Moreover, there are other locations where it has also been introduced and naturalized but never only cultivated [37,38]. For this reason, samples from Spain (Murcia and Alicante provinces), Algeria (Mostaganem, and Mascara provinces) and Morocco (Berkane, Agadir, and Taroudant provinces) were included to make the predictive suitability analysis more robust.

## *Presence data*

Twenty-seven records were downloaded from the Global Biodiversity Information Facility (GBIF), an open-access network that includes occurrence records of any species around the world (https://www.gbif.org/es/). This database was completed by adding coordinates obtained from a bibliographic search in Morocco [9,31,39], Algeria [13,40], and Spain [41]. All the occurrence site records represent the natural and naturalized distribution of *A. spinosa* in The WGS84 coordinate system (see Appendix 1).

## *Selecting environmental data*

Nineteen bioclimatic variables and the elevation model (SRTM) were obtained from WorldClim data portal [42]. The SRTM was used to calculate three topographic variables: slopes, hillshade and aspect.

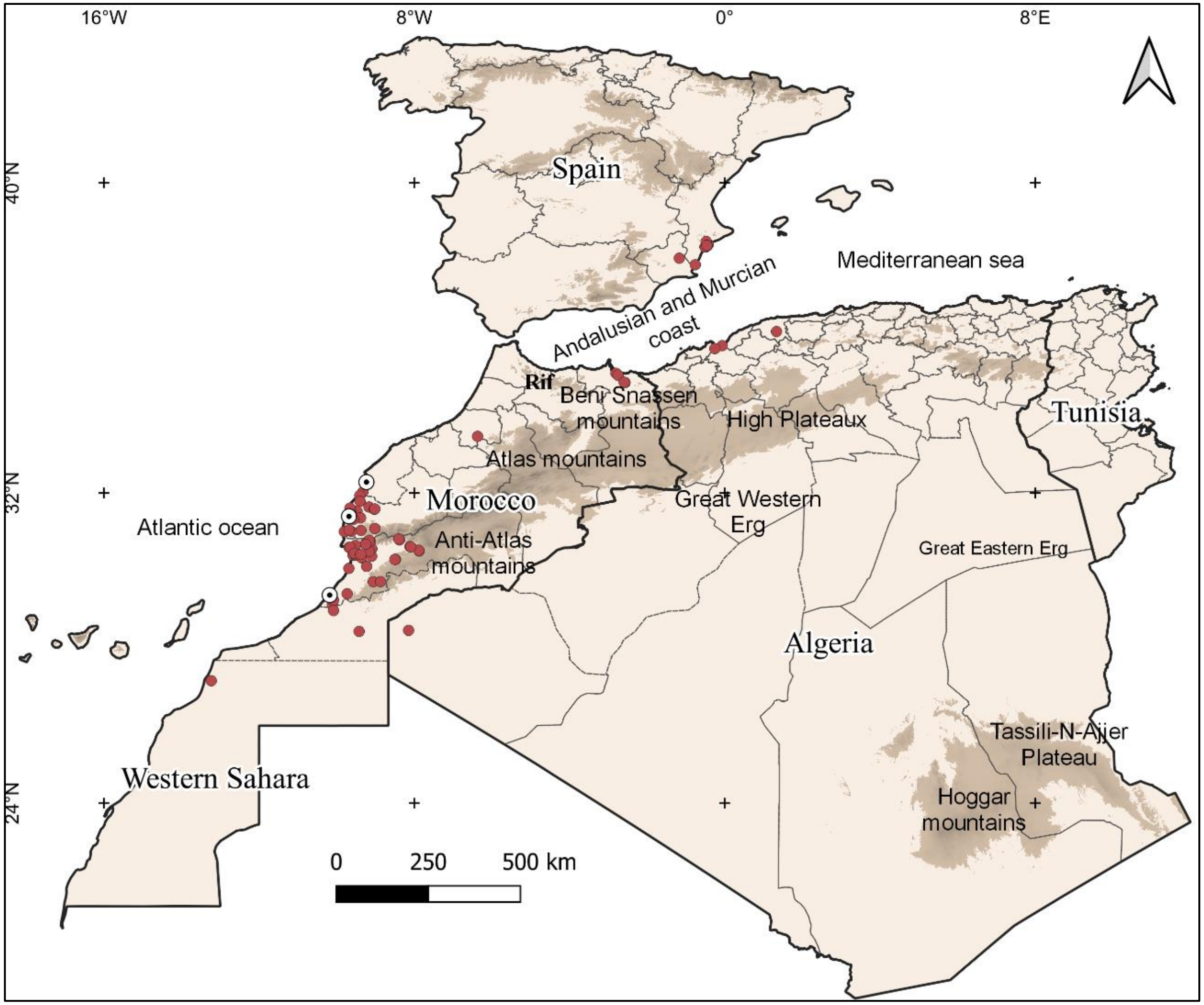

**Figure 1** Study area in Morocco, Western Sahara, Algeria, Tunisia, and Spain. Datum WGS84.Darker areas show mountain ranges above 1000 m. Red dots represent the argan occurrence points, and black dots the wind stations

In addition, two indicators of aridity were included: The Global Aridity Index and the Potential Evapotranspiration [43]. The NDVI is a vegetation index that serves as an indicator of the contrast between vegetation and bare soil. To include the state of the earth's surface where argan is found, four satellite images of NDVI and Land Surface Temperature during April and July 2002 were extracted from the standard MODIS Land products that combine data records from Terra and Aqua satellites (https://daac.ornl.gov/). Daily wind speed and direction data from the Global Surface Summary of the Day (GSOD) stations were download from 1973-1999 period using the Weather Data Client of GSOD in R (GSODR) package [44]. In total, the 3 stations located at the closest airports to the study area were used: Safi, Essaouira, and Sidi Ifni (Figure 1).

The Twenty-seven climatic and environmental variables were compiled in raster (grid) format with 1 km$^2$ spatial resolution. The data file was generated using the *point sampling tool* QGIS plugin.

Additionally, the argan preference for basic soils is known so (https://pfaf.org/), although soil pH has not been considered for statistical analysis, the global gridded of pH soil information at 0-5 cm depth has been included as a restrictive layer to refine the results of the suitability model.

The complete environmental dataset was classified into seven categories (Table 1) and were managed and overlapped using the Quantum Geographic Information System (QGIS) and MaxEnt [45].

**Table 1** Climatic and environmental variables collected for this ecogeographic study

| Category | Code | Variables | Reference |
|---|---|---|---|
| Temperature | Temp | Annual Mean Temperature | [42] |
| | Tmdr | Mean Diurnal Range | |
| | Tist | Isothermality | |
| | Tsst | Temperature Seasonality | |
| | Tmwm | Max Temperature of Warmest Month | |
| | Tncm | Min Temperature of Coldest Month | |
| | Tmar | Temperature Annual Range | |
| | Tmwq | Mean Temperature of Wettest Quarter | |
| | Tmdq | Mean Temperature of Driest Quarter | |
| | Tmwq | Mean Temperature of Warmest Quarter | |
| | Tmcq | Mean Temperature of Coldest Quarter | |
| Precipitation | Prec | Annual Precipitation | [42] |
| | Pmwm | Precipitation of Wettest Month | |
| | Pmdm | Precipitation of Driest Month | |
| | Psst | Precipitation Seasonality | |
| | Pmwq | Precipitation of Wettest Quarter | |
| | Pmdq | Precipitation of Driest Quarter | |
| | Pmwq | Precipitation of Warmest Quarter | |
| | Pmcq | Precipitation of Coldest Quarter | |
| Topographic | Elev | Elevation (SRTM) | [42]QGIS |
| | Hshd | Hillshade | |
| | Aspt | Aspect | |
| | Slop | Slope | |
| Land Surface | LSTa | Land Surface Temperature April 2002 | MODIS Land products |
| | LSTj | Land Surface Temperature July 2002 | |
| | NDVIa | Normalized Difference Vegetation Index April 2002 | |
| | NDVIj | Normalized Difference Vegetation Index July 2002 | |
| Dryness | Dai | Global Aridity Index | [43] |
| | Devt | Potential Evapotranspiration | |
| Wind | Wspd | Wind speed | [44] |
| | Wdtn | Wind direction | |
| Restrictive | Rph | pH soil information at 0-5 cm depth | [46] |

## Ecological requirements

The frequency distribution of the sampling points ($n = 51$) was studied in the environmental variables of the blocks of temperature, precipitation, topography, and dryness, to characterize the values that represent the ecological optimum for the species and its ecological amplitude concerning each environmental variable [47].

The ecological profiles display the distribution of the values of each variable and reveal their mean values, variance (ecological amplitude), and possible adjustments to gaussian, bimodal, or exponential distribution curves. To compute and illustrate the histograms of the observations the generic R function *hist* was used. Thus, the height of each rectangle is proportional to the number of points with the same value [48].

The role of the wind is essential in determining the presence of fog, and it is necessary to consider both direction and speed. Due to the nature of the wind data, the *time Average* function of the *openair* R package was applied to calculate the monthly and annual average value of wind speed ($u_i$) and orientation ($\theta_i$) at each station [49]. This function calculates the east-west ($Vn_i = u_i * Cos\,\theta_i$) and north-south ($Ve_i = u_i * Sin\,\theta_i$) components of all observations ($N$), then gets an average of each component ($Vn = \frac{1}{N}\sum Vn_i$) and ($Ve = \frac{1}{N}\sum Ve_i$) to finally calculate the resultant mean wind speed ($U = \sqrt{Vn^2 + Ve^2}$) and direction ($\tan\theta = \frac{Ve}{Vn}$). To convert the vector direction (towards which the wind is blowing) into the meteorological direction (from which the wind is blowing) add 180° to the direction if $\theta < 180°$ or subtract 180° if $\theta > 180°$.

## *Habitat heterogeneity*

In order to be able to apply the different multivariate analyses using data that have different units of measurement, a Z-score method (the difference between the input value and the mean is divided by the standard deviation of each variable) has been used.

*K*means Clustering was applied as a non-hierarchical clustering technique to establish groups from the partition of the set of n = 51 observations in *k* groups in which each observation belongs to the group whose mean value is closer. It was calculated using the *kmeans* function in the *stats* R package [50]. It began by creating *k* random clusters. Then, the mean of each group and the distances of each individual. The individuals were placed within new resulting groups with the minimum intraspecific variance and maximum interspecific variance (members most similar to each other and the most different from those of other classes).

Then a principal component analysis was applied to summarize the large number of raster layers or descriptors and easily understand their role in the formation of the groups. The pre-selection of the descriptors followed two criteria: a) More contribution of each one to the new dimension and b) less correlation between them.

The Partial Least Squares Discriminant Analysis (PLS-DA) is used to determine heterogeneity in environmental systems [51]. The function *plsda* of the R package mixOmics v2.7-1 was applied to obtain the Variable Importance in Projection (VIP) score and select the variables with the higher discriminating power (VIP >1 criterion) [52].

In addition to this, the $R^2$ (measures the degree of fit of data) and $Q^2$ (measures the predictive power) values describe the quality of the PLS-DA model. $Q^2$ is an estimation of the predictive ability of the model and is calculated via cross-validation (CV). In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares or PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the $R^2$. Good predictions will have low PRESS or high $Q^2$ [53].

## *Modelling habitat suitability*

The MaxEnt algorithm was used to construct the potential distribution model of *A. spinosa* to North Africa and the Iberian Peninsula [45,54]. This algorithm represents the distribution of the species by the probability function *P* over a set *X* of locations in the studied area. *P* assigns a positive value to every place *x* so that the sum of P(x) is the unit. In addition to the occurrence points used in the statistical analysis, we have added records of the presence of specimens introduced in Spain and Algeria, using the seven bioclimatic variables selected in the previous section and the soil pH layer. The output obtained predicts the suitability of the argan habitat satisfying all the associated bioclimatic limits and maintaining the distribution to its maximum entropy. The lowest suitability areas are symbolized by 0, while 1 symbolizes areas with maximum suitability [20,35,36]. The ROC curve (receiver of characteristics) graphically represents the discriminative capacity of the model from all possible cut-off points and how adjusted this distribution is expressed by the value of the area under the curve (Poor 0.75 AUC > 0.95 Excellent) [55].

The function *ENMevaluate* from *ENMevalin* R package (version 1.3.1093), help in selecting model setting that balance model goodness-of-fit and complexity [56].

The analyses carried out to evaluate the effect of the bioclimatic variables extracted from the values associated with the points with the presence of the species in their spatial distribution are summarized in Figure **2**.

### *Potential limitations*

The scope of such studies depends on the quality of the input data. In our case, free access data (both occurrence points and explanatory variables) have been used, for this reason a one-by-one coordinates check and filter was made and a biasfile has been calculated [57]. This type of data has resolution and precision limitations that could create biases in the results. For example, it does not identify topographic or climatic features occurring in specific areas smaller than 1km$^2$.

On the other hand, the wind station data belong to the global airport network and the ones closest to the study area were selected with data available in the period 1973-2000.

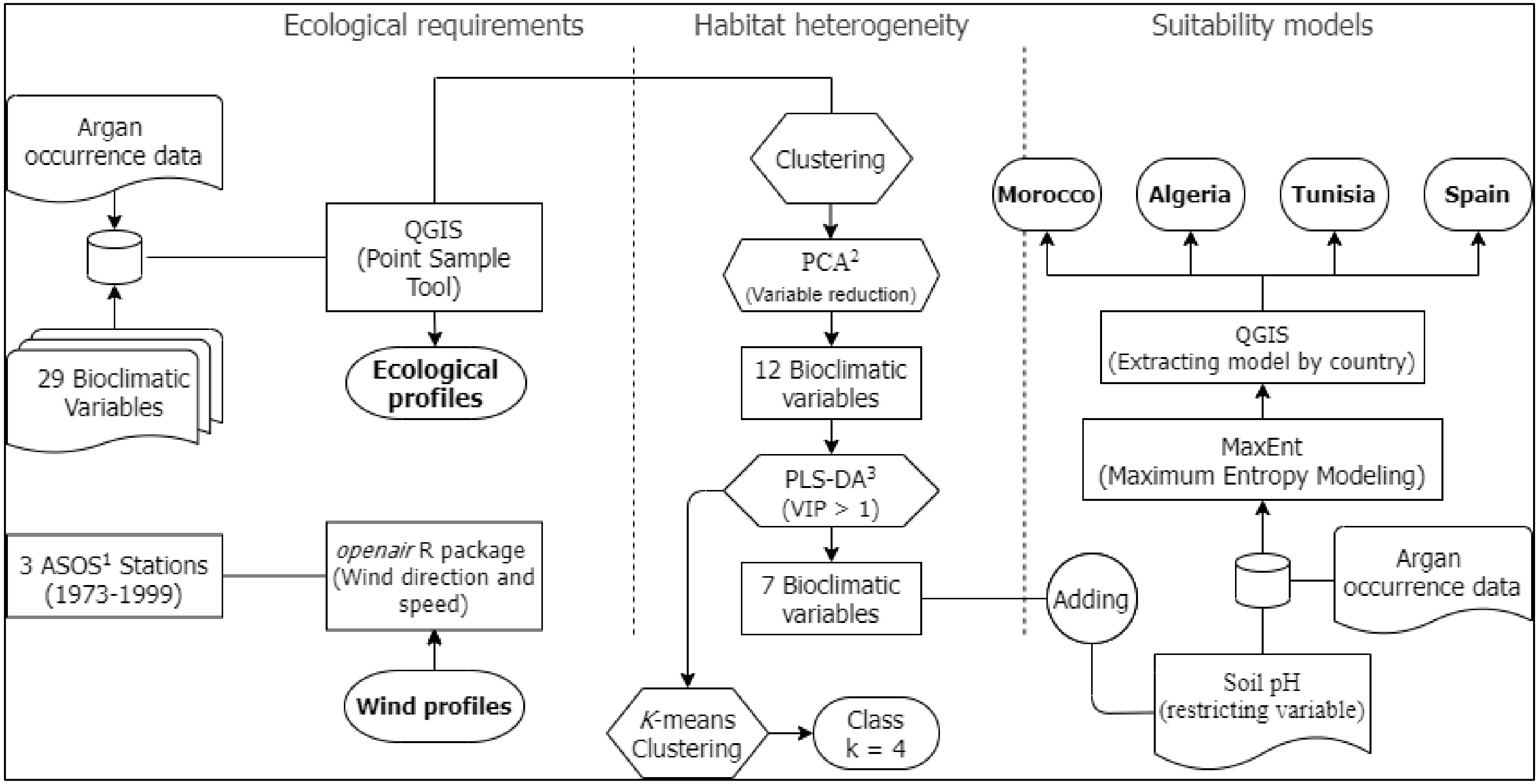

**Figure 2** Flowchart summarizing the methodology used in this ecogeographical study. [1]Automated Surface Observing System. [2]Principal Component Analysis. [3]Partial Least Squares - Discriminant Analysis

# Results

The ecological amplitude of the species (Figure 3, Appendices 2,3) showed clear evidence of the argan adaptation to xeric climates, by observing the low annual precipitation (200 - 400 mm) (Figure 3i), the high land surface temperature (up to 40°C) (Figure 3r), and the aridity index ranging between 0.01 - 0.24 (Figure 3s), typical from arid and semi-arid ecosystems in the sub-Saharan regions of Morocco and Algeria.

The thermometric variables show a clear trend of having a binomial distribution (Figure 3). The 3a, 3c, 3e, and 3g revealed a very centred distributions with just 4°C between the minimum and maximum values. At the same time, the driest quarter of the year (Figure 3e) is strictly centred between 22 - 24°C while the coldest quarter (Figure 3h) has a wider range between 8 - 16°C. Argan tolerates minimum temperatures in a wide range (Figure 3d) from 1 to 10°C and a narrow diurnal thermal range (Figure 3b) between 10 - 12°C.

A Gaussian distribution with 40 - 60 mm (precipitation of wettest month) is showed in Figure 3j, while the precipitation of driest month (Figure 3k) concentrates between 0 - 2 mm. The precipitation of wettest quarter has a maximum between 120 -160 mm, which seems to indicate that the rain is concentrated in that period. In summary, 50% of the rain may occur during the wettest and coldest quarter in the argan habitats, but it is close to zero in the warmest and driest quarter. These findings further support the idea of the argan capacity to acquire humidity from wet wind condensation and fog-harvesting.

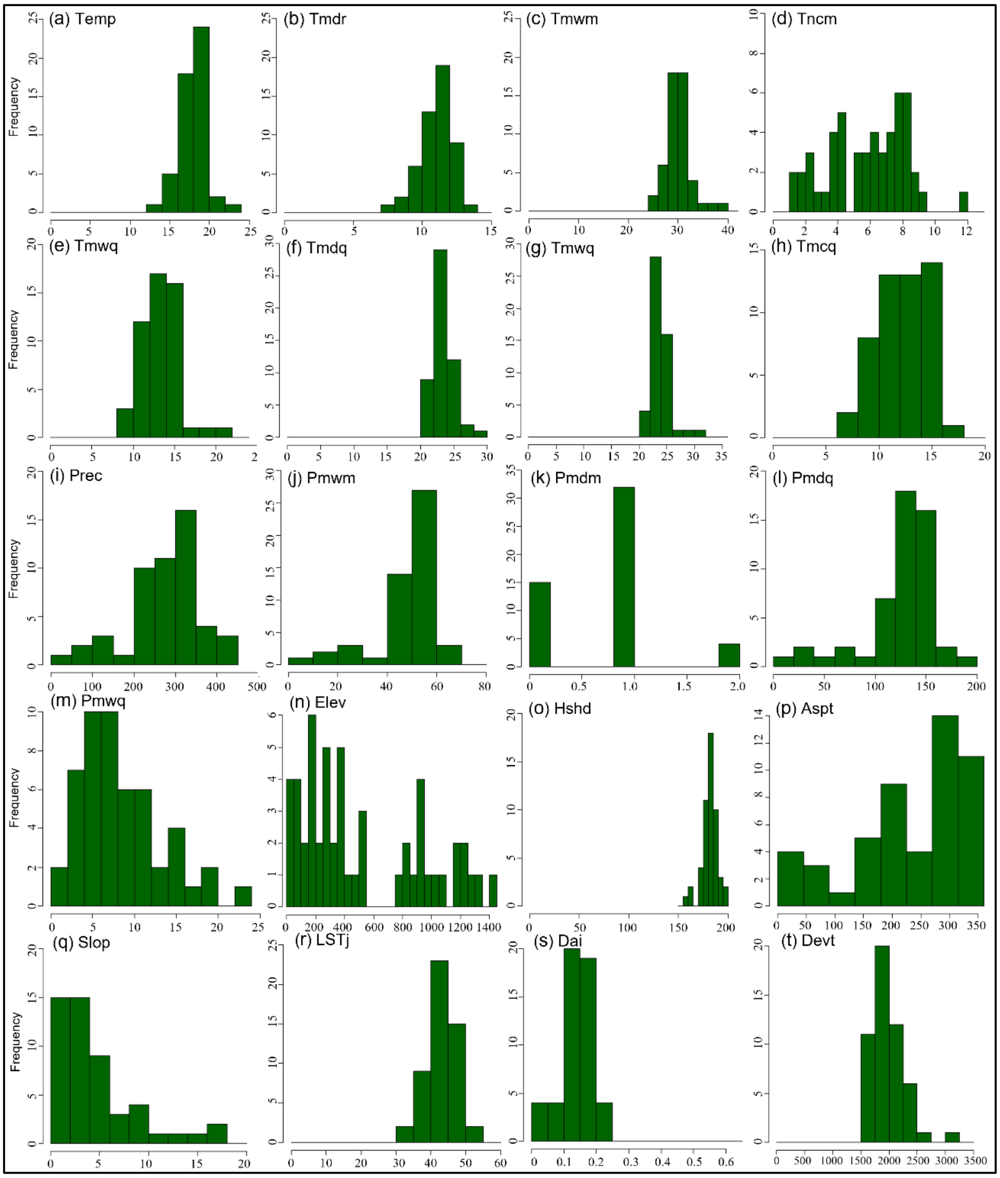

**Figure 3**Frequency distribution of bioclimatic variables used in the statistical analysis in North Africa (n = 51). **(a)** Annual mean temperature. **(b)** Mean diurnal range temperature. **(c)** Max temperature of warmest Month. **(d)** Mean Temperature of coldest month. **(e)** Mean temperature of wettest quarter. **(f)** Mean temperature of the driest quarter. **(g)** Mean temperature of warmest quarter. **(h)** Mean temperature of coldest quarter. **(i)** Annual precipitation. **(j)** Precipitation of wettest month. **(k)** Precipitation of driest month. **(l)**Precipitation of driest quarter. **(m)**Precipitation of warmest quarter. **(n)** Elevation. **(o)** Hillshade. **(p)** Aspect. **(q)** Slope. **(r)** Land surface temperature july. **(s)** Global Aridity Index. **(t)** Potential Evapotranspiration

Evapotranspiration shows a gaussian distribution with values between 1500 and 2200 mm/year (Figure 3t). The elevation (Figure 3n) shows a clear multimodal distribution, recognizing the existence of three populations: a coastal is distributed between 0 - 400 m, an inland one located in the mountains of the Saharan and Grand Atlas, with altitudes concentrated to the 900 m, and another one with higher altitudes between the 1200 and 1300 m, being able to rise in the Saharan Atlas above the 1500 m. The hillshade graph indicates values from 180 - 190°, and Figure 3p shows a clear preference for the N-NW orientations. The logistic distribution of the slopes (Figure 3q) shows the preference of argan for flat areas (slope 15%), littoral plains, open plateaus, and *oueds*.

At the Sidi Ifni station, prevailing winds with a WNW-NNW direction are observed in the months of April, May and June and speeds between 1.5 and 3 m/s (Figure 4a-c). However, in the northern stations of Safi (Figure 4d-f) and Essaouira (Figure 4g-i) the dominant humid winds occur during the summer months (July, August, September) in this case with NNW - NE direction and speeds between 2 and 6 m/s.

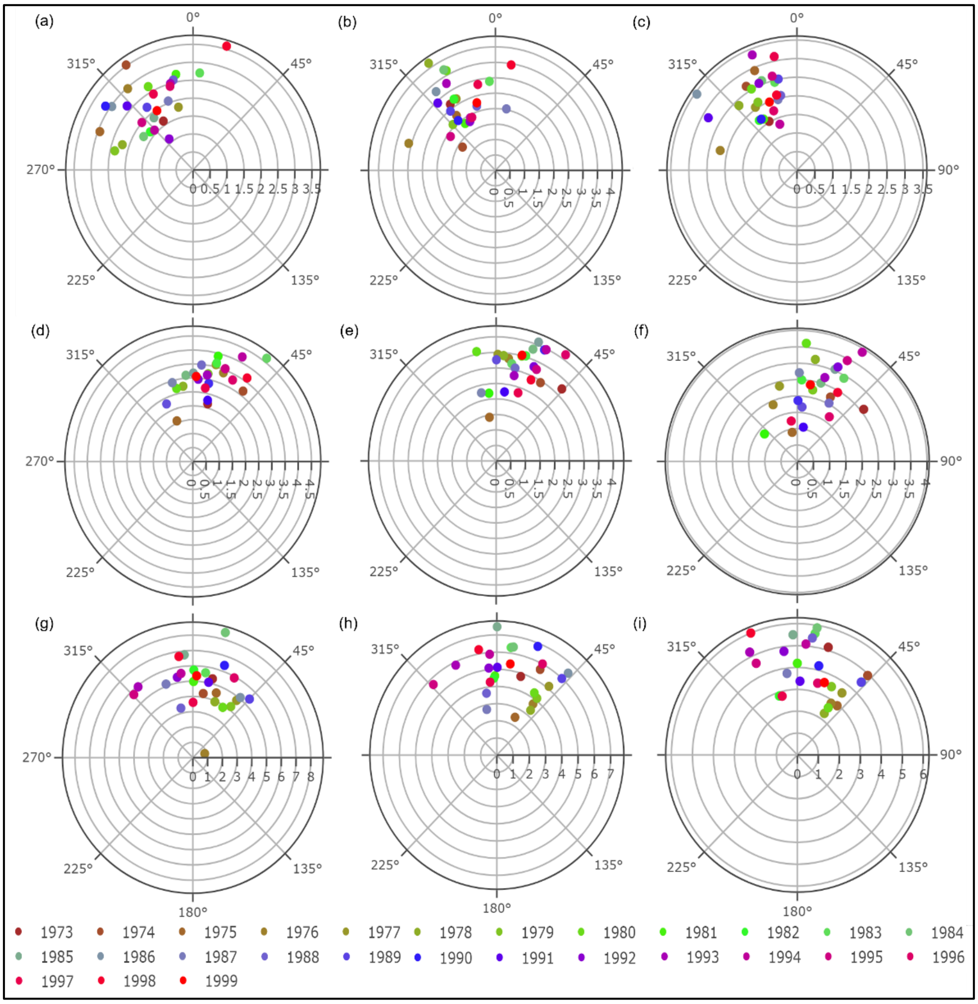

**Figure 4** Mean speed (m/s) and direction (degree) of the predominant winds (period 1973-1999). **(a, b, c)** Sidi Ifni station April-June. **(d, e, f)** Safi station July-September. **(g, h, i)** Essaouira station July-September. Rest of the month showed in the Appendices 4-6

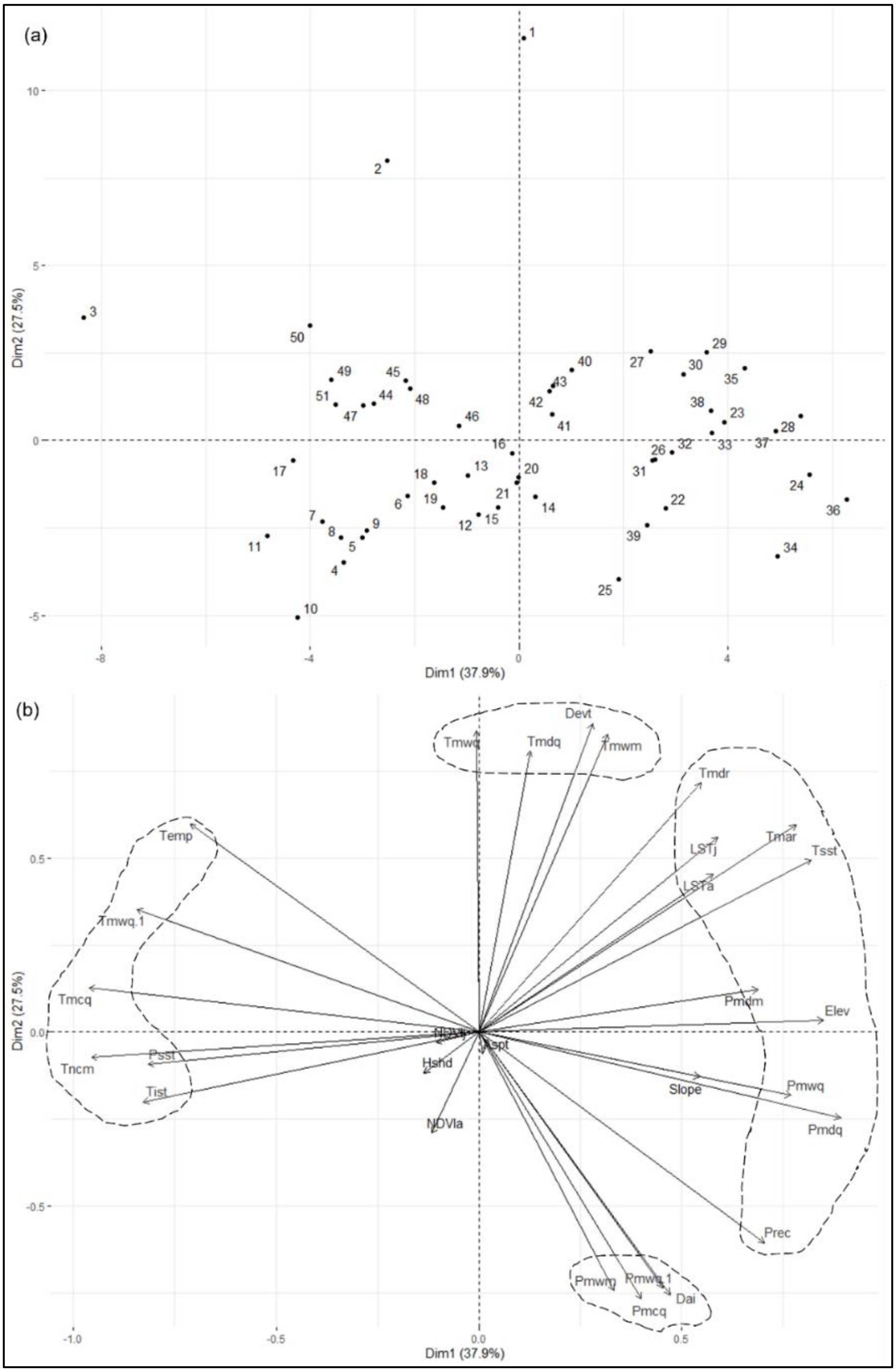

**Figure 5** Principal Components Analysis using 29 bioclimatic variables. **a)** Individuals between dimension 1 and dimension. **b)** Variables between dimension 1 and dimension 2

The non-hierarchical clustering analysis shows four main groups applying all the 29 bioclimatic variables (see Appendix 7) and the PCA analysis shows the distribution of the variables (Figure 5).

The first three dimensions explain 74.59% of the total variability. Principal component 1 (PC1) explains 37.93% of the variability and is related to precipitation, temperature, and elevation variables (Temp, Tncm, Tmar, Prec, Pmdm, Pmdq, Elev). On the other hand, PC2 describes 27.46% with the more extreme values such like high temperatures, aridity index and evapotranspiration (Tmdr, Tmwm, Pmwm, Dai, Devt). The weighting loadings indicate the importance of each variable in the construction of the principal components (see Online Resource 8).

The variable distribution reveals a significant difference in the dimension 1 between a group with higher elevation and annual precipitation during the driest month and quarter, besides a wide temperature range in contrast with another group that is affected by higher annual mean temperatures and lower temperatures during the coldest month. The dimension 2 separates the groups considering stress variables that get down localities with high aridity index, precipitation during the wettest month and quarter and get up localities with higher diurnal range, maximum temperature of the warmest month and evapotranspiration (Figure 5).

The 12 variables that better discriminate the four main classes are Temp, Tmdr, Tmwm, Tncm, Tmar, Prec, Pmwm, Pmdm, Pmdq, Elev, Dai, and Devt. Due to these predictors, we have called the groups: Desert (1), Littoral (2), Mountain (3), and Sublittoral (4) (Figure 6).

### *Desert group (1)*

In this group the annual and mean diurnal range of the temperature varies between 12 to 14°C and 28 to 34°C, respectively. The localities of Tindouf (01) and Assa Zag (02) have the higher temperature during the warmest month which causes more evapotranspiration. Along with the Laayoune (03), Tiznit (51) and Guelmim (49, 50) localities, all together comprise a geographical area influenced by the Sahara, with a hyperarid climate and annual rainfall above 70 mm and less precipitation during the wettest month and the driest quarter. It is important to mention that these three last samples have less evapotranspiration and a lower temperature range, that is the reason why they are classified in the sublittoral group.

### *Littoral group (2)*

Due to the Atlantic influence, the Littoral group of 18 localities has the least range temperature, this means, the lowest temperature during the warmest month and the highest temperature during the coldest month. This area that also include the Mediterranean coast localities, has less evapotranspiration, and is considered as Arid climate. In this class, the elevation ranges between 26 to 300 m except for two more inland localities (14, 15) that reach 500 m. The recorded annual rainfall is between 200 and 328 mm and becomes lower during the wettest month in a range of 47 to 62 mm. Besides, the precipitation does not exceed 8 mm during the driest quarter.

### *Mountain group (3)*

This group is made up of 18 localities characterized by having lower temperature values both in the annual mean (14 - 18°C) and in the minimum of the coldest month (1.30 - 5.70°C). Likewise, annual precipitation varies in a range of 200 to

427 mm with lower precipitation during the wettest month (35 - 69 mm) and the driest quarter (5 - 16 mm). The altitudinal range of this group is the highest with elevations from 750 to 1500 m. This group also includes the two isolated localities from Oued Grou (39) and Chlef (35) that have lower elevation and higher temperatures than the others.

*Sublittoral group (4)*

The sublittoral group consisting of 13 localities corresponds to a more inland and meridional area located between 88 - 488 m, not reach higher altitude zones. This group is characterized by having intermediate climatic conditions (Tmdr = 11°C, Tmar between 21.2 – 24.8°C) higher than in the Mountain group, but lower than in the Littoral group.  The precipitation of the driest quarter varies in a range of 2 - 5 mm. It is the group with the lowest aridity index after the desert group.

The most important variables (VIP > 1) identified by the PLS-DA method were Tmdr, Pmdm, Tncm, Elev, Tmar, Dai, and Pmdq and the boxplots show the distribution of the VIP variables in each group (see Appendix 12). The nonuniform concentration of the desert group samples coincides with the similarities between the southernmost sublittoral localities (Laayoune, Tiznit, and Guelmim), even so, for most variables the remaining three groups are well differentiated.

In total, 66 occurrence records of *A. spinosa* and the 7 most important environmental layers were used in this suitability analysis, including the Soil pH in $H_2O$ (Dph) as a restricting variable (see Appendix 13).

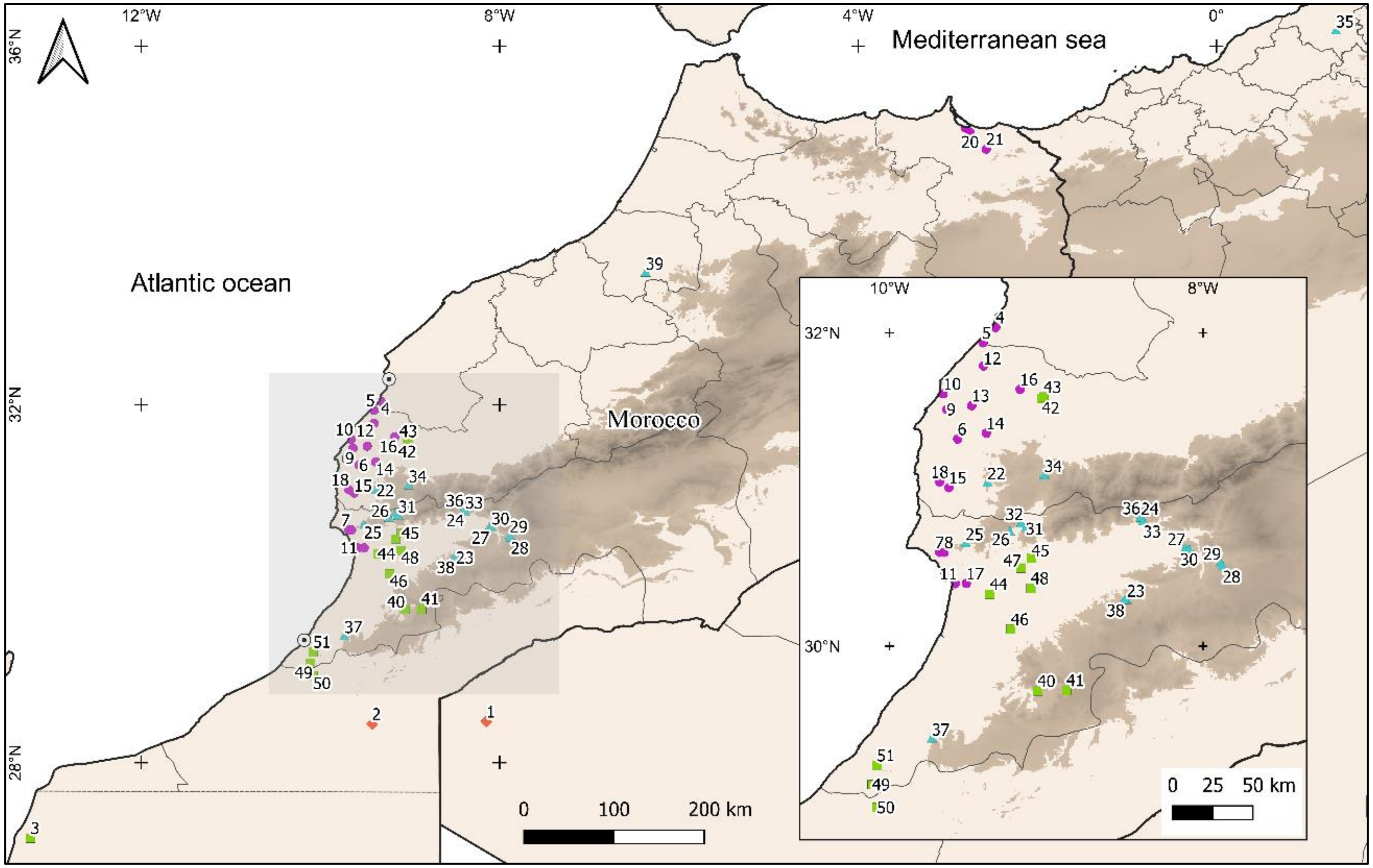

**Figure 6** Clustering results using k-means in the native area. The desert group is represented by orange rhombuses, littoral by magenta circles, mountain by blue triangles, and sublittoral by green squares. Darker areas show mountain ranges above 1000 m.

Ten MaxEnt runs were executed to determine the distribution model resulting in a robust and stable model (see Appendix 12). The mean AUC value of 0.98 indicates that the distribution explains very well from the environmental variables.

The MaxEnt model's jackknife test of variable importance indicated that the aridity index (Dai) is the most effective single variable for predicting the distribution of argan in the considered area. However, the elevation (Elev) is not (by itself) useful for estimating the distribution of the species. Omitting the minimum temperature of the coldest month (Tncm), temperature annual range (Tmar) or Dai lightly decreases the predictive performance, which therefore appears to have the most information that was not present in the other variables (see Appendix 13).

The response curves (see Appendix 14) plot the dependence of the predicted suitability model both on the selected variable and on dependencies induced by correlations between the selected variable and other variables. Taking as reference the values for $P = 0$ (absence) and $P \geq 0.50$ (presence), these graphs substantiate the analysis of ecological profiles.

Areas with a high probability of occurrence are shown in red, gradually decreasing to low probability in blue (Figure 7). Figure 7(a, c, e) shows the coherent zones that the model considers ideal for the cultivation of argan in all the studied territories. In any case, the introduction of the pH of the soil considerably reduces the potential area of argan cultivation, giving the model even more coherence Figure 7(b, d, f).

In Morocco, the suitability model is clearly more restrictive when including the pH layers especially as regards the level of probability of success, but there is little change in the area given the extensive presence of non-acid soils with pH

≥7 in most of the Moroccan territory and the extensive area of distribution of argan in the SW of the country.

The model in Algeria shows the limited potential area for argan cultivation, reduced to the western Mediterranean coast. Further south, some level of suitability reappears, coinciding with the Hoggar and Tassili massifs.

In the Peninsular Spain, there is a cultivable area in the SW in the province of Huelva and the ideal zone is mainly spread over the Iberian S, SE, and E always looking for the most thermal areas at the same time as close to the sea as possible and without a special preference for mountainous areas, with greater probability in the province of Almeria. This is also observed in the Balearic Islands.

In the Canary Islands, it is not surprising the high probability of success for the cultivation of argan, considering the presence of *Sideroxylon canariense,* a Macaronesian endemic species close to *A. spinosa*, which lives in phytosociological communities, equivalent to arganin Morocco, in shrubs of succulent plants dominated by species of *Euphorbia* (tabaibos and cardones), *Kleinia*, *Ceropegia*, *Dracaena* and other species of Macaronesian distribution.

Finally, regarding Tunisia, the model recognizes the suitability of the flat coastal areas that appear on the eastern slopes of the small mountains and Tunisian reliefs. Towards the interior, despite the presence of relief of the Keirouan mountains, the model is not wrong in recognizing their suitability as a hyperarid orographic system, where only *Phoenix dactylifera* palms developed at the bottom of the drainage systems. The suitability model that includes pH reduces the area of success in the northernmost part of the country by removing acidic soils. For more detailed description (see Appendices 15, 16, 17).

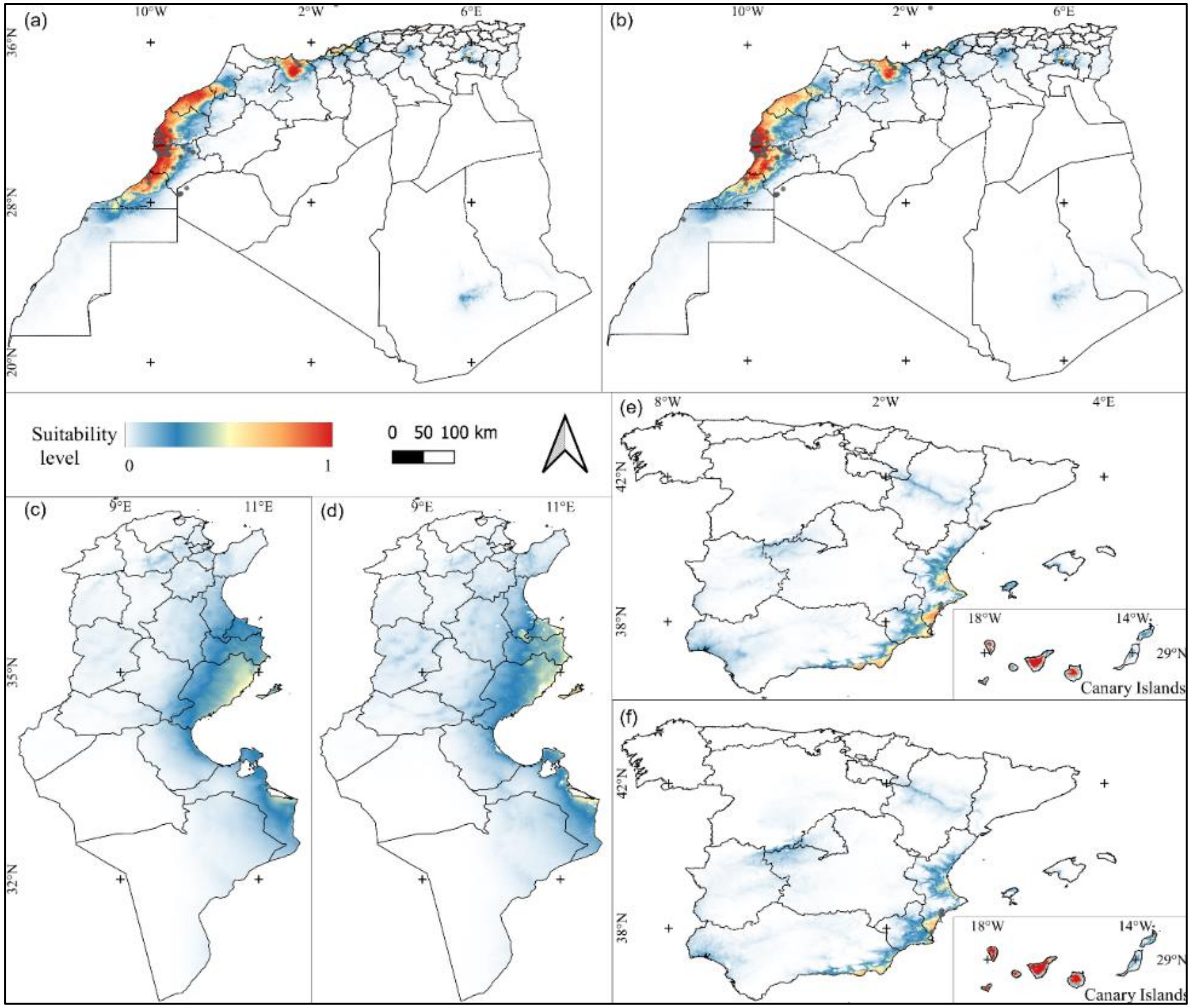

**Figure 7** Potential distribution of *A. spinosa* generated by MaxEnt ecological niche modeling using 7 (excluding pH) and 8 (including pH) environmental variables. Mean diurnal range (Tmdr), minimum temperature of the coldest month (Tncm), temperature annual range (Tmar), precipitation of the driest month and quarter (Pmdm, Pmdq), elevation (Elev), aridity index (Dai) and Soil pH in H2O (Dph). Morocco and Algeria (**(a)** excluding pH.**(b)** including pH), Tunisia (**(c)** excluding pH.**(d)** including pH), and Spain (**(e)** excluding pH, **(f)** including pH). Bluer shades represent less suitability and redder ones represent more suitability

## Discussion

The multimodal distributions of the ecological profiles demonstrate the different ecological optimum of argan in its distribution area and confirm the existence of diverse habitats, something already mentioned in studies on argan plant communities [58]. Thus, the elevation and mean precipitation of the driest month are trimodal and the minimum temperature of the coldest month is quadrimodal, suggesting that the argan tree lives in frost-free areas and adopts strategies to compensate for extreme climatic conditions, for example, being located at the bottom of *oueds* and taking advantage of the moisture supply from the fog and dew regimes in the more desert-like areas.

It is well-known that the Atlantic influence in the argan area located between 27° - 32°N, partly compensates its xericity at the littoral habitat [9,12,26]. According to Hammou [59] and Nerd [60] the warm winds of the Sahara (named *Chergui*) and the amount of water available affect the flowering of argan trees in southwestern Morocco. This area benefits from the oceanic influence whose atmospheric circulation is dominated by the Azores anticyclone with N-NE winds that drag summer fog inland.

The argan drought-avoiding strategy has been studied in the areas of its ecological optimum, according to Zunzunegui[61], deep soil layers are its main source of water. However, 130 km to the southwest of there (sublittoral zone) where more extreme conditions occur, fog and dew events become more relevant. This dependence on fog as water supply has been studied with other xerophytic species in hyperarid coastal zones like Namib (*Welwitschia mirabilis*) and Atacama (*Tillandsia* spp.) deserts [62,63].

The advection fog appears in southwestern Morocco when moist air from the Atlantic Ocean flows inland, where it meets the relief of the region. The wind speed (1.2 m/s - 2.4 m/s) and orientation (WNW - NNW) form the ideal conditions for generating dense fog layers [64,65]. These conditions coincide with those observed in the months of April, May, June in Sidi Ifni (SW), and July, August, and September in both more to the north Safi and Essaouira.

According to Marzol and Sánchez-Mejía [64], although there are more fog days on the coast than inland, the collected amount of water could be higher in the mountains (perhaps due to adiabatic cooling, a phenomenon typical of mountain fogs). A correlation between the fog events, an efficient condensation, and the presence of phenological phases sensitive to water deficit could be suspected of this outcome. In fact, the months with most fog events inland coincide with the flowering period of some argan populations (April, May, and June) [60].

These argan abilities to adapt to the different habitats are achieved thanks to its polymorphism, a character that has been demonstrated by numerous authors at molecular level [7,10,28,29,66,67]. At the same time, the differences observed in their phenology are also correlated with this polymorphism [59,68].

Surprisingly, the model does not recognize suitability in Tindouf (west Algeria) where the presence of argan is naturalized or may be even spontaneous, and in any case a cultivated species [15,69]. The unusualness of the environmental values of this area of southwestern Algeria is not very representative with respect to other larger areas, making these characteristics outliers and do not effectively train the suitability model. As in many other species of economic interest, the presence and human footprint are so ancient that often confounds interpretation whether they are palaeophytes or relict species in certain locations.

However, although samples from Tunisia were not used, the model recognizes suitability in the provinces of Tunis, Nabeul, Sfax, Sousse and Medenine named in recent studies [70,71]. The same occurs in Canary Islands where there is evidence of argan introduction in some locations (https://www.atlasdesemillasdecanarias.org).

Finally, the identification of the ecological euroicity of argan justifies the comprehensiveness of the suitability models obtained in this study and makes it possible to find solutions to mitigate the effects of climate change on natural argan populations.

## Conclusions

The simultaneous application of three complementary quantitative analysis methods (ecological profiles, multivariate analysis, and suitability models) has allowed identifying the ecological requirements of *A. spinosa*, analysing the heterogeneity of its habitat and establishing the successful potential areas for cultivation of argan in the western Mediterranean Basin.

Outcomes from this ecogeographic study further support the polymorphic character of argan and provide insights into the specific adaptation strategies that it has allowed growing in littoral, sublittoral, mountainous, and desert habitats.

In addition to its natural range in Morocco and Algeria, potential argan cultivation areas have been characterized as more suitable in Spain than in Tunisia. The Mediterranean coast of south-eastern Spain represents the area that best will resemble the ecological requirements of the argan tree and can be considered an ideal choice as a cultivation area and climatic refuges to improve the conservation of this multipurpose species.

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# Capítulo III

## Assessing argan tree (*Argania spinosa* (L.) Skeels) *ex situ* collections as a complementary tool to *in situ* conservation and crop introduction in the Mediterranean basin

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# Abstract

*A. spinosa* (L.) Skeels, hereafter argan, is a tree species naturally distributed in Morocco and Algeria, introduced mainly for productive purposes in countries such as Tunisia, Israel, and Spain. This promising species has a more extensive potential cultivation and use due to its economic prospects in human food, and cosmetics. These reasons and its great aridity adaptation have raised the strategic value of argan and its *ex situ* collections, compared to other more sensitive to climate change crops. From this perspective, this study aims to evaluate the genetic diversity of an *ex situ*, 10 years-old collection on more than 600 specimens raised in southern Iberian Peninsula, and to promote its cultivation in the most suitable regions for its introduction throughout the Mediterranean basin. To this end, the genetic and morphological diversity of a subset of selected specimens was compared, and six MaxENT models were trained using 96 occurrence points in both wild and cultivated localities (*ex situ* collections), together with six bioclimatic variables in a current time frame and under two climate change scenarios (optimist and pessimist). Surprisingly, this Iberian collection's genetic diversity was highly representative of the wild population's diversity in their natural range. Given this representativeness, these cultivars could be a complementary conservation tool as well as a starting point for domestication, breeding, and cultivation programs in a wide environmental range in these territories. The natural distribution of argan will be considerably reduced and shift towards northern habitats by 2050 and 2080, where climatic refuges and new cultivation areas could be established.

**Keywords**: argan tree, *Argania spinosa*, climate change, *ex situ* conservation, genetic diversity, suitability models.

# Introduction

The argan tree is a species native to south-western Morocco and western Algeria. It is the main component of the Arganeraie, a forest formation declared a Biosphere Reserve by UNESCO and protected by Moroccan legislation. Even, the United Nations proclaimed May 10 as International Argan Tree Day [1,2]. Therefore, their management, which is particularly committed to their conservation, must adopt an ecosystemic perspective in these territories, compatible with their use as an ancestral natural resource. But, beyond its natural range, the species has also been introduced over the centuries, in countries such as Tunisia, Israel, Iran, Libya, Mauritania, and Spain [3–8], and more recently in others such as Australia, the United States, Kuwait, and Mexico [9,10], generally seeking to cultivate and exploit it. The situation is different in each of these countries. This introduction, almost always carried out for productive purposes, has been able to generate processes of selection and genetic breeding, so that these germplasm collections could represent an alternative for *ex situ* conservation, especially in the case of old introductions and specimens kept in botanical gardens and arboreta.

This species, adapted to different habitats (littoral, sublittoral, mountainous, and desert) inhabits a wide variety of vegetal formations and landscapes together with other species, depending on locality and ecological conditions, such as *Quercus ilex* subsp. *rotundifolia*, *Pistacia lentiscus*, *Olea marocanna*, *Ziziphus lotus*, *Periploca laevigata, Tetraclinis articulata*, *Juniperus phoenicea*, *Whitania frutescens*, *Genista tricuspidata*, *Globularia alypum*, *Rhus tripartita*, *G. ifniense*, *Ziziphus lotus*, *Lavandula dentata*, *Ephedra nebrodensis*, or even *Buxus balearica* [11,12]. It is also altitude-independent, as it occupies from the coastal areas up to 1,800 m where penetrates the valleys forming oueds (wadis) that rise from the High Atlas and the Anti-Atlas Mountains.

A very singular case of the presence and persistence of these ancient crops in the territories occupied by Al-Andalus, an ancient region comprising the southern and eastern two-thirds of the Iberian Peninsula and over which Muslim domination extended for centuries, is sufficiently documented [13,14]. Specifically, texts from the Middle Ages on agronomy, botany and pharmacognosy suggest its cultivation and oil production (Ibn Ŷulŷul (10th c.), Abū l-Jayr: (12th c.) and Ibn al-Bayṭār (13th c.) in García Sánchez [14]. Even in these days, 800 years later, there are still scattered trees in some localities in the provinces of Murcia and Alicante and some cultivated trees in different gardens [5,15,16].

From an agronomical point of view, argan has high economic prospects due to its applications in the cosmetic and human food industries. If we add this to its great aridity adaptation, we are faced with a complementary or alternative crop for warm and low-rainfall areas, where other woody crops such as olive, pistachio, and almond trees may already be in decline because of the progressive climate change in circum-Mediterranean, North African and Middle East countries. However, the techniques of domestication, propagation, and cultivation of argan have not yet been effectively resolved and the area of exploitation is almost exclusively limited to its natural range.

On the other hand, *in situ* conservation measures may be insufficient to reverse the regression of argan natural communities [2]. The genetic diversity in these habitats has been extensively studied for decades with different molecular techniques [4,17–27]. Most of these studies were mainly aimed at assessing the amount and partitioning of genetic diversity among natural argan populations to understand to what extent genetic patterns are linked to geographic distributions and to establish what measures should be taken in the future for the conservation of this important species based on its genetic structure. Mouhaddab [24–26] have even identified core collections to preserve the total genetic diversity.

The remarkable genetic and phenotypic diversity observed in *Argania spinosa* has generally been related to the diversity of habitats in which it occurs [12,28–31]. This polymorphism could explain to a large extent why the species is able to occur in a relatively wide range of habitats, sometimes taking advantage of Atlantic advection fogs in the coastal regions of SW Morocco [32] and sometimes occupying the bottoms of drainage rills, reaching higher altitude mountain environments with higher precipitation, relieved by orographic fogs on the southern slopes of the western High Atlas and the northern slopes of the Anti-Atlas, or even living close to the Mediterranean in Beni-Snassen mountains in the Eastern Rif. These different ecosystems show a floristic composition of relative diversity, loaded with endemisms, made up of Mediterranean floristic components (with *Olea europea, Pistacia atlantica, P. lentiscus, Buxus balearica, Quercus ilex, Tetraclinis articulata*, or *Juniperus communis*), Macaronesian (*Kleinia, Aeonium, Euphorbia* and *Astydamia* species, even *Dracaena ajgal* and some fern such as *Davallia c*anariensis) or Afrotropical (*Acacia* spp.). All these communities are considered by phytosociology school [11,31]as Mediterranean and sclerophyllous plant communities, included in the order *Acacio-Arganietalia*. In them, the argan tree is in direct competition with a few tree species (such as *Tetraclinis articulata, Acacia gummifera, Pistacia atlantica, P. lentiscus, Ceratonia siliqua* - probably an archaeophyte - or even *Buxus balearica* or *Quercus ilex* at the limits of its ecological range). It may also compete with some thorny shrubs with intricate branching, with oleoid to microbuxoid leaves (the same range of variation as the argan tree) such as *Olea europea, Ziziphus lotus, Lycium sp., Genista* spp. *Periploca angustifolia*). However, it probably does not compete with other physiognomically very different species, such as crassulents of

Macaronesian, nor with the Mediterranean small shrubs of *Teucrium, Sideritis, Thymus, Lavandula* or *Globularia*.

In this ecosystem framework, the argan tree has "open" ecological niches in which it can compete, undergoing favorable physiognomies with respect to some of its competitors. For this reason, Argan probably has very different growth habit and branching forms, very different levels of spinescence, polymorphic leaves (from oleoid to microbuxoid), fruit types and production, phenology, and pollination systems (ambophily). In this scenario, we must also consider, as a complementary justification for its polymorphism, the hypothesis that argan has developed an "i" strategy (diversity cenotic strategy described by Blandin [33]) as well as other species in Mediterranean ecosystems, as a form of adaptation of the late Tertiary flora to Quaternary mediterraneanization. This strategy promotes infraspecific variability, compensating the "s" diversity deficit (strategy of occupying ecological niches with species of different taxonomic nature).

Whatever the reason for the outstanding polymorphism of this species, it is clear that any attempt at cultivation or reintroduction in other regions should play on a sufficiently representative genetic diversity. The identification of localities and small *ex situ* collections of argan, especially if they comprise enough genetic diversity, makes feasible to create suitability models that may expand its potential distribution for cultivation. These *ex situ* collections may facilitate argan domestication and cultivation and be an alternative way of conservation facing the future risks that wild populations may have.

Taking into account the above considerations, the main objective of this study was directed towards the evaluation of *ex situ* collections as a complementary tool to *in situ* conservation and to search for possible climatic refugia, developing for this purpose three more specific objectives: 1) To evaluate the molecular and morphological diversity of existing *ex situ* argan collections in the S of the Iberian

Peninsula in comparison with that already evaluated in their natural distribution; 2) To draw the most successful areas for argan cultivation in the Mediterranean basin (and nearby archipelagos) using probabilistic models; 3) To identify the most effective areas for future argan cultivation, in the same Mediterranean geography and under two foreseeable scenarios of climate change (2050 and 2080).

## Materials and methods

### *Natural range of argan*

Argan naturally occurs in Mediterranean Africa, extending over 1,076,000 ha [34]in south-western Morocco and 96,940 ha in south-western Algeria (Tindouf) [35,36]. In this work, we have therefore studied argan's natural distribution extended to the rest of the Mediterranean Basin, between 15°N and 50°N latitude and 20°W and 50°E longitude as study area. We selected this study area, considering how climate change trends affect this area and that the argan formations are ecologically Mediterranean (infra-Mediterranean bioclimatic ground, in *Acacio-Arganetalia* formations of *Quercetea ilicis* for sigmatist phytosociology).

### *Experimental crop collections. A case study in south Spain*

Beyond its natural area, there are localities around the Mediterranean basin where argan cultivation has been tested for productive and reforestation purposes. Such are the cases of some cities in Algeria, Tunisia, Israel, and Spain where argan is located in small experimental cultivations, commercial nurseries, botanical gardens and scattered old specimens (Table 1).

**Table** There are other documented records of individuals introduced in Libya [3,37]. The genetic diversity of these *ex situ* localities has not yet been evaluated, except for a minor experimental crop in Spain [38].Therefore, in this work we have attempted to assess diversity for what may be the argan collection with the largest number of specimens in Spain, located in the municipality of Aguilar de la Frontera (Andalusia, Spain) with about 600 trees under cultivation, planted by seeds in 2013 and transplanted in 2015 (Figure 1).

**Table 1**. *Ex situ* localities (experimental crops and ancient trees) of *Argania spinosa* in the Mediterranean basin

| **Status** | **Country** | **Locality** | **Plantation year** | **Latitude (º)** | **Longitude (º)** | **References** |
|---|---|---|---|---|---|---|
| Experimental crops | Algeria | Adrar-ville | 2003 | 27.88 | -0.28 | [35,39] |
| Experimental crops | Algeria | Bechar | 2003 | 31.63 | -2.22 | |
| Experimental crops | Algeria | Timimoun | 2003 | 29.25 | 0.24 | |
| Experimental crops | Algeria | Tindouf-ville | 2003 | 27.67 | -8.11 | |
| Experimental crops | Israel | Ktura and Ramat | 1994 | 29.97 | 35.07 | [8] |
| Experimental crops | Tunisia | Gabés | Early sixties | 33.51 | 10.15 | [4,40] |
| Experimental crops | Tunisia | Nabeul | | 35.82 | 10.57 | |
| Experimental crops | Tunisia | Sfax | | 35.07 | 10.18 | |
| Experimental crops | Tunisia | Tunis | | 36.84 | 10.19 | |
| Experimental crops | Spain | Aguilar de la Frontera | 2013 | 37.48 | -4.73 | |
| Experimental crops | Spain | Santomera | No data | 38.11 | -1.04 | [5,6,41,42] |
| Ancient trees | Spain | Agaete | - | 28.10 | -15.70 | |
| Ancient trees | Spain | Alicante | - | 38.39 | -0.46 | |
| Ancient trees | Spain | Alicante | - | 38.37 | -0.50 | |
| Ancient trees | Spain | Alicante | - | 38.36 | -0.51 | |
| Ancient trees | Spain | Jijona | - | 38.49 | -0.48 | |
| Ancient trees | Spain | Molina de Segura | - | 38.06 | -1.17 | |
| Ancient trees | Spain | Orihuela | - | 37.90 | -0.76 | |
| Ancient trees | Spain | Tenerife | - | 28.15 | -16.52 | |

The black line represents the higher elevation group, and the red line represents the lower elevation group. Red dots represent the coastal subpopulation, purple dots represent the western subpopulation, blue dots represent the central and upper elevation subpopulation, and green dots represent the subpopulation that is closest to the drainage.

**Figure 1** Landscape view of the experimental crop collection with about 600 trees located in the municipality of Aguilar de la Frontera (Andalusia, Spain)

Sample size's selection was based on the criteria used in the consulted genetic diversity studies, around 20 individuals per locality [19,21,23,26]. In these cases, the aim was to compare natural populations consisting of hundreds or thousands of individuals. However, there are not so many localities in the Iberian Peninsula where adults or old specimens of argan trees are preserved, and they are also made up of a very small number of individuals. Therefore, a stratified sampling design was carried out to ensure the presence of the different physiognomic and morphological characters observed in this single locality of Aguilar de la Frontera with around 600 specimens. A total of 210 specimens located along the transects defined in the field were carefully examined. Ten descriptors were defined to explain the phenotypic variability present (D: level of development; F: physiognomy shoot angle; R: branching; Fx: phyllotaxis; FH: leaf shape; CH: leaf colour; NF: fruit quantity; CF: fruit colour; MF: fruit maturity; FF: fruit shape). Therefore, 10% of the specimens examined (n= 21) were chosen as representative of the different combinations of characters found. The rule of 20 individuals per locality was maintained.

## *Morphological diversity*

Morphological diversity has been studied by different authors who have evaluated the germplasm of this species from an agronomic perspective and identified different morphotypes for both the fruit and the morphologic aspect of the tree. In this study, we selected six morphological and four fruit characters (Figure 2).

A cluster analysis was performed to obtain a first approximation of differentiable morphotypes in the germplasm collection under cultivation chosen in this study. This method clustered the individuals according to their similarity in terms of the different types of modalities within the ten selected qualitative categories. The result of the clustering is shown in the dendrogram obtained by applying the Jaccard similarity index. The Multiple correspondence analysis (MCA) function of the FactoMineR package was used to recognize the traits associated with each group and individual [43].

### *Molecular diversity*

Simple Sequence Repeat (SSR or microsatellite) markers are ideal for genetic studies of population structure [44]. Due to their co-dominance, and high reproducibility, these markers are far enhanced to the commonly used Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), and Amplified Fragment Length Polymorphism (AFLP) [45]. SSR markers previously used by Yatrib [19], El Bahloul [21], Majourhat [23] and Mouhaddab [26] were used to assess the genetic diversity of this argan crop collection in south Spain. Additionally, we correlate some morphological characters and the molecular identity of the individuals, based on its high polymorphism. This correlation would provide a tool for predicting the performance (e.g., productivity) in the crop of the different cultivated specimens.

Accordingly, a pre-selection was made of the 15 primers with the highest polymorphism information content (PIC), which had shown good discrimination of loci, and heterozygosity when analysing diversity between individuals and populations of argan (ASMS01, ASMS20, ASMS2012-04, ASMS2012-34, ASMS2012-37, MH07, MH04, MH06, ME11, MH20, MH08, ME05, MH12, MH17, MH22), of which six showed amplification capacity in the 21 argan samples studied.

DNA was extracted from 20 mg of leaf sample previously sprayed with liquid nitrogen, using the QIAGEN Mini Kit plant Dneasy. Quantification was done by absorbance spectrophotometry at a wavelength of 260 nm using a NanoDrop-Lite spectrophotometer, for which a 1/10 dilution of the extracted DNA samples dissolved in 200 µl of TE had to be performed.

The PCR programme consisted of an initial step of 6 min at 94°C followed by 35 cycles of 30s at 94°C, 30s at 55°C for PCR I, and 57°C for PCR II, 30s at 72°C and a final extension step of 20 min at 72°C. PCRs were performed at the Central Service for Research Support (SCAI) of the University of Cordoba with the Biometra T3 Thermocycler and the HorsePower-Taq DNA Polymerase Kit (Canvax).

Genetic analysis was performed by capillary electrophoresis with Genetic Analyzer ABI3130 XL (Applied Biosystems) and GeneMapper v 4.0 analysis software. For which 2-2.5 µl of amplified product mixed with 6µl of formamide and 0.15 µl of ROX 400 size standard and denatured 2 min at 95°C were loaded.

The genetic diversity was estimated by the number of alleles (Na) per locus SSRs and farther genetic information of the codominant SSR markers was determined by the effective number of alleles (Ne), observed heterozygosity (Ho), and expected heterozygosity (He) [46] calculated applying the GenAlEx package [47].

### *Suitability models and climate change scenarios*

The five bioclimatic variables that better explain the current argan distribution area according to Labarca-Rojas [32] were obtained from the WorldClim data portal [48]. It contains information about Mean Diurnal Range Temperature (Tmdr), Min Temperature of Coldest Month (Tncm), Temperature Annual Range (Tmar), and Precipitation of Driest Month and Quarter (Pmdm, Pmdq), and Elevation from 1960 to 1990. In addition, the global gridded of pH soil information at 0-5 cm depth was included to the dataset [49]. All of them with a spatial resolution of 30s (~1 $km^2$).

To test whether the introduction of *ex situ* localities widens the range of argan distribution, two current suitability models were estimated: the first one using only *in situ* localities and the other combining *in situ* and *ex situ* localities (see Figure 2). These combined localities were used to calculate the future geographical distribution based on the Hadley Centre Global Environment Model (v. 2). Four models were obtained in two different periods 2050 (mean values from 2041 to 2060) and 2080 (the mean values from 2061 to 2080) applying two scenarios of the Intergovernmental Panel on Climate Change (IPCC): Representative Concentration Pathway (RCP) 4.5 and 8.5 (Figure 2). The four scenarios were predicted using the MaxEnt algorithm that represents the distribution of the species by the probability function P over a set $X$ of locations in the studied area.

Given the diversity of ecosystems in which the argan is found, all sites considered natural have been used (see supporting data). On the other hand, to reinforce the suitability models, sites from other countries such as Algeria, Tunisia, Israel, and Spain in which the species has been successfully introduced outside its natural range have been added. These *ex situ* localities with known coordinates are summarised in Table 1.

In total, eighty-seven occurrence points from both *in situ* and *ex situ* localities were used to train the models. The obtained output predicts the suitability of the argan fulfilling all the associated bioclimatic limits and maintaining the distribution to its maximum entropy. The lowest suitability areas are symbolized by 0, while 1 symbolizes areas with maximum suitability [50,51]. The ROC curve (receiver of characteristics) graphically represents the discriminative capacity of the model from all possible cut-off points and how adjusted this distribution is expressed by the value of the area under the curve (AUC) (Poor 0.75 <AUC > 0.95 Excellent) [52].

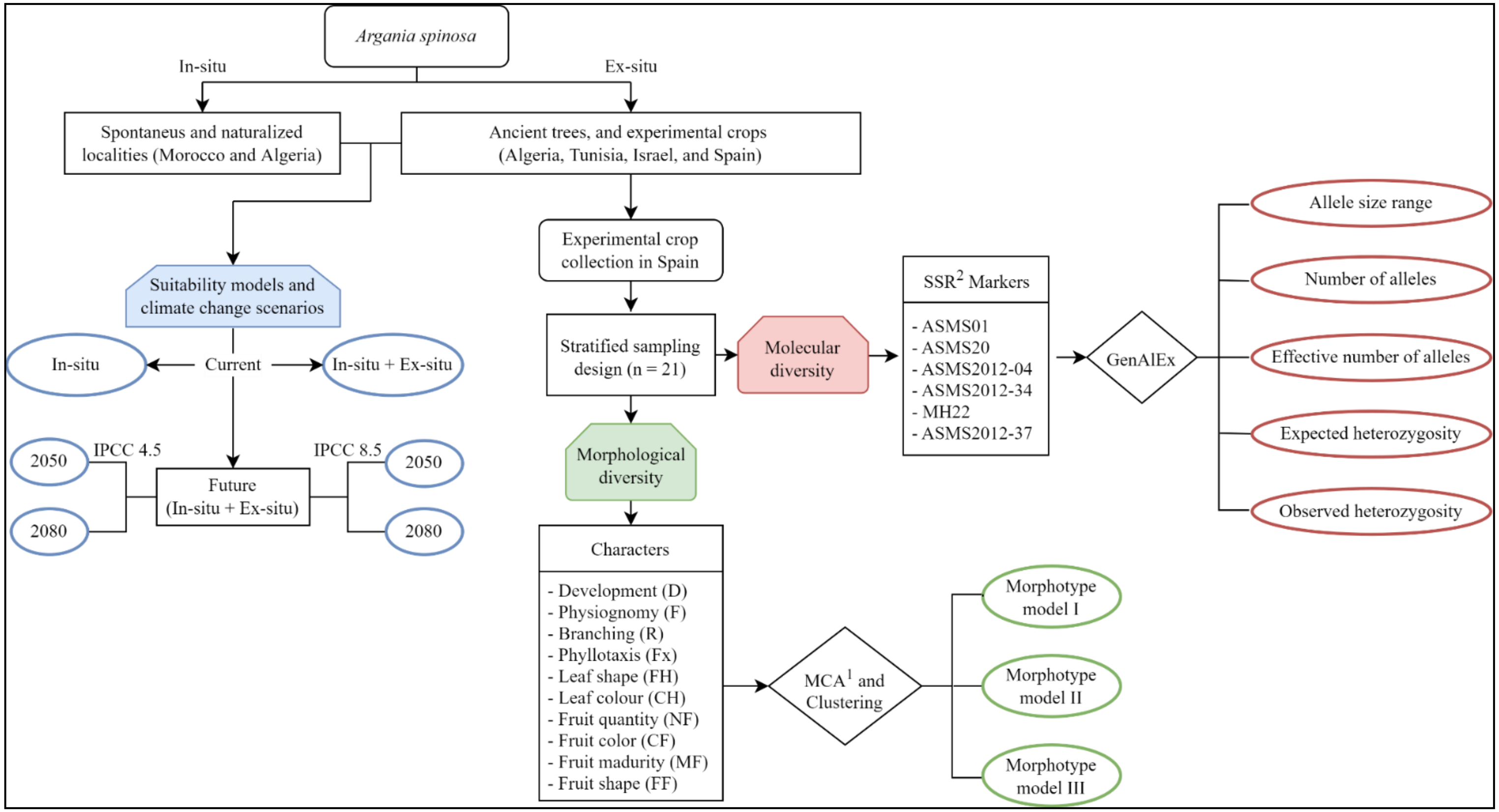

**Figure 2** Flowchart summarizing the methodology used in this study. [1]Multiple Correspondence Analysis. [2]Simple Sequence Repeats

# Results

## *Molecular diversity*

These results reveal the genetic diversity of 18 individuals out of 21. Three individuals, 9, 15, and 20, did not amplify with any of the primers used. A total of 55 alleles were detected among 18 *A. spinosa* trees (Table 2). The number of alleles per locus (Na) varied from 4 (ASMS2012-04) to 14 (ASMS01), with an average of 9.17. While the effective number of alleles per locus (Ne) ranges from 2.41 (ASMS2012-04) to 10.623 (ASMS01).

**Table 2.** Characterization of 6 SSR loci with 18 individuals of *A. spinosa*

| **Locus** | **Bp** | **$N_a$** | **$N_e$** | **$H_e$** | **$H_o$** | **$X^2$** |
|---|---|---|---|---|---|---|
| ASMS01 | 141-175 | 14 | 10.62 | 0.91 | 1.00 | NS |
| ASMS20 | 202-220 | 9 | 3.16 | 0.68 | 0.67 | NS |
| ASMS2012-04 | 303-315 | 4 | 2.41 | 0.59 | 0.50 | NS |
| ASMS2012-34 | 197-247 | 12 | 8.42 | 0.88 | 0.83 | NS |
| MH22 | 191-209 | 6 | 5.49 | 0.82 | 0.44 | * |
| ASMS2012-37 | 191-219 | 10 | 7.28 | 0.86 | 0.78 | NS |
| Total | - | 55 | - | - | - | |
| Mean | - | 9.17 | 6.23 | 0.79 | 0.70 | |
| SE | - | 1.52 | 1.29 | 0.05 | 0.09 | |

Bp: allele size range; Na: number of alleles; Ne: effective number of alleles; He: expected heterozygosity; Ho: observed heterozygosity; ***X**2*: Chi-Square Tests for Hardy-Weinberg Equilibrium; NS: no significant difference. *Significant (*P<0.05*) between He and Ho according to ***X**2 test.*

No significant differences were observed between Ho and He for five loci, indicating that the sample is close to Hardy–Weinberg equilibrium. Nevertheless, locus MH22 shows a He higher than Ho, indicating a slight lack of heterozygosity. This might be due to the presence of null alleles.

## *Morphological diversity*

Table 3 shows the results of the characteristics taken for each individual studied in the experimental farm.

The cluster analysis establishes, as shown in the dendrogram (Figure 4), the distribution of the 21 individuals analyzed through qualitative characters, identifying three large groups: a morphotype I that gathers in the centre of the dendrogram the seven most productive individuals (Figure 5), a morphotype II on the bottom with nine individuals of medium fruiting (Figure 6), and the morphotype III on the top of the dendrogram with the five sterile individuals (Figure 7).

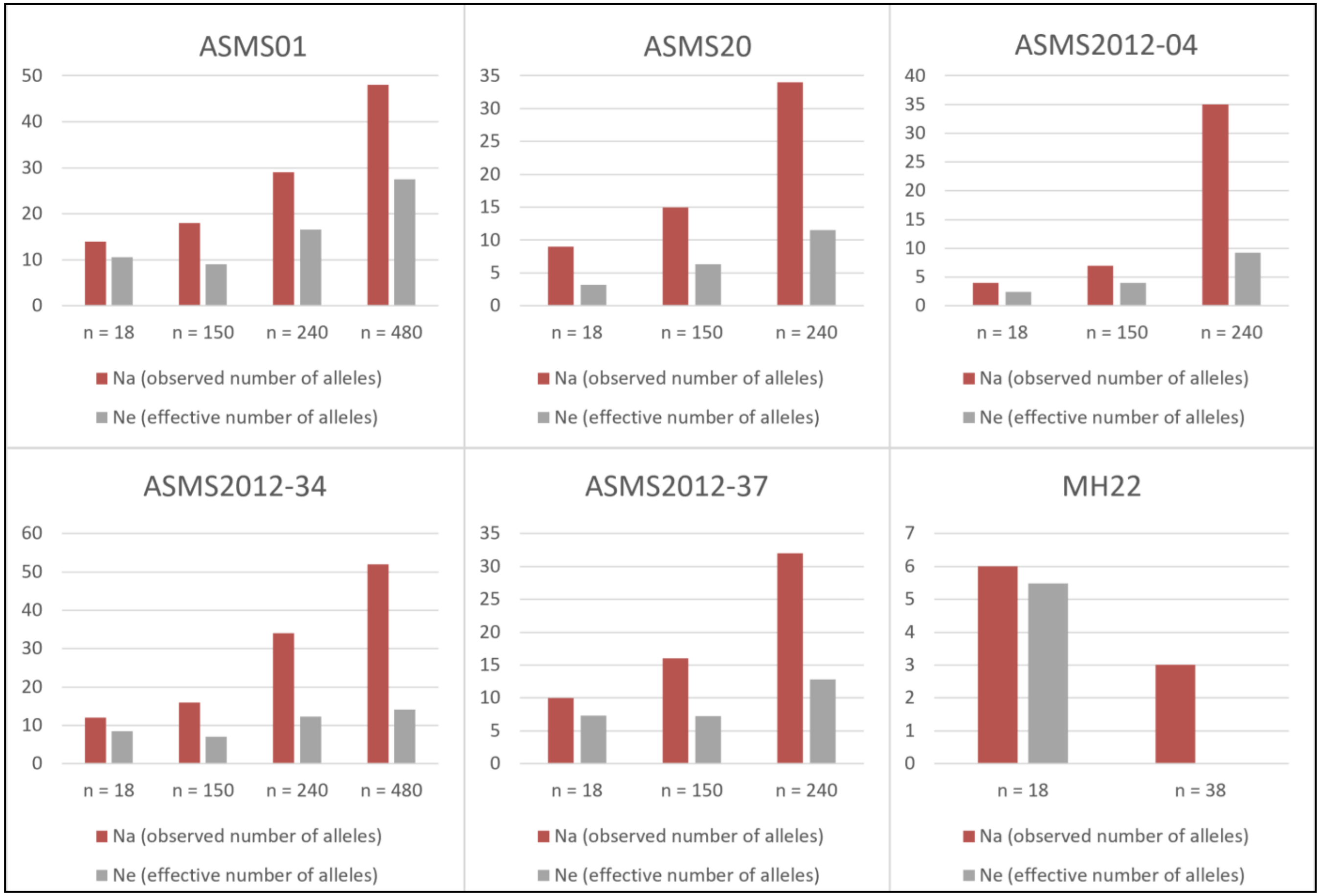

**Figure3** Comparison of the number of detected and effective alleles for each locus with other studies. N = 38 [23]. N = 150 [21]. N = 240 [19]. N = 480 [26]

**Table 3**Morphological categories used to group 21 argan specimens from an experimental crop collection in Andalusia (Spain)

| ID | D | F | R | Fx | FH | CH | NF | CF | MF | FF |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | High | Upright-patent | Medium | Fasciculate | Buxoid | Green | Abundant | Yellow | Medium | Piriform |
| 2 | High | Upright-patent | High | Fasciculate | Oleoid | Light green | Scarce | Yellow | Full | Piriform |
| 3 | High | Upright-patent | Medium | Fasciculate | Buxoid | Green | Abundant | Yellow | Full | Apiculate |
| 4 | Medium | Decumbent | Low | Fasciculate | Oleoid | Green | Scarce | Yellow-red | Full | Rounded |
| 5 | High | Upright-patent | Medium | Fasciculate | Oleoid | Green | Abundant | Yellow | Medium | Fusiform |
| 6 | High | Upright-patent | High | Fasciculate | Buxoid | Green | Abundant | Yellow | Full | Rounded |
| 7 | High | Decumbent | Medium | Solitary | Myrtoid | Green | Scarce | Yellow | Full | Apiculate |
| 8 | Medium | Decumbent | Medium | Fasciculate | Buxoid | Green | Scarce | Brown | Full | Apiculate |
| 9 | Medium | Decumbent | High | Fasciculate | Oleoid | Green | Abundant | Yellow | Full | Apiculate |
| 10 | Medium | Patent | Medium | Fasciculate | Oleoid | Green | Abundant | Yellow-red | Full | Apiculate |
| 11 | Low | Decumbent | Medium | Solitary | MicroBuxoid | Dark green | - | - | - | - |
| 12 | Medium | Patent | Medium | Fasciculate | Oleoid | Green | Abundant | Yellow | Full | Oval |
| 13 | Medium | Patent | Medium | Fasciculate | Oleoid | Green | Scarce | Yellow | Low | Fusiform |
| 14 | High | Decumbent | High | Fasciculate | MicroBuxoid | Dark green | - | - | - | - |
| 15 | High | Decumbent | High | Fasciculate | Buxoid | Green | Abundant | Yellow | Low | Fusiform |
| 16 | Medium | Decumbent | Medium | Solitary | MicroBuxoid | Green | - | - | - | - |
| 17 | High | Patent | High | Fasciculate | Oleoid | Light green | - | - | - | - |
| 18 | High | Patent | High | Solitary | Buxoid | Green | Scarce | Yellow | Full | Oval |
| 19 | Medium | Decumbent | Medium | Solitary | Buxoid | Dark green | Scarce | Yellow | Full | Apiculate |
| 20 | Low | Patent | Medium | Solitary | Rounded | Dark green | - | - | - | - |
| 21 | Medium | Patent | Medium | Fasciculate | Oleoid | Green | Scarce | Amarillo | Full | Piriform |

D: level of development; F: physiognomy shoot angle; R: branching; Fx: phyllotaxis: FH: leaf shape: CH: leaf colour; NF: fruit quantity; CF: fruit colour; MF: fruit maturity; FF: fruit shape.

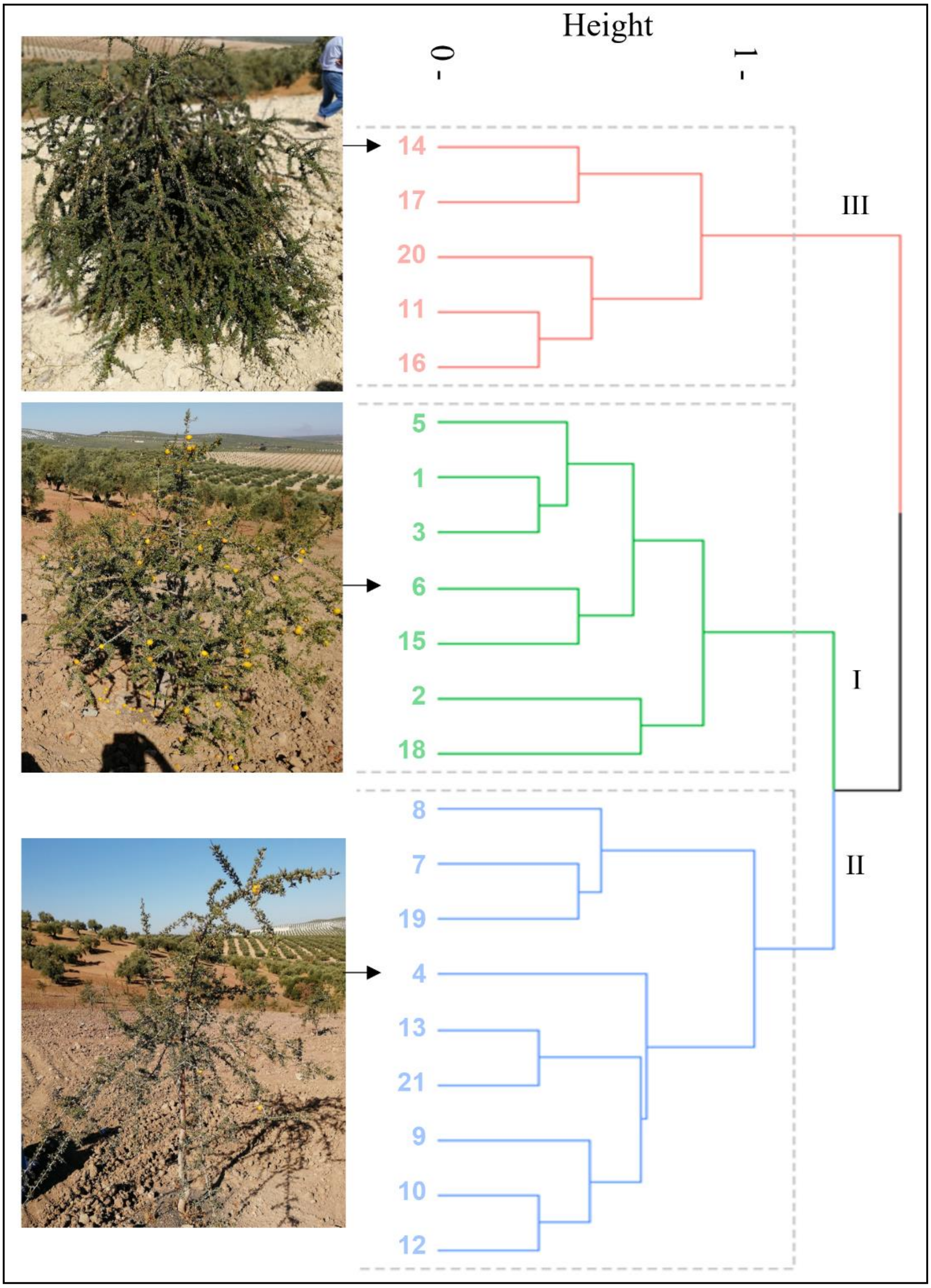

**Figure 4**Dendrogram of qualitative characteristics among 21 collected trees of *A. spinosa* based on Jaccard similarity coefficient and ward method

**Figure5** Seven individuals of the Morphotype I model: well-developed trees, with erect-patent branching, fasciculate leaves, and buxoid or oleoid shape, light green colour, with abundant fruiting and yellow pyriform or fusiform fruit during maturity

**Figure6** Nine individuals of the of the Morphotype II model: more or less well-developed trees, with patent or somewhat decumbent branching, with leaves in fasciculate arrangement, myrtoid in shape and green colour, medium or scarce fructification with round or oval fruits of reddish or brownish colour

**Figure 7** Five individuals of the of the Morphotype III model:poorly developed trees with patent or decumbent branches, solitary, alternate leaves, microbuxoid shape, and intense green colour, without fruits

The MCA method was applied to group the 21 sampled specimens by similar morphological characters (Figure 8). This algorithm differentiates three different groups. The non-productive group include individuals with no fruit, low development, microbuxoid leaves, and dark green color. The second group include individuals with erect-patent branches, large development, abundant fruit, pyriform or fusiform in shape, and less intense green colour of the leaves. The third group includes individuals with low branching, few oval fruits, and full maturity.

Specifically, three models of argan trees have been distinguished: model I: well-developed trees, with erect-patent branching, fasciculate leaves, and buxoid or oleoid shape, light green color, with abundant fruiting and yellow pyriform or fusiform fruit during maturity (see Figure 5); model II: more or less well-developed trees, with patent or somewhat decumbent branching, with leaves in fasciculate arrangement, myrtoid in shape and green color, medium or scarce fructification with round or oval fruits of reddish or brownish colour (see Figure 6), and model III: poorly developed trees with patent or decumbent branches, solitary, alternate leaves, microbuxoid shape, and intense green color, without fruits (see Figure 7). Morphotype I could be probably very close to the ideotype desired by any farmer.

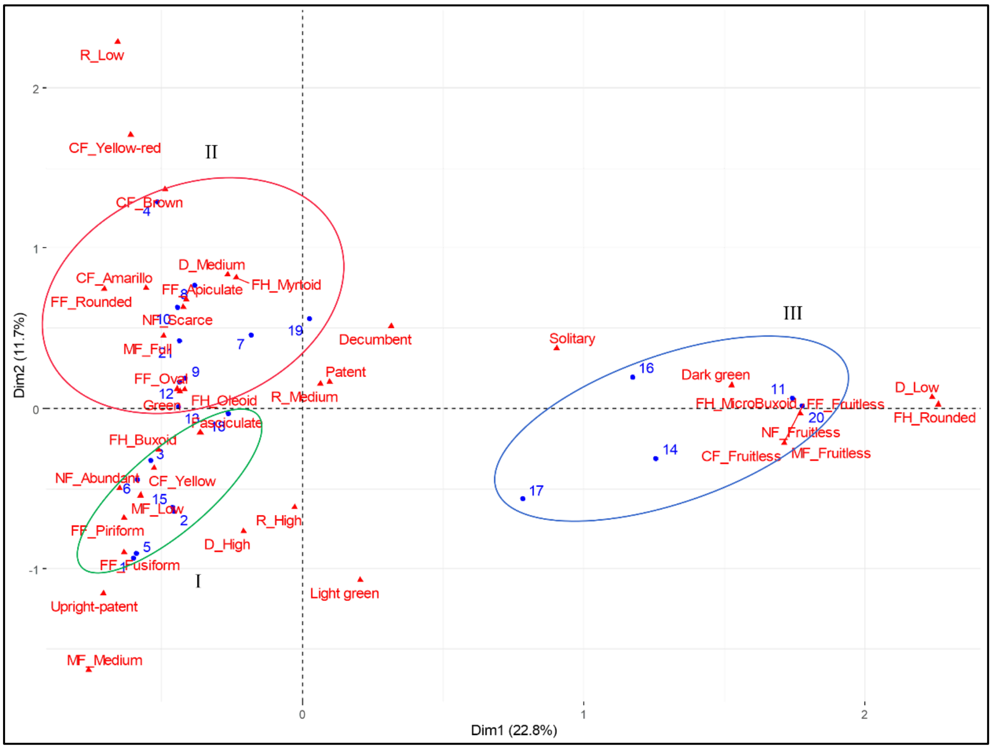

**Figure 8** Biplot of the 21 sampled argan *ex situ* collection grouped applying the Multiple Correspondence Analysis (MCA) method

### *Suitability model and climate change scenarios*

The current geographical distribution of argan was modelled in Maxent. Analysis of the AUC values of the training and test data indicated acceptable values of 0.95 and 0.94, respectively. These results explain the excellent discrimination ability of the model. The Jackknife test reveals that both variables Mean Diurnal Range Temperature (Tmdr) and Min Temperature of Coldest Month (Tncm), had the highest contribution in the model. Although the remaining bioclimatic variables used were considered necessary in the model (Figure 9).

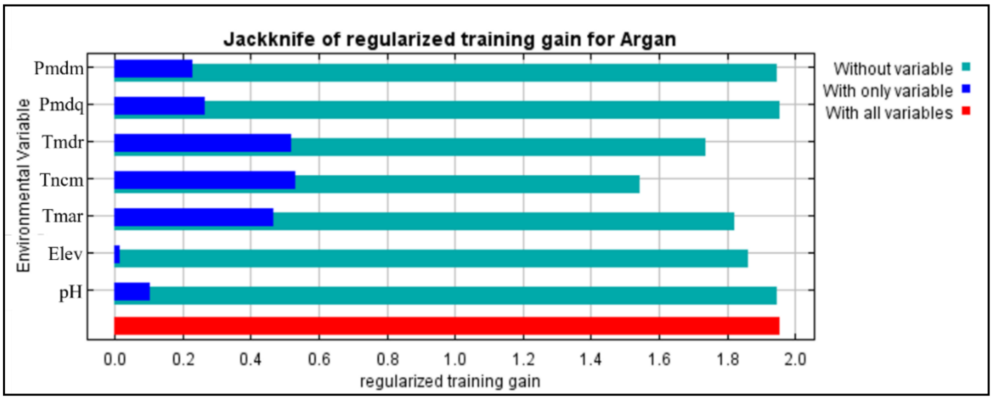

**Figure 9** Jackknife test of the importance of individual climatic and environmental variables (blue bars) in the development of the MaxEnt model relative to all climatic and environmental variables (red bar). Mean Diurnal Range Temperature (Tmdr) and Min Temperature of Coldest Month (Tncm), had the highest contribution in the model

As can be seen in the current scenario, suitability extends to locations where argan could be successful beyond its natural range. Table 4 and Figure 10 show the extent of the area estimated by the MaxEnt algorithm in six different models; combining current climate conditions with only *in situ* locations (Figure 10a) and with *in situ* and *ex situ* locations (Figure 10b), two IPCC scenarios were used to calculate the remaining four models, the most optimistic (RCP 4.5) by 2050 (Figure 10c), and 2080 (Figure 10d), and the pessimistic one (RCP 8.5) during the same periods 2050 (Figure10e), and 2080 (Figure 10f), this time using all locations.

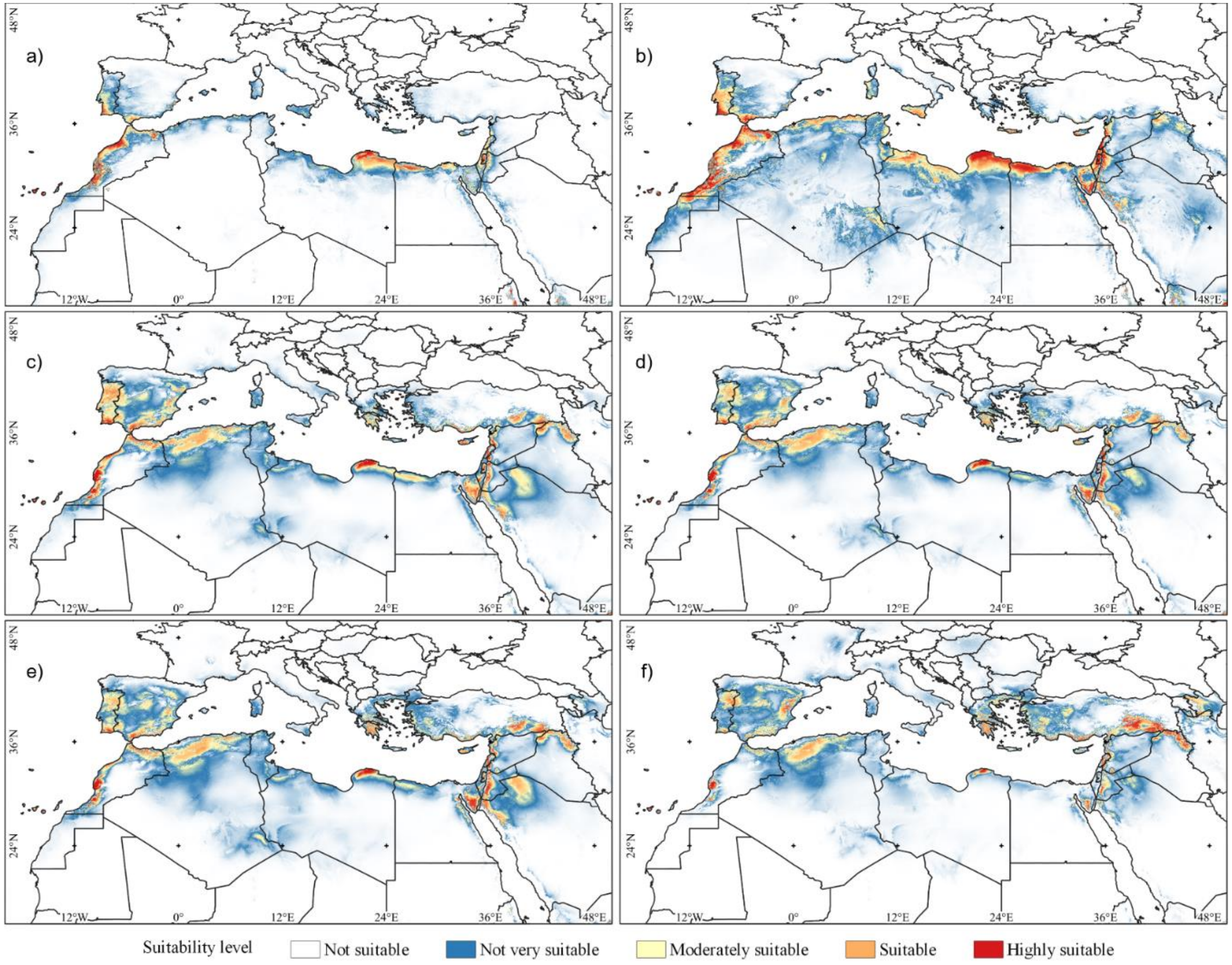

**Figure 10** Suitability areas of *A. spinosa* in the Mediterranean basin. Current geographical distribution using *in situ* **(a)** and *in situ* + *ex situ* **(b)** collections. Potential geographical distribution by 2050 and 2080 according to the climate scenarios RCP 4.5 **(c, d)** and RCP 8.5 **(e, f)** Suitability class range: 0 means not suitable (white color), 0–0.25 means not very suitable (blue color), 0.25–0.50 means moderately suitable (yellow color), 0.50–0.75 means suitable (orange color), and 0.75–1 means highly suitable (red color)

A large part of the study area does not have the most suitable conditions for argan occupation (more than 80%). The IPCC scenarios for 2050 and 2080 shift the area of distribution and/or cultivation towards the N, with a large part of the current area of its wild populations in Morocco disappearing and refuges and new areas of cultivation appearing on the N side of the Mediterranean Basin (Iberian, Italic and Balkan Peninsulas and the Tyrrhenian Islands).

**Table 4.** Current suitability areas of *A. spinosa* in the Mediterranean basin and its potential distribution area in 2050 and 2080 in the IPCC scenarios RCP 4.5 and RCP 8.5

| **Range** | **Class** | **Current** | | **RCP 4.5** | | | | **RCP 8.5** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **2050** | | **2080** | | **2050** | | **2080** | |
| | | **Km²** | **%** | **Km²** | **%** | **Km²** | **%** | **Km²** | **%** | **Km²** | **%** |
| 0 | Not suitable | 17,864337 | 72 | 19,567786 | 79 | 20,182163 | 82 | 19,148483 | 78 | 20,021218 | 81 |
| 0.00 - 0.25 | Not very suitable | 4508312 | 18 | 3137074 | 13 | 2816728 | 11 | 3471106 | 14 | 3057667 | 12 |
| 0.25 - 0.50 | Moderately suitable | 1252317 | 5 | 1158390 | 5 | 1006092 | 4 | 1301601 | 5 | 1008325 | 4 |
| 0.50 - 0.75 | Suitable | 612,113 | 2 | 585,496 | 2 | 485,431 | 2 | 545,071 | 2 | 399,521 | 2 |
| 0.75 - 1 | Highly suitable | 408,108 | 2 | 187,537 | 1 | 145,869 | 1 | 170,022 | 1 | 149,552 | 1 |
| | **Total (km²)** | **24,645187** | **100** | **24,636283** | **100** | **24,636283** | **100** | **24,636283** | **100** | **24,636283** | **100** |

RCP: Representative Concentration Pathway

# Discussion

The level of genetic representativeness achieved with the management of 21 individuals from the south Iberian crop collection is enough to compensate for possible diversity losses in wild populations in North Africa, as shown in Table 2 and Figure 3. The ratio between effective and detected alleles indicates a better distribution of allele frequency among our sampled individuals compared with the results obtained by El Bahloul [21], Yatrib [19], and Mouhaddab [26] with a sample size of 150, 240 and 480 individuals, respectively. Figure 3 shows how the rise in effectiveness decreases with increasing sample size, especially with ASMS2012-34 and ASMS2012-37 loci. It is relevant to mention that Mouhaddab [26] calculated a core collection integrated by 92 individuals, increasing the number of effective alleles from 14.55 to 19.60.

Results from Mouhaddab [26] show the possibility to establish a core collection of only 13 individuals using ISSR techniques and 96 using SSR. With only two SSR markers (ASMS01 and ASM2012-34) they obtained a mean Ne = 20.81 with a sample of 480 trees while using the same markers in the crop collection we have obtained a mean Ne = 9.52 i.e., 45.75 % of the total diversity of the 24 natural localities of argan in Morocco, so the number of alleles detected is highly significant. Likewise, our sample has a mean Na = 9.80, which exceeds those results obtained by Bnikkou [38], who got a mean Na = 6.80 and 4.60, respectively when evaluating a Spanish locality (Alicante) and a Moroccan one (Essaouira).

This genetic study has made it possible to identify individuals with higher heterozygosity; we have observed that the individuals 1, 5, 6, 13, 16 and 17 have the highest heterozygosity across loci (H > 0.83) and individual 3 has the lowest values (H = 0.33). This information may be interesting for the improvement of the species. It can be stated that the genetic diversity detected is very representative, because the allelic distribution has high effectiveness, and the level of heterozygosity is very close to the expected one. Only one of the six markers deviated significantly from HWE (Ho < He), the same pattern was observed in El Bahloul [21]. The opposite is true for other species such as *Pistacia* *vera* and *Olea europaea* which have presented loci with significant deviation from HWE when assessing their genetic structure using SSR markers [53,54].

In this assessment, it has been considered essential to ensure the management of a sufficiently representative genetic diversity in the collections and cultivation trials carried out.

On the other hand, it has not been possible to establish a correlation between molecular and morphological diversity since the markers used have been employed in various studies of similarity between populations in all localities of the argan natural habitats. Similarly, Majourhat [23] was also unable to correlate fruit types with genotype when comparing the results obtained with RAPD and SSR techniques, observing in their results that accessions of different morphotypes had, in some cases, more genetic similarity than accessions with the same morphotype.

Our results confirm the high degree of argan polymorphism at both genetic and morphological levels as shown in [26,55]. This high diversity is justified not only by the habitat heterogeneity [30], but also probably by the development of an “i” diversity cenotic strategy [33], a typical adaptation strategy for species that appear dominant in ecosystems with low “s” diversity. Such is the case of the Arganeraie ecosystem where the lack of species capable of occupying the different ecological niches allows argan to conserve and express a high genetic diversity, thus achieving better resilience at the ecosystem level.

Despite ongoing reforestation programs and because of changing climatic conditions, the argan natural area occupancy continues to decrease each year [2]. This trend is evident in the climate scenarios modelled in this study, with a clear decrease in the most suitable area. Similarly, experiences in more southerly latitudes demonstrate the successful introduction of argan to northern Morocco and Algeria [35,56], thus corroborating the observed shift of the most suitable conditions for this species to thrive towards higher latitudes (Figure 10). Therefore, the introduction of new *ex situ* localities increases the potential area of distribution of argan in the Mediterranean basin, extending the range of action with a higher probability of cultivation in countries such as Tunisia, northern Algeria, Libya, Egypt, Israel, Portugal, and Spain (Figure 10). In the Iberian Peninsula, the model not only incorporates localities on the SE Mediterranean coast where argan specimens have been preserved [5,15,16], but also others that have now disappeared, on the SW Atlantic coast, where the historical record shows that it was also successfully cultivated [57,58]

## Conclusions

The historical presence and introduction of the argan tree in diverse regions of the Mediterranean basin allow us to consider these *ex situ* materials as a complementary tool for the conservation of the genetic diversity of this species. This tool supports the implementation of domestication, improvement, and cultivation programs in a very wide range of environmental variables in these territories.

The genetic diversity of the argan cultivated collection studied in the south of the Iberian Peninsula is highly representative of the total diversity estimated for the wild populations of Morocco and can therefore be considered as a collection of *ex situ* diversity. Furthermore, as a core collection, it can be used for the selection of propagation and cultivation materials for productive purposes. Within these 600 specimens, three different morphotypes have been identified with distinguishable characters in terms of growth habit, branching, size, shape and colour of leaves and fruits.

The suitability models generated from the native distribution localities of the argan tree demonstrate the possibilities of its cultivation in a coastal area of variable width along the circum-Mediterranean areas, especially in the Iberian and North African regions. By extending the occurrence data with *ex situ* localities where it is successfully cultivated or conserved, its ecological range is increased and the possibility of cultivation over larger areas is demonstrated.

Under foreseeable climate scenarios according to IPCC models (RCP4.5 and RCP8.5), the distribution and/or cultivation area of the argan tree shifts towards the N, disappearing a large part of the current area of its wild populations in Morocco, and refuges and new cultivation areas appear on the N side of the Mediterranean basin (Iberian and Balkan Peninsulas and islands such as Sicily and Sardinia).

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## Data availability statement

The data that support the findings of this study are openly available in figshare repository at http://doi.org/10.6084/m9.figshare.18680201.

# Capítulo IV

## Recovery of neglected species with cloud water micro condense capacity as a response to climate change: The case of sclerophyllous boxwoods of *Buxus balearica* Lam. In the Southern Spanish Mediterranean

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## Abstract

Understanding the survival needs of neglected or underutilized species (NUS) in agroforestry can offer valuable insights to mitigate climate change and biodiversity loss. This paper analyses the habitat heterogeneity of *Buxus balearica* populations (palaeorelict of the Mediterranean Basin) using a methodology that integrates four approaches: ecological profiling, multivariate analysis, and the clustering of populations according to environmental variables and suitability. The habitat analysis was conducted on 240 occurrence points, integrating open-source databases and germplasm collection field work. Results reveal that the distribution of *B. balearica* in the south of the Iberian Peninsula is mainly conditioned by thermal variations, the humidity regime, and microtopography, which makes it very vulnerable to current and future climate change scenarios. The analysis of habitat heterogeneity demonstrates its capacity to estimate genetic diversity, which provides a solid basis for future research and conservation actions. The rapid extinction process of these boxwoods is evident by comparing the suitability model under different climate scenarios. There is an urgent need to improve the current status of the species and associated landscapes, considering *B. balearica* as an underutilized species, and establishing a sound management plan to reinforce its populations and protect its natural habitat.

**Keywords:** ex situ conservation; habitat heterogeneity; neglected species; NUS

# Introduction

The NUS (neglected and underutilized species) paradigm has become one of the most promising tools in the last two decades for policies to increase agrodiversity, sustainability, food autonomy, agricultural innovation, and adaptation to climate change [1]. The species and varieties that have the character of NUS, both neglected and forgotten, salvageable from ancient food and agricultural cultures, as well as underutilized, reduced in their use to small geographical or cultural areas, below their potential capacities, make up a block of forgotten genetic resources that are, in many cases, at a risk of genetic erosion or disappearance [2]. Their conceptual framework goes beyond the agri-food horizon to include hundreds of ornamentals, industrial, medicinal, and forestry species. Among the latter, we include those that, due to their ecosystemic role, can reduce water pollution, prevent soil erosion, reduce the $CO_2$ footprint, or improve aquifer recharge. For the last of these functions, important plant communities such as Laurel forests, Yungas, Guarani Atlantic forests, and Afromontane forests contribute to precipitating atmospheric water vapor [3–7]. Also, under Mediterranean climates and especially in their geographical environment, i.e., relict formations, species from the Late Tertiary vegetation (Messinian to Pliocene) survive, which retain a cryptoprecipitating character thanks to their evergreen, glabrous, microlauroid leaves, with oleoid, buxoid or myrtoid forms. They belong to genera such as *Pistacia*, *Buxus*, *Rhamnus*, *Phyllirea*, *Coriaria*, *Maytenus*, *Osyris*, *Arbutus*, *Olea,* or *Quercus*. *Buxus balearica* Lam. is one of these species [8].

It is, therefore, a residual species that continues to suffer a sharp reduction in its distribution area, frequency and abundance, a species that is disappearing along with other constituent elements of its thermophilic thickets (*Maytenus senegalensis* (Lam.) Exell., *Osyris quadripartite* Salzm. ex A.DC., *Cneorumtricoccon* L.), related to the lost laurel forests of the Mediterranean coastal mountains of the late Cenozoic. The profound impact of human presence on the environment of the riparian regions of the Mediterranean basin, forest fires and changes in the use and management of the forest environment, the disappearance of vectors related to their dispersal [9,10], and the accelerated climate change, generating the increase in summer water deficit, make the current existing protection measures insufficient. The areas declared as protected do not encompass all the small populations of boxwood in the Balearic Islands. While ex situ conservation measures such as seed banks aim to supplement these efforts by preserving boxwood accessions for the long term, the absence of reforestation or reintroduction plans for the species within its native habitats renders significant progress unattainable. *B. balearica* is not the only species of the genus threatened with extinction. This is also the case, for example, with *B. Vahlii* Baill., a species endemic to Puerto Rico and Santa Cruz [11], or *B. sinica* (Rehder & E.H. Wilson) M. Cheng in China [12].

The leaf morphology of species such as *B. balearica* suggests the ability to condense moisture from fog and dew. Recent studies have demonstrated the influence of the Atlantic fog regime on the distribution of populations of *Argania spinosa* (L.) Skeels, with similar leaf morphology, in their natural range (mainly in Morocco), shows remarkable habitat heterogeneity [13,14]. A similar phenomenon is observed in *B. balearica*, according to the data and experience we have acquired in the last 30 years after monitoring its populations and studying the habitat and ex situ conservation of this species in the south of the Iberian territory [15–17]. The relict character and its presence in two different climatic floors, the thermo- and lower meso-Mediterranean, have been already confirmed by its phytosociology [18,19]. There is also evidence of genetically different populations at the molecular level associated with this phytogeographical heterogeneity [15,20].

*B. balearica* still occurs in the Western Mediterranean, in Sardinia, Balearic Islands (in Cabrera and Mallorca and in the Sierra de Tramontana), and very locally in N. Morocco (Rif, Middle Atlas, Saharan Atlas, and High Atlas). We also find possible historical references in other Tyrrhenian territories, as the commentary of Theophrastus (3rd century BC) refers to various localities and uses of boxwood, speaks of the very large and splendid boxwoods of Cirno (Corsica), and indicates how those of this island are more robust than those of any other place, which leads us to think that it may be *B. balearica*.

In the Iberian Peninsula, *B. balearica* is found exclusively in Andalusia (the provinces of Malaga, Granada, and Almeria). It occupies some coastal populations and other more inland ones until 1.900 m.a.s.l., between the mountains of Cázulas, Los Guajares, Tejeda, Almijara, and Alhama. It is also found in the Sierra de Gádor [17,21–24]. The area is subject to a climate that varies from the semi-arid Mediterranean (below 500 m.a.s.l.) to the sub-humid Mediterranean (above 600 and 700 m). Average monthly temperatures range from 11 °C to 26 °C and can reach up to 40 °C during the summer. Rainfall ranges from 450 to 500 mm in coastal areas and over 1000 mm in some mountain areas.

The Mediterranean influence is characterized by the interaction of warm and humid sea air with the land surface, leading to the condensation of water vapor and the formation of coastal fog. The region’s abrupt topography further shapes this meteorological phenomenon, acting as a natural barrier that compels the moist air from the Strait of Gibraltar and the Alboran Sea to ascend, cool, and condense, giving rise to orographic fogs [25,26]. Also, the cloudy fronts that reach these coastal mountains from the peninsular interior, pushed by winds of the west, create when descending after overcoming summits and water dividers, another type of orographic fog that generates humidity at the highest altitude levels.

The present study aims to identify and corroborate the presence of *B. balearica* boxwoods in the two types of habitats mentioned in the Iberian area studied, one sheltered at high altitudes and the other one that is more thermophilic and coastal. Furthermore, we aim to assess the significance of advection fogs in facilitating the survival of this species, particularly in coastal environments.

Advection fogs, a meteorological phenomenon of interest, form when the prevailing warm and dry westerly winds from the interior, locally referred to as “terral”, cease and push warm surface waters towards the Alboran Sea. This, coupled with the subsequent thermal inversion, condenses substantial humidity masses when the moist air comes into contact with a colder surface. These condensed fogs are transported inland, affecting coastal regions [25,26]. Remarkably, these fogs have been documented for millennia, with Phoenician colonies inhabiting this territory from the 8th to 3rd centuries BC. The Phoenicians referred to these fogs as “taró”, a term that has persisted up to the present day. These ancient mariners made offerings to the goddess Malak (Malaga, formerly Phoenician Malaka, lies within the sphere of influence of taró) in hopes of securing success in coastal fishing—a tradition that continues to hold economic and cultural significance in the region.

This article undertakes a comprehensive statistical and probabilistic analysis of the environmental factors that define the Andalusian region. Our goal is to present a thorough examination of the distribution patterns of *B. balearica*. Building on the suitability study previously conducted [17], we have integrated new climate change scenarios to account for periods of temperature fluctuations that would result in a greater water stress for the species. This information will inform the development of an *ex situ* conservation program that takes into account the assessed genetic diversity.

# Materials and methods

## *Case study species and its ex situ conservation program*

The fieldwork consisted of collecting thirty-seven new locations of *B. balearica* that were included in a germplasm collection programme for ex situ conservation and analyzing the limits of the areas currently protected by regional and national legislation in Andalusia and Spain. The thirty-seven accessions are preserved in a seed bank using cold storage techniques. These accessions were collected over the last ten years and based on a variability criterion by selecting individuals from as many types of habitats as possible [27,28].

Since the presence of fog significantly increases atmospheric saturation, relative humidity data have been obtained from the ASOS (Automated Surface Observing System) network, using the station closest to the study area that is located in Málaga airport at latitude 36.67N and longitude 4.49W (LEMG) (https://mesonet.agron.iastate.edu (accessed on 17 October 2021)). Although other stations within the study area are part of the national observation networks operated by the State Meteorological Agency (AEMET), we exclusively relied on the ASOS station with 30 min data availability. This specific time resolution is essential to discern anomalies in records potentially linked to abrupt shifts in humidity that could result from fog events.

## Source Data

Habitat analysis of *B. balearica* has been carried out using 240 occurrence points in the south of the Iberian Peninsula (provinces of Malaga, Granada, and Almeria) obtained from the GBIF and REDIAM databases, and mainly the accessions collected for their subsequent long-term ex situ conservation in the Andalusian Plant Germplasm Bank.

The information layers have been downloaded from the environmental information portal in Andalusia (https://portalrediam.cica.es/descargas, accessed on 30 July 2021), from which 20 environmental variables of temperature, precipitation, topography, and insolation have been obtained (Table 1).

**Table 1** Climatic and environmental variables collected for this ecogeographic study

| Code | Variable | Description |
|---|---|---|
| TMC | Mean temperature of the warmest month | Mean value in August* |
| TMMC | Maximum temperature of the warmest month | Maximum value in August* |
| TMF | Mean temperature of the coldest month | Mean value in January* |
| TMMF | Minimum temperature of the coldest month | Minimum value in January* |
| PT01 | Mean precipitation of the first quarter | January–February–March* |
| PT02 | Mean precipitation of the second quarter | April–May–June* |
| PT03 | Mean precipitation of the third quarter | July–August–September* |
| PT04 | Mean precipitation of the fourth quarter | October–November–December* |
| MDT | Elevation from the Digital Elevation Model | DEM of Andalusia |
| ORI | Aspect | Calculated from DEM |
| SLP | Slope | |
| DIST | Euclidean distance to the drainage network | Calculated from water bodies |
| MDI1 | Intermediate insolation model | Between December–March |
| MDI2 | Insolation model during spring equinox | On 21 March |
| MDI3 | Intermediate insolation model | Between March–June |
| MDI4 | Insolation model during summer solstice | On 21 June |
| MDI5 | Intermediate insolation model | Between June–September |
| MDI6 | Insolation model during autumn equinox | On 21 September |
| MDI7 | Intermediate insolation model | Between September–December |
| MDI8 | Insolation model during winter solstice | On 21 December |

* The period from 1971 to 2000

The variable values for each occurrence point were extracted using the QGIS point sampling tool plugin that combines the information layers and the points of presence in the Coordinate Reference System: ETRS89 UTM 30N, with a pixel size of 75m for each variable.

To perform the three quantitative analyses, we standardized the input data using the z-score formula, $((Xi - X)/s)$, where $Xi$ represents a specific data point, $X$ is the sample mean, and $s$ represents the sample's standard deviation.

### *Numerical Ecology Approach*

### Ecological Profiles

The ecological profiles allow us to check the ecological requirements of the study species by analyzing the frequency distribution of the environmental data layers. The comparison of the maximum and minimum values for the entire range allows for the identification of ecological behaviors and preferences. Each variable acts as a descriptor, and for its representation, the generic function of R has been used, hist() [29].

### Multivariate Analysis

The correlation analysis between the environmental variables has been achieved by applying the principal component analysis (PCA), which determines the variance explained by these few independent main axes and the contribution of the variables in defining these axes. The variables that provide a more significant variance in the construction of the axes have been used in the clustering analysis.

A hierarchical fusion method has been selected to find groups of individuals with similar demands and to differentiate populations. Clusters were built using the

Ward.D2 method that was executed with the hclust() function of the STATS package of R version 1.3.1093.

## Suitability Models

Climate change models used to predict the potential species distribution were the Local Climate Change Scenarios of Andalusia (ELCCA) updated to the 4th Report of the Intergovernmental Panel on Climate Change (IPCC). These models are available in the catalogue of data for Andalusia in three climatic periods, i.e., 2011–2040, 2041–2070, and 2071–2099, calculated from the combination of the general circulation models of the atmosphere (CGCM2 and ECHAM4) and different emission scenarios (A2 and B2). These data were downloaded from the environmental information portal in Andalusia. The climate model used was the BCM2 in the sra2 scenario corresponding to average temperature values of 16.7 °C, 17.3 °C and 18.9 °C in the periods 2011–2040, 2041–2070, and 2071–2099, respectively [30].

The three potential scenarios have been modelled with the maximum entropy algorithm of MaxEnt software version 3.4.3, November 2020 [31,32]. The occurrence points provide the values of each variable in both the current and future scenarios described above. In this way, it is possible to predict the regions with the highest probability of suitability that meet the maximum entropy values. The suitability scale is represented between 0 (lowest suitability) and 1 (highest suitability), and the area under the ROC curve (receiver of characteristics) shows the discriminative capacity of the calculated model and presents a tighter distribution if greater than 0.75 (poor $0.75 < AUC > 0.95$ excellent). The model configuration parameters have been calculated using the ENMevaluate function of the ENMeval package of R (v. 2.0.3) [33].

# Results

The process of collecting accessions for ex situ conservation has enabled the incorporation of the accessions listed in Table 2 into the collections of the Bank of Andalusian Plant Germplasm (Junta de Andalucía) collections.

**Table 2** Accessions of *Buxus balearica* (Andalusian Seed Bank (BG), Spain)

| BG Accession Number | Collection Locality | Province | Type of Collection |
|---|---|---|---|
| 14779 | Nerja | Málaga | Base |
| 15596 | Frigiliana | Málaga | Base |
| 15636 * | Nerja | Málaga | Base |
| 16208 | Nerja | Málaga | Base |
| 16662 | Vélez de Benaudalla | Granada | Base |
| 17667 | Vélez de Benaudalla | Granada | Base |
| 17779 | Almijara | Málaga | Base |
| 18579 | Almuñécar | Granada | Base |
| 19213 | Motril | Granada | Base |
| 19214 | Lobres | Granada | Base |
| 19215 | Cómpeta | Málaga | Base |
| 19216 | Almijara | Málaga | Base |
| 19217 | Cómpeta | Málaga | Base |
| 19218 | Cómpeta | Málaga | Base |
| 19219 | Nerja | Málaga | Base |
| 19220 | Cómpeta | Málaga | Base |
| 19221 | Cómpeta | Málaga | Base |
| 19222 | Cómpeta | Málaga | Base |
| 19223 | Cómpeta | Málaga | Base |
| 19224 | Nerja | Málaga | Base |
| 19225 | Ragor | Almería | Base |
| 19226 | Cázulas | Granada | Base |
| 19227 | Otívar | Granada | Base |
| 19238 | Arroyo de la Miel | Málaga | Base |
| 19520 | Güájares (Los) | Granada | Base |
| 20593 | Vélez de Benaudalla | Granada | Base |
| 22061 | Vélez de Benaudalla | Granada | Base |
| 50949 | Guadalfeo (ex situ) | Granada | Active |
| 51043 | Guadalfeo (ex situ) | Granada | Active |
| 51439 | Guadalfeo (ex situ) | Granada | Active |

Base = −20 °C storage condition. Active = −5 °C storage condition. * Accession 15636 is stored in Active and Base collections

From 2004 to 2021, during the warm months of June, July, and August (refer to Figure 1 and Table 3), a total of 13,177 accumulated hours of relative humidity above 70% were recorded. Additionally, there were only 740 days with humidity exceeding 90%, with August showing the highest humidity in both cases. This observation may align with the increased fog frequency in the Alboran Sea during the warm period (June–August). It is asserted that during periods of heightened water stress, there is a noteworthy contribution of humidity in the area.

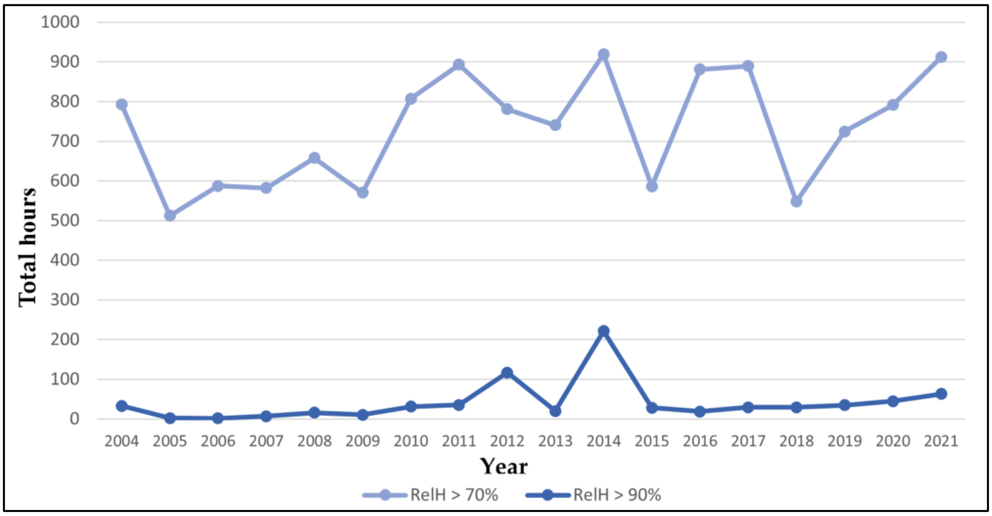

**Figure 1** Graph of historical (2004–2021) total hours in June, July, and August against relative humidity obtained from the LEMG station (ASOS network 36.67 N, 4.49 W) above 70% (comfort point) and 90% (condensation point)

Observations reveal cyclical fluctuations occurring approximately every 7 years. Margalef [34,35] also references these climatic cycles in connection to the occurrence of colder winters and their impact on fishing yields along the Mediterranean coasts. This pattern could potentially be associated with advection fog events.

**Table 3** Historical (2004–2021) summary of records of total hours in June, July, and August with relative humidity obtained from the LEMG station (ASOS network 36.67 N, 4.49 W) above 70% (comfort point) and 90% (condensation point)

| | **RelH> 70%** | | | | | | **RelH> 90%** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | **Records** | **June** | **July** | **August** | **Total Hours** | **%** | **Records** | **June** | **July** | **August** | **Total Hours** | **%** |
| 2004 | 1586 | 434 | 676 | 476 | 793.0 | 37 | 65 | 14 | 9 | 42 | 32.5 | 2 |
| 2005 | 1025 | 279 | 285 | 461 | 512.5 | 24 | 4 | 3 | 0 | 1 | 2.0 | 0 |
| 2006 | 1175 | 350 | 317 | 508 | 587.5 | 27 | 3 | 0 | 0 | 3 | 1.5 | 0 |
| 2007 | 1164 | 276 | 420 | 468 | 582.0 | 27 | 14 | 1 | 4 | 9 | 7.0 | 0 |
| 2008 | 1316 | 361 | 430 | 525 | 658.0 | 30 | 31 | 1 | 24 | 6 | 15.5 | 1 |
| 2009 | 1141 | 352 | 421 | 368 | 570.5 | 26 | 21 | 19 | 0 | 2 | 10.5 | 0 |
| 2010 | 1615 | 369 | 545 | 701 | 807.5 | 37 | 62 | 38 | 18 | 6 | 31.0 | 1 |
| 2011 | 1786 | 656 | 480 | 650 | 893.0 | 41 | 70 | 30 | 5 | 35 | 35.0 | 2 |
| 2012 | 1561 | 433 | 489 | 639 | 780.5 | 36 | 233 | 91 | 38 | 104 | 116.5 | 5 |
| 2013 | 1481 | 372 | 431 | 678 | 740.5 | 34 | 40 | 17 | 14 | 9 | 20.0 | 1 |
| 2014 | 1838 | 460 | 620 | 758 | 919.0 | 43 | 443 | 74 | 177 | 192 | 221.5 | 10 |
| 2015 | 1173 | 215 | 580 | 378 | 586.5 | 27 | 56 | 16 | 40 | 0 | 28.0 | 1 |
| 2016 | 1763 | 506 | 521 | 736 | 881.5 | 41 | 37 | 14 | 16 | 7 | 18.5 | 1 |
| 2017 | 1779 | 471 | 574 | 734 | 889.5 | 41 | 58 | 9 | 32 | 17 | 29.0 | 1 |
| 2018 | 1096 | 271 | 312 | 513 | 548.0 | 25 | 59 | 39 | 8 | 12 | 29.5 | 1 |
| 2019 | 1449 | 404 | 426 | 619 | 724.5 | 34 | 69 | 37 | 3 | 29 | 34.5 | 2 |
| 2020 | 1583 | 305 | 744 | 534 | 791.5 | 37 | 89 | 19 | 29 | 41 | 44.5 | 2 |
| 2021 | 1824 | 483 | 581 | 760 | 912.0 | 42 | 126 | 36 | 18 | 72 | 63.0 | 3 |
| **Total** | **26,355** | **6997** | **8852** | **10,506** | **13,177.5** | | **1480** | **458** | **435** | **587** | **740.0** | |

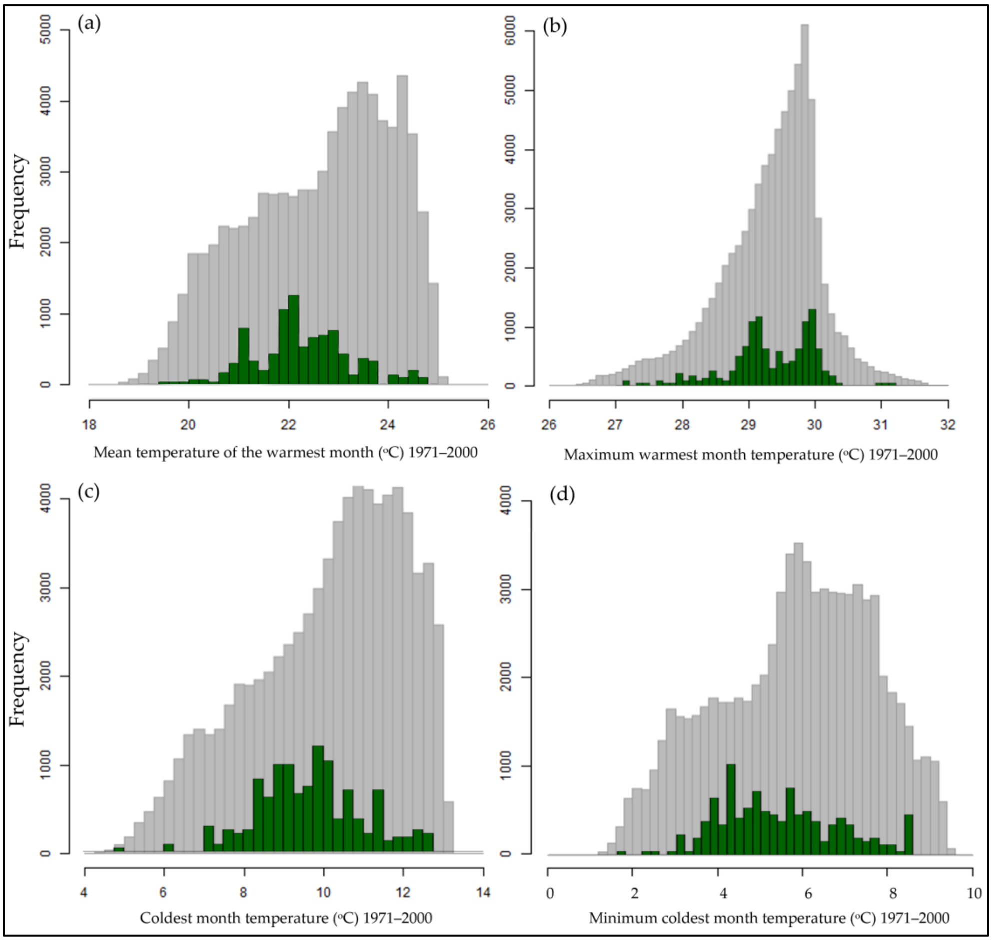

**Figure 2** Ecological profiles of thermometric variables of *Buxus balearica* during the period of 1971–2000: (**a**) tmc (mean temperature of the warmest month), (**b**) tmmc (maximum warmest month temperature), (**c**) tmf (coldest month temperature), and (**d**) tmmf (minimum coldest month temperature). The gray bars in the background represent the mean values of the territory, and the green bars represent the mean values of the species

As can be seen in the temperature profiles (Figure 2), in the areas with a range of 4–16 °C, the species prefers lower temperatures, between 8–10 °C, and dropping to 4 °C during the coldest month. Regarding higher temperatures, there are no significant differences when compared to the rest of the territory.

The bimodal distributions of the frequencies related to temperatures (Figure 2), precipitation (Figure 3), altitude (Figure 4), and insolation models (Figure 5) show the existence of two populations.

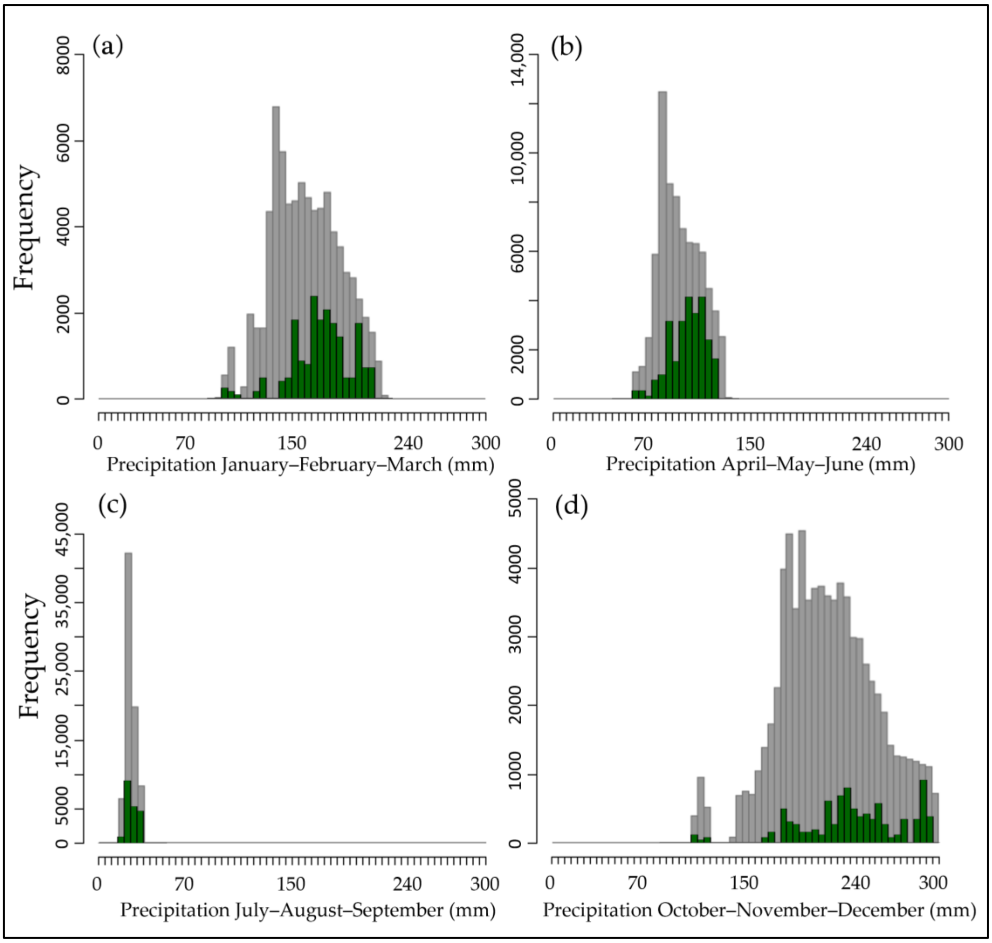

**Figure 3** Ecological profiles of pluviometric variables of *Buxus balearica* during the period of 1971–2000. **(a)** PT01 (mean precipitation of the first quarter).**(b)** PT02 (mean precipitation of the second quarter.**(c)** PT03 (mean precipitation of the third quarter).**(d)** PT04 (Mean precipitation of the fourth quarter). The gray bars in the background represent the mean values of the territory, and the green bars represent the mean values of the species

The precipitation graphs by quarters (Figure 3) reveal a distinction between winter and autumn, suggesting that the existence of drier localities is possibly related to Almería. Notably, there is a preference for areas with higher precipitation. During winter, the species aims to accumulate the greatest amount of water (200–300 mm). Remarkably, in the summer, the species is found in areas with lower rainfall, indicating a resistance to drought or reliance on alternative sources of humidity beyond precipitation.

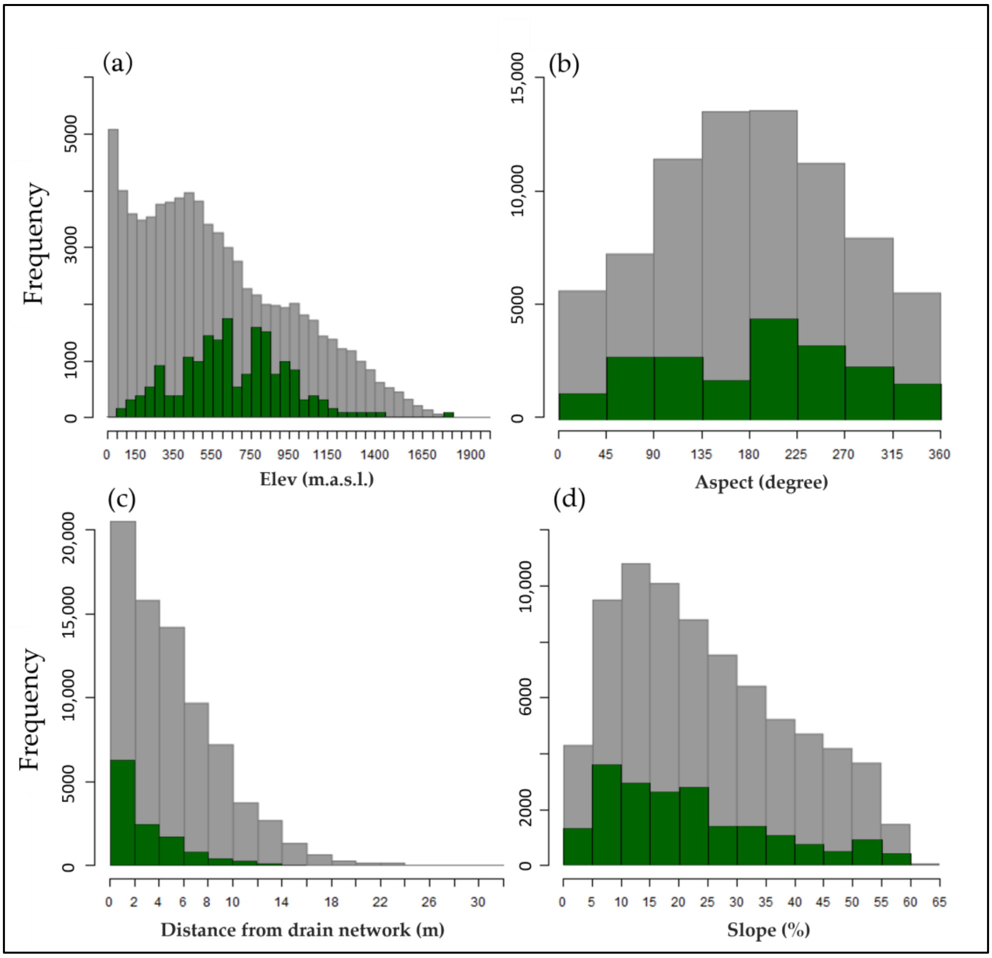

**Figure 4** Ecological profiles of topographic variables of *Buxus balearica* during the period of 1971–2000. **(a)** MDT (elevation from the digital elevation model), **(b)** ORI (Aspect), **(c)** DIST (Euclidean distance from drainage network), and **(d)** SLP (slope). The gray bars in the background represent the mean values of the territory, and the green bars represent the mean values of the species

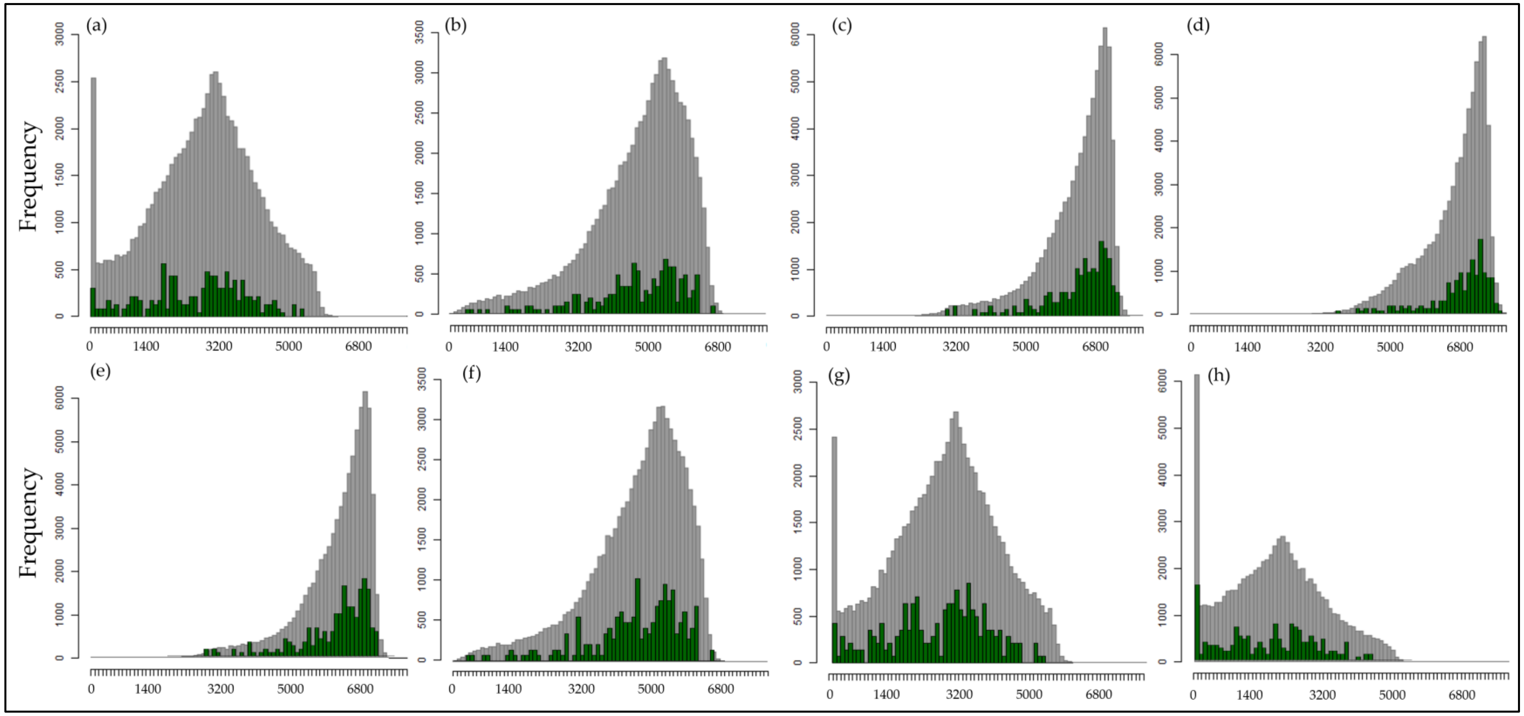

**Figure 5** Ecological profiles of the insolation models of *Buxus balearica* during the period of 1971–2000. **(a)** MDI1 (insolation model between December–March).**(b)** MDI2 (insolation during spring equinox).**(c)** MDI3 (insolation model between March–June).**(d)** MDI4 (insolation model during summer solstice).**(e)** MDI5 (insolation model between June–September).**(f)** MDI6 (insolation model during autumn equinox).**(g)** MDI7 (insolation model between September–December).**(h)** MDI8 (insolation model during winter solstice). The gray bars in the background represent the mean values of the territory, and the green bars represent the mean values of the species

As shown in Figure 4, altitude presents a Gaussian distribution, tending to be binomial, with a decrease in frequency between 300 and 500 m. This suggests the existence of populations favoring coastal areas and others with an optimum of around 800 m, disappearing above 1200 m. Orientation and slope show a slight preference, or a better preservation, with southern orientations.

The distance from the axis of the drainage network indicates that the plant tends to thrive in areas near ravines, streams, and rivers. This preference is likely due to lower incident radiation, reduced evapotranspiration, and enhanced defense against forest fires.

During equinoxes, the distribution exhibits a greater variance (Figure 5), allowing for a better diagnosis of heterogeneity. However, a stronger correlation with the distribution of localities is observed during solstices. Specifically, boxwood localities prefer areas with fewer hours of sun in winter (minimum sun elevation) and more sun in summer (maximum sun elevation). The MDI4 and MDI8 models best explain the distribution of populations.

There is a distinct behavior in the variables MDI12 and MDI22 compared to MDI0, evident in the factorial study. The ecological profile reveals that during days close to the winter solstice, numerous localities do not receive direct sunlight. Conversely, the profile during the summer solstice should feature many localities with a high number of sunshine hours, aligning with the equinox profiles. The October–November profile is intermediate.

These results correspond to those observed in the factorial study and the cluster of the species' locality distribution. Specifically, the temperature frequency distributions show a clustering of localities in a thermophilic coastal sub-population and in another of a montane character, occupying the meso-

Mediterranean level. The species does not seem to tolerate frost (or at least does not respond well to it due to competition with other species), disappearing below the mean temperatures of the coldest month below 7 °C and mean minimum temperatures of the coldest month below 3 °C.

According to PCA analysis, component 1 explains 38% and comprises the variables of the insolation models, with the spring and autumn equinoxes contributing the greatest weight (Figure 6). In contrast, the variables MDI4 and MDI8 contribute 0.26 and 0.32, respectively, to the same component (Table 4).

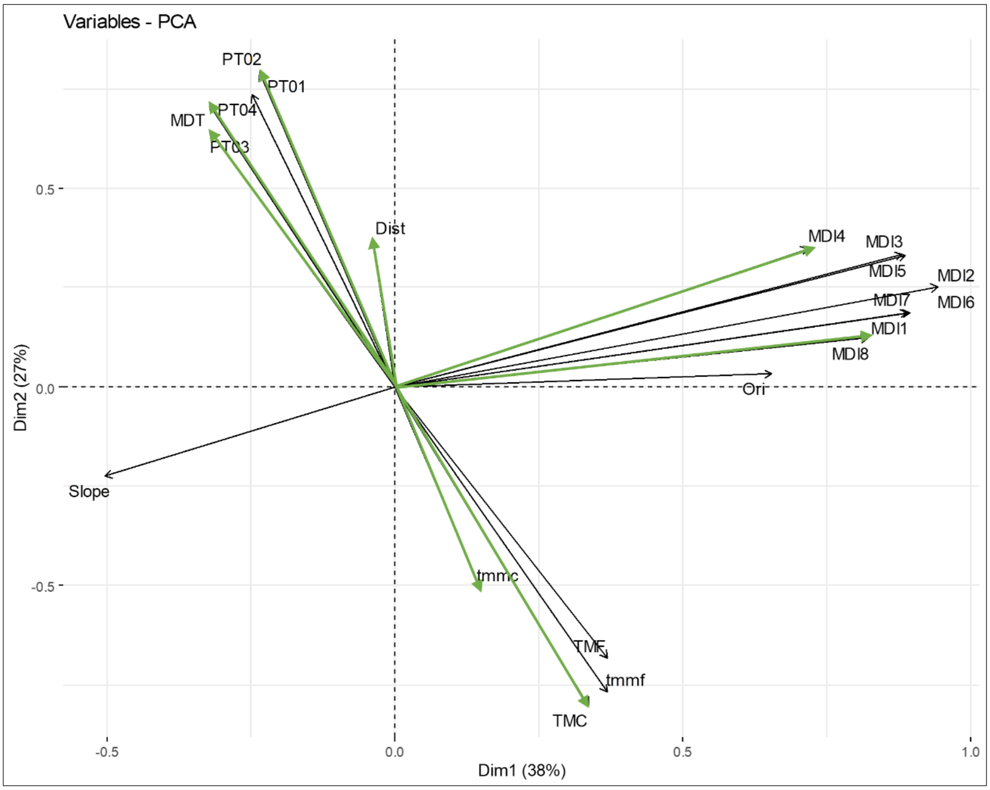

**Figure 6** Principal component analysis between dimensions one and two using 20 bioclimatic variables of *Buxus balearica* during the period of 1971–2000. Green arrows represent the eight variables included in the cluster analysis

**Table 4** Loadings and the accumulated variance explained with principal components

| Code | Variable | Principal Component (PC) | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| TMC | Mean temperature of the warmest month | 0.12 | −0.34 | 0.07 |
| TMMC | Maximum temperature of the warmest month | 0.05 | −0.22 | 0.40 |
| TMF | Mean temperature of the coldest month | 0.13 | −0.29 | 0.35 |
| TMMF | Minimum temperature of the coldest month | 0.13 | −0.33 | 0.05 |
| PT01 | Mean precipitation of the first quarter | −0.09 | 0.34 | 0.26 |
| PT02 | Mean precipitation of the second quarter | −0.09 | 0.34 | 0.20 |
| PT03 | Mean precipitation of the third quarter | −0.12 | 0.28 | 0.36 |
| PT04 | Mean precipitation of the fourth quarter | −0.09 | 0.32 | 0.37 |
| MDT | Elevation from the Digital Elevation Model | −0.12 | 0.31 | −0.37 |
| ORI | Aspect | 0.24 | 0.01 | −0.04 |
| SLP | Slope (%) | −0.18 | −0.10 | −0.21 |
| DIST | Euclidean distance to the drainage network | −0.01 | 0.16 | −0.33 |
| MDI1 | Intermediate insolation model | 0.32 | 0.08 | −0.09 |
| MDI2 | Insolation model during spring equinox | 0.34 | 0.11 | −0.04 |
| MDI3 | Intermediate insolation model | 0.32 | 0.14 | 0.06 |
| MDI4 | Insolation model during summer solstice | 0.26 | 0.15 | 0.12 |
| MDI5 | Intermediate insolation model | 0.32 | 0.14 | 0.06 |
| MDI6 | Insolation model during autumn equinox | 0.34 | 0.11 | −0.04 |
| MDI7 | Intermediate insolation model | 0.32 | 0.08 | −0.09 |
| MDI8 | Insolation model during winter solstice | 0.30 | 0.05 | −0.10 |
| | Standard deviation | 2.76 | 2.32 | 1.57 |
| | Proportion of Variance | 0.38 | 0.27 | 0.12 |
| | Cumulative Proportion | 0.38 | 0.65 | 0.77 |

Component 2 groups in one direction the temperatures of the warmest month and the minimum temperatures of the coldest month and in another the precipitation from January to June with an accumulated variance of 27%. Component 3 only contributes 12% but absorbs the topographic variables (MDT and Dist), the precipitation from July to December (PT03 and PT04), the maximum temperatures of the warmest month (TMMC), and the mean of the coldest month (TMF).

The first three axes together account for 77%. Axis 1 is highly correlated with the degree of insolation (and shielding in the opposite direction) that the vegetation receives, especially with respect to the number of hours of sunshine at the summer and winter equinoxes. Axis 2 is mainly configured according to the extreme temperatures recorded (minimum in the coldest months and maximum in the hottest months). The configuration of axis 3 is dominated by precipitation in the second half of the year (August onwards) together with altitude.

The 12 variables that contribute the most variance (>|0.33|) to the three principal components are TMC, TMMC, TMF, TMMF, PT01, PT02, PT03, PT04, MDT, DIST, MDI2, and MDI6 (Table 4). However, those with a correlation higher than |0.75| (TMF, TMMF, MDI2, and MDI6) have been removed. Due to the contribution of the insolation models during the two solstices (MDI4 and MDI8) and given that there is no correlation between these variables, the eight chosen variables are TMC, TMMC, PT01, PT03, MDT, DIST, MDI4, and MDI8 (Figure 6).

The variables included in the cluster analysis were PT01, PT03, TMC, TMMC, MDT, DIST, MDI4, and MDI8 (Figure 6). There are two sets of well-differentiated localities, those of the lower meso-Mediterranean and upper thermo-Mediterranean levels that are very thermal and free of frost with average temperatures of the coldest month above 11 °C and those of the upper thermo-Mediterranean meso-Mediterranean with average temperatures that drop to 7 °C.

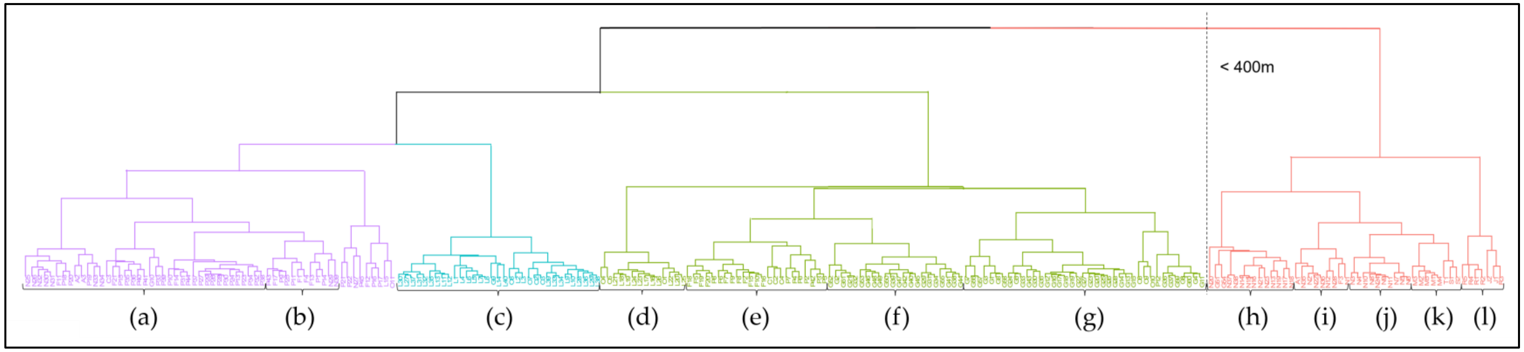

**Figure 7.** Dendrogram of habitat heterogeneity of *Buxus balearica* in Andalusia using eight variables (PT01, PT03, TMC, TMMC, MDT, DIST, MDI4, and MDI8). Each color represents a subpopulation, and it is correlated to the Figure 8 representation. **(a)** Cómpeta. **(b)** Chillar riverbasin.**(c)** Lentejí>600m. **(d)** Lentejí<400m. **(e)** Frigiliana, South Cómpeta. **(f)** South Guájares. **(g)** North Guájares. **(h)** Middle Nerja.**(i)** Cerro Gordo, Arroyo de la Miel. **(j)** Low Chillar. **(k)** Guadalfeo, Molvízar. **(l)** Almería

According to Figure 7, within the coastal and thermal sub-population, there are five different nuclei: two more eastern ones (Rágol and Guadalfeo–Molvízar), another central one (Cantarriján), another western one (Nerja and lower Frigiliana), and finally the westernmost nucleus (Cómpeta). Within the mountain sub-population, we can distinguish the nucleus of Lentejí and Otívar, Competa and upper Nerja, and Los Guájares.

In these results (Figure 8), the Balearic populations were immediately differentiated from those of the Iberian Peninsula, and within these, the eastern population of the Sierra de Gádor (Almeria) was separated from the Malacitano-Granadina populations, where again the coastal populations were separated from the inland mountains, and in turn, these were separated in a western block (Sierra de Almijara: Nerja-Frigiliana) from the eastern populations of the Sierras de Cázulas and Guájares (Guadalfeo-Molvízar).

Different models of potential presence calculated for *B. balearica* using Maxent in the periods 2011–2040, 2041–2070, and 2071–2099 (Figure 9) were obtained with a reliability of 0.98, according to the analysis of the AUC values of each one. The algorithm effectively determines habitats, particularly under different climate change scenarios.

In the 1961–2000 scenario, the potential distribution extends west of the current central core through the Malacitan mountains. The 2011–2040 scenario shows a slight variation, despite demographic regression in current populations. The 2040–2070 scenario reduces the density of presence in its central core in Málaga–Granada, and its potentiality decreases more drastically in the western potential core. In the 2070–2100 scenario, *B. balearica* landscapes disappear completely from their western and eastern potential cores, while their presence in the current central core becomes anecdotal. The species and its landscapes disappeared almost entirely from the Iberian Peninsula.

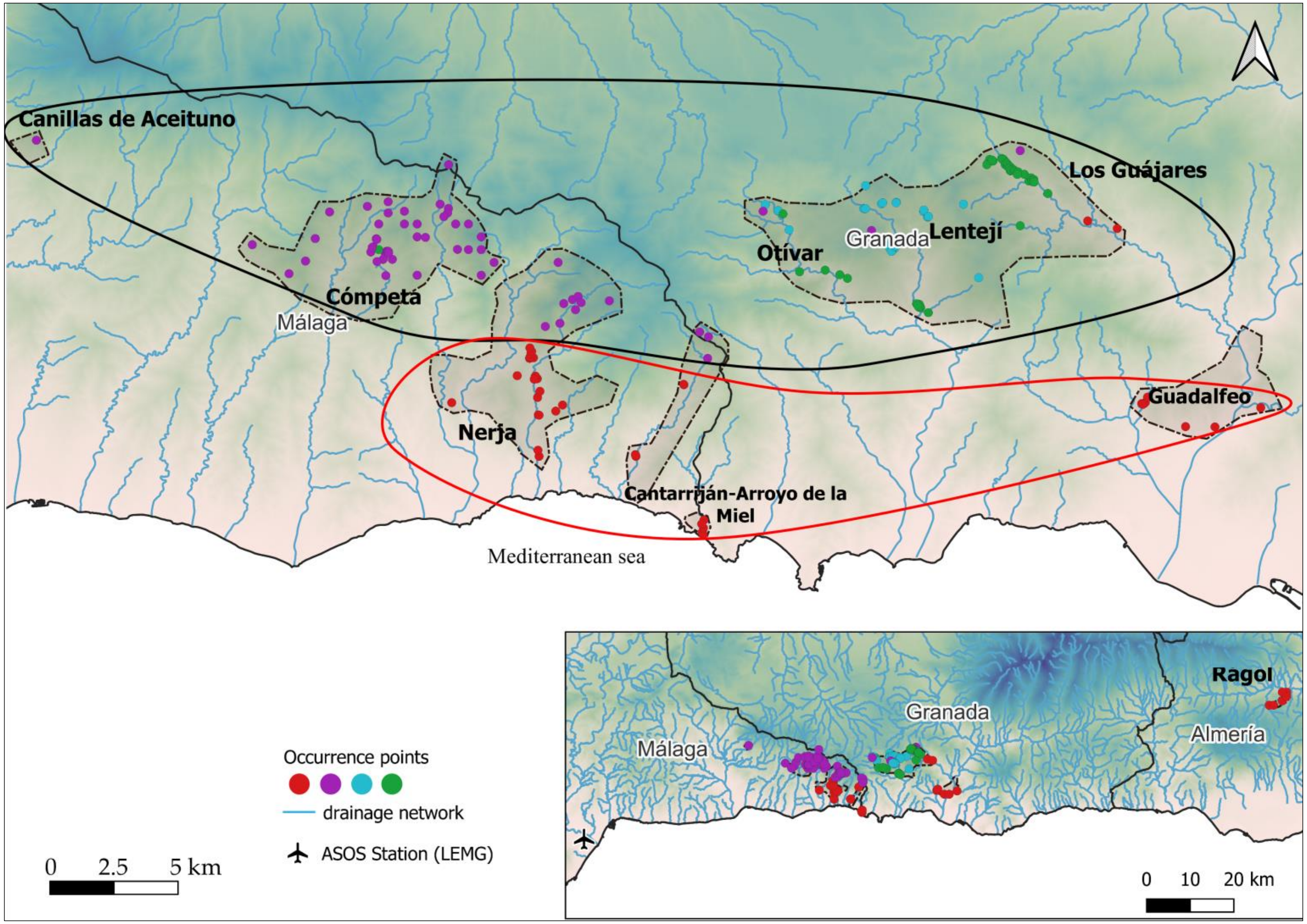

**Figure 8** Representation of the clusters obtained based on habitat heterogeneity of *Buxus balearica* in Andalusia using eight variables (PT01, PT03, TMC, TMMC, MDT, DIST, MDI4, and MDI8).

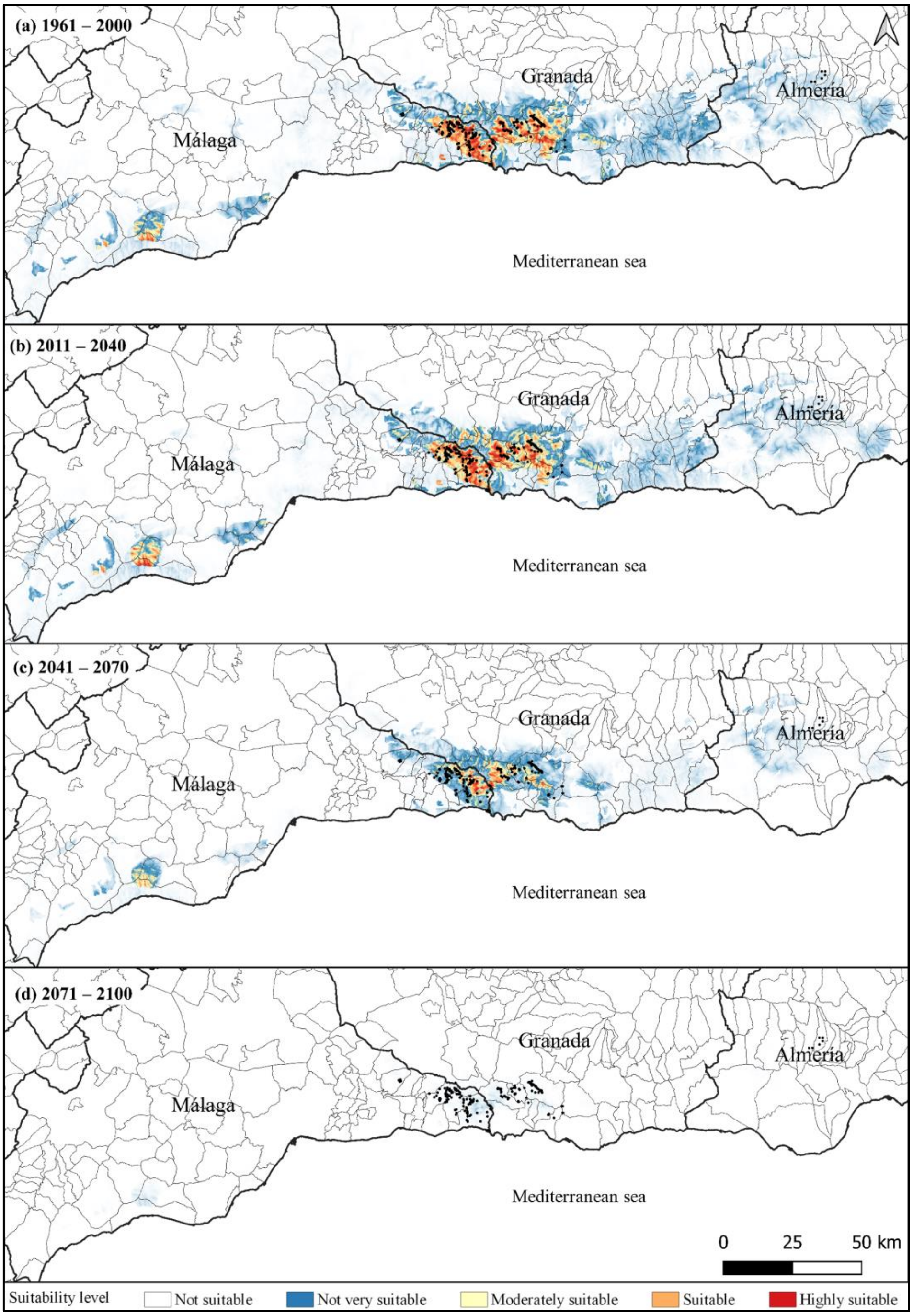

**Figure 9** Potential distribution of *Buxus balearica* generated by MaxEnt ecological niche modelling using eight environmental variables: Dist, Lit, MDT, pH, prec, tmc, tmf, tmmc, and tmmf. Bluer shades represent less suitability and redder ones represent more suitability. **(a)** Potential model during the period of 1971–2000. **(b)** Potential model during the period of 2011–2040. **(c)** Potential model during the period of 2041–2070. **(d)** Potential model during the period of 2071–2100

## Discussion

The study of a species' habitat was, for a long time, the traditional method of assessing genetic diversity in forest sciences until the advent of molecular techniques. The difficulty of conventional genetic methods and the longer life cycle of most forest species justified this form of assessment. Recent studies [26,36] with *Argania spinosa* have already demonstrated the enormous usefulness of this approach, which is again evidenced here with *B. balearica*. Our strategy simultaneously and synergistically uses several numerical methods, from the classical study of ecological profiles (statistical analysis) and clustering and principal component methods (multivariate analysis), together with predictive models that include different climate change scenarios (Maxent), allowing us to compare and correlate results with those obtained from the study of genetic diversity at the molecular level [16,20,37].

The frequency distribution of the reticules in which the species appears does not correlate with the frequency of precipitation in all the reticules studied, except in the summer quarter, where its presence seems related to a low precipitation (between 20 and 40 mm). These localities receive an annual rainfall of > 500 mm, with about 110 mm in the second quarter. Therefore, the species seems to require a certain reserve of rainwater in the soil, to which a quantity not yet estimated should be added, coming from leaf condensation. Given the climatic variation observed in the region over the last few decades, this water supply may be becoming increasingly scarce.

The habitat of *B. balearica* in the Southern Spanish Mediterranean suggests the existence of two very different sets of localities: those of the lower thermo-Mediterranean floor, very thermal, frost-free, with average temperatures in the coldest month above 11 °C, and those of the upper thermo- and meso-

Mediterranean with temperatures that even drop to 7 °C. Indeed, within the coastal and thermal sub-population, an eastern nucleus (Guadalfeo-Molvízar) can be clearly distinguished from two others, a central one (Cantarriján and Arroyo de la Miel) and a more westerly one (lower Nerja). Within the mountain sub-population, several nuclei can be distinguished: (a) the nucleus of Otívar, probably characterized by the high slopes (the boxwoods are on vertical walls); (b) the upper eastern nucleus of Guájares-Cázulas; (c) the central nucleus of the upper valley of the river Chíllar; (d) the western nucleus of the valleys above Cómpeta, including that of Cázulas; and (e) the small nucleus of Canillas de Aceituno (Figure 8).

Furthermore, it is corroborated that the species only occurs on limestone soils with a pH > 7. In the provinces of Málaga and Granada, they are always found on kakiritized marmoreal limestones; and in the Almería locality of Rágol, they are also found on limestones, although not marmoreal.

Similarly, due to the low dependence on topographic variables, it can be deduced that the species tends or is tending to take refuge in those areas where it has a low-temperature oscillation and optimum humidity without following any strictly altitudinal or latitudinal pattern, as is the more general case of other species.

It remains to be demonstrated whether the habitat diversity is only due to the disappearance of boxwood in intermediate situations, between 300 and 500 m altitude, due to external aspects (greater occupation or human pressure), or whether these two phenotypically and genotypically differentiated populations exist.

The resulting clusters coincide with those obtained by other investigations at the molecular level using RAPDs [16,20], estimating practically the same grouping of populations. The easternmost population in Almería (Rágol) is differentiated from that of the Malacitano-Granadino group, and within these, a coastal nucleus was distinguished from a montane (meso-Mediterranean) nucleus. Within the latter, there were also differences between the eastern localities (Lentejí, Otívar, Guadalfeo, and Molvízar) and the western ones (Cómpeta). The genetic diversity observed by other authors [16,20] was greater at the intra-population level than at the inter-population level (because of the cross-population nature of the species). However, a significant genetic difference was observed between the populations of the meso-Mediterranean group.

Concerning the potential distribution of *B. balearica* in future climate change scenarios, the habitat of *B. balearica* would not only be reducing and fragmenting, but also it would be lost in the strict sense of the term. The climatic conditions that allowed the survival of a forest made up of myrtoid, ramnoid, or buxoid leaf species, which are coriaceous, glabrous, non-spiny, and capable of achieving cloud water interception by condensing the microclimatic humidity of the soft mists and sea breezes that occur even in the middle of summer, would disappear with them. This species was not and is not the only species with this function, as others within the same community, such as *Rhamnus alaternus* L., *Pistacia lentiscus* L., *Coriariamyrtifolia* L., or *Quercus coccifera* L., are species with leaves or leaflets like those of *Buxus* [8,18,36].

The application of the model in several consecutive future climate scenarios shows a process of progressive and rapid extinction of the boxwoods of *B. balearica* in the south of the Iberian Peninsula. The area of its potential presence under the climatic conditions of the first quarter of the 21st century is drastically reduced in only twenty more years, becoming almost completely extinct in the eastern half of its current area. By 2070, their extinction would be already profoundly affecting the western half as well, and by the end of the century, their extinction would be total. Unfortunately, climate change predictions are coming true faster than forecasts based on probability calculations [38]. As Schultes [39] said, in relation to the loss of traditional knowledge associated with biodiversity and the need for urgent action, also with regard to the *in situ* and *ex situ* conservation of *B. balearica* as with many other NUS species, *it will soon be too late*.

All these boxwoods constitute cultural landscapes formed by *B. balearica,* and many of the species mentioned above are phytosociologically associated with it. These are NUS (neglected or underutilized species) of a forestry nature that, in the past, were used not only for their firewood, wood, mastic, and other resins but also for a wide range of aromatic, medicinal, pickling, dyeing, melliferous, and condimentary uses. In addition to these uses, the important ecosystemic function is represented by its crypto-precipitating capacity, condensing fog (advection and orographic), which represents a capacity to recharge aquifers that has unfortunately largely disappeared today.

As a result of the habitat diversity study that was carried out, 65 doses of Balearic boxwood seeds from 20 different localities in the provinces of Granada, Malaga, and Ameria have been collected and conserved in the Andalusian Plant Germplasm Bank (Table 2). Ex situ cultivation has already generated new accessions in the Royal Botanical Garden of Cordoba. This set constitutes a genetic reserve that ensures its conservation and possible future *in situ* actions using reintroduction techniques. The study could also seriously correct the limits of the current area protected by the declared Natural Park “Sierras de Tejeda, Almijara, and Alhama” since many of the localities studied and collected for *ex situ* conservation are outside these limits. This makes *ex situ* and *in situ* conservation measures essential to ensure the future of these boxwoods and other rare and threatened species and plant communities in the Mediterranean region [40,41]. This involves implementing a comprehensive plan that includes expanding the boundaries of protected natural areas, choosing specific locations for population reinforcement or reintroduction, and, crucially, fostering a nuanced understanding of these ecosystems' value. Not only do they serve as cultural landscapes, but also, they play a pivotal role in cloud water interception and water management within the Andalusian Mountain range.

## Conclusions

This comprehensive study of *Buxus balearica*, a species integral to the Mediterranean's highly fragmented cultural landscape, underscores its status as a neglected and underutilized species (NUS) with significant forest interest. Facing threats of extinction due to anthropogenic pressures, marginalization, and climate change, *B. balearica*'s conservation is crucial. The *ex situ* conservation efforts have been successful, with 31 new accessions from diverse localities like Nerja, Cómpeta, Cantarriján, and Guadalfeo, including a novel entry from Rágol, Almeria.

The study's habitat heterogeneity analysis, building upon previous genetic diversity estimates, reveals critical insights into the environmental needs of *B. balearica*. It highlights the species' distinct distribution in the Iberian Peninsula, separating into two sets of localities: those from the lower thermo-Mediterranean level influenced by summer water compensation through advection fogs and those from the high-Mediterranean and meso-Mediterranean levels, dependent on orographic humidity at higher altitudes. This distinction is vital in understanding the species' adaptation and survival strategies.

Significantly, the study shows the species' sensitivity to summer water stress and its reliance on microclimatic phenomena, highlighting its vulnerability to the ongoing climate change in the region. The detailed ecological profiles, including thermometric and pluviometric variables, provide a nuanced understanding of the species' preferred environmental conditions, showing a preference for areas with higher precipitation and lower temperatures.

The findings also reveal a bimodal distribution in the species' frequency related to temperature, precipitation, altitude, and insolation models, suggesting the existence of two distinct populations. This is further elucidated through the analysis of principal components, which sheds light on the interplay between various ecological factors like insolation, temperature extremes, and precipitation patterns in shaping the species' distribution.

The potential distribution models for *B. balearica*, developed using MaxEnt ecological niche modeling (software version 3.4.3, November 2020), indicate a significant shift in the species' habitat under future climate scenarios. The predictive models, with high reliability, show a worrying trend of habitat contraction and loss, particularly in the species' western and eastern potential cores, with a marked reduction in the central Málaga–Granada area.

In summary, this study not only contributes to the understanding of *B. balearica*'s ecological requirements and its response to climatic changes but also highlights the urgency for targeted conservation strategies to preserve this valuable Mediterranean species.

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# Capítulo V

## Discusión general

En esta tesis se han investigado dos especies NUS que responden a intereses agrícolas, forestales, industriales y/o ecosistémicos en un escenario de cambio climático donde la recuperación de usos y conocimientos tradicionales (CTs) se convierte en herramienta de adaptación y/o mitigación.

Establecida esa hipótesis, la metodología de la tesis aplica el estudio de las exigencias ecológicas de los cultivos, especies y formaciones estudiadas, así como su potencial recuperación o introducción mediante la aplicación simultánea de varios métodos de ecología numérica y análisis espacial (SIG): perfiles ecológicos, análisis multivariable y modelos probabilísticos de idoneidad en diferentes escenarios climáticos. Incluso se ha manejado la diversidad morfológica o molecular de las poblaciones estudiadas, según el caso de *Argania spinosa* en el capítulo III de esta tesis. De esta manera se maneja la mencionada recuperación de CTs relativos a la extracción y uso de sus semillas, frutos, aceites, maderas y, especialmente, a su capacidad de recarga de acuíferos a partir de fuentes infrautilizadas como son las nieblas de advección y las orográficas.

En el caso del argán, ***Argania spinosa***, que vive en una región del NW de África, se beneficia de la influencia oceánica y de una circulación atmosférica dominada por el anticiclón de las Azores generador de vientos desde el N-NE que arrastran nieblas estivales hacia el interior. Es bien conocido que esta influencia atlántica compensa en parte la xericidad del hábitat litoral en el que vive la especie [1–3]. El almacenamiento del agua en capas profundas del suelo y la capacidad de extracción que tiene el argán de este recurso en sendos niveles contribuye en la eficacia que el argán demuestra en su adaptación para evitar y/o tolerar el estrés hídrico [4].

A pesar de los programas de reforestación en ejecución en la península ibérica y, debido a las condiciones climáticas cambiantes, la ocupación del área natural de argán sigue disminuyendo cada año [5]. No obstante, las experiencias en latitudes más meridionales demuestran el éxito de la introducción del argán en el norte de Marruecos y Argelia [6,7], corroborando el desplazamiento observado (capítulo II de esta tesis) de las condiciones más adecuadas para que esta especie prospere hacia latitudes más altas. Por tanto, la introducción de nuevas localidades ex situ aumentaría el área potencial de distribución del argán en la cuenca mediterránea, ampliando el radio de acción con mayor probabilidad de cultivo en países como Túnez, norte de Argelia, Libia, Egipto, Israel, Portugal y España.

En este sentido, aunque los datos de presencia del argán no incluyeron en nuestro modelo localidades ya conocidas en el área tunecina, el modelo de idoneidad obtenido sí reconoce su potencialidad para algunas comarcas de Túnez (Figura 7 del capítulo II de esta tesis) como las de Nabeul, Sfax, Susa y Medenine, nombradas en estudios recientes [8,9]. Sorprendentemente, el modelo no reconoce la idoneidad en Tinduf (oeste de Argelia), donde la presencia de argán puede ser resultado de una muy antigua introducción y posterior naturalización [10,11], como ha sucedido en otras localidades de la zona occidental de la cuenca mediterránea. Esto sucedió en la península ibérica, donde el modelo calculado incorpora no solo localidades en la costa SE mediterránea, territorios en los que efectivamente se han conservado ejemplares de argán [12–14], sino también otras donde la especie desapareció, como en la costa SO atlántica, según cuenta el registro histórico [15,16]. Lo mismo ocurre en Canarias, donde existen evidencias de introducción del argán en algunas localidades [URL (Consultado 05/12/23): https://atlasdesemillasdecanarias.org/atlas/ficha.php?ID=89].

El nivel de representatividad genética alcanzado con el manejo de 21 individuos de la colección de cultivo del sur de la península ibérica ha sido suficiente para compensar las posibles pérdidas de diversidad en las poblaciones silvestres del norte de África (Tabla 2 y Figura 3 del capítulo III de esta tesis). La relación entre alelos efectivos y detectados indica una mejor distribución de la frecuencia alélica entre los individuos muestreados en campo en los trabajos de esta tesis y los árboles cultivados en el Cortijo de Frías (T.M., Aguilar de la Frontera, Córdoba) respecto a otros trabajos que, no obstante, demostraban la posibilidad de establecer una colección núcleo de tan solo 13 individuos utilizando técnicas ISSR y 96 utilizando SSR. El estudio genético realizado ha permitido identificar individuos con elevado nivel de heterocigosis, información que puede ser interesante para la mejora de la especie. Se puede afirmar que la diversidad genética detectada es muy representativa, ya que la distribución alélica tiene una alta efectividad, y el nivel de heterocigosis ha resultado ser muy cercano al esperado. La población del Cortijo de Frías constituye, por lo tanto, una valiosa colección de diversidad genética ex situ a partir de la cual podrán obtenerse diversas selecciones para la difusión del cultivo en Andalucía y en otras regiones de España y del oeste mediterráneo.

La visión sintética y multidisciplinar en lo conceptual y posteriormente metodológico aplicada al estudio de *Argania spinosa,* donde los resultados obtenidos son utilizados en la propuesta de medidas de conservación *ex situ*, *in situ*, o como apoyo a futuros procesos de domesticación, había venido siendo ya ensayada por el equipo de investigación en el que surge esta tesis doctoral, a través del estudio de una muy singular especie silvestre de la flora forestal ibérico-balear, en proceso de regresión en los paisajes forestales andaluces, *Buxus balearica*.

La distribución potencial de *B. balearica* en futuros escenarios de cambio climático muestran como su hábitat no sólo se estaría reduciendo y fragmentando, sino que incluso llegaría a perderse por completo (Figura 9 del capítulo IV de esta tesis). Desaparecerían con ellas las condiciones climáticas que permitieron la supervivencia de un bosque formado por especies de hojas mirtoides, ramnoides o buxoides, coriáceas, glabras, no espinosas y capaces de lograr la interceptación del agua de las nubes condensando la humedad microclimática de las suaves brumas y brisas marinas que se producen incluso en pleno verano [URL (consultado 30/01/24): https://www.malagahoy.es/malaga/taro-Malaga_0_1697232231.html]. El área de su presencia potencial bajo las condiciones climáticas del primer cuarto del siglo XXI se reduce drásticamente en sólo veinte años más, extinguiéndose casi por completo en la mitad oriental de su área actual. En 2070, su extinción afectaría ya profundamente también a la mitad occidental y, a finales de siglo, su extinción sería total. Desgraciadamente, las predicciones sobre el cambio climático se están cumpliendo más rápido que las basadas en cálculos de probabilidades [17]. Debemos advertir que esta especie no es la única con esta función dentro de su comunidad, pues parecen participar de la misma función de condensación otras como *Rhamnus alaternus* L., *Pistacia lentiscus* L., *Coriaria myrtifolia* L., *o Quercu scoccifera* L. [18–20].

Todas estas bojedas constituyen paisajes culturales formados por *B. balearica*, junto con otras muchas de las especies mencionadas anteriormente que coexisten en su hábitat. Se trata de especies NUS de carácter forestal que, en el pasado, fueron utilizadas no sólo por su leña, madera, almáciga y otras resinas, sino también para una amplia gama de usos aromáticos, medicinales, encurtidos, tintóreos, melíferos y condimentarios. Además de estos usos, la importante función ecosistémica está representada por su capacidad condensadora de nieblas (advección y orográfica) y, por lo tanto, de recarga de acuíferos hoy desgraciadamente muy desaparecida.

Como resultado del estudio de diversidad de hábitats realizado, se han recogido 65 dosis de semillas de boj balear procedentes de 20 localidades diferentes de las provincias de Granada, Málaga y Almería, que se conservan en el Banco de Germoplasma Vegetal Andaluz. El estudio también podría corregir seriamente los límites de la actual zona protegida por el Parque Natural declarado "Sierras de Tejeda, Almijara y Alhama" ya que muchas de las localidades estudiadas y recolectadas para su conservación *ex situ* se encuentran fuera de estos límites. Esto implica la aplicación de un plan integral que incluya la ampliación de los límites de las áreas naturales protegidas, la elección de lugares específicos para el refuerzo o la reintroducción de poblaciones y, sobre todo, el fomento de una comprensión matizada del valor de estos ecosistemas. No sólo sirven como paisajes culturales, sino que también desempeñan un papel fundamental en la interceptación del agua de las nubes y en la gestión del agua dentro de la cordillera andaluza.

Los hallazgos de esta tesis doctoral refuerzan la oportunidad de desarrollo sostenible que las especies NUS representan para nuestros sistemas agrícolas y forestales. Mediante la aplicación de la metodología contrastada en los capítulos II, III y IV, es posible sentar las bases de medidas de recuperación necesarias para construir sistemas más resilientes empleando especies con mejores mecanismos de adaptación al cambio climático acelerado.

Como decía E. Schultes [21], en relación con la pérdida de los conocimientos tradicionales asociados a la biodiversidad y la **necesidad de una acción urgente**, ***pronto será demasiado tarde***.

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# Capítulo VI

## Conclusiones

(1) *Argania spinosa* y *Buxus balearica* son especies NUS clave en sus respectivos paisajes culturales, con muy diferente uso tradicional pero coincidentes en su capacidad condensadora del agua de las nieblas marítimas de advección y orográfricas. La elección de estos dos estudios de caso nos ha permitido evaluar el grado sensibilidad, problemas de conservación y perspectivas de uso y explotación de estas formaciones vegetales en diferentes escenarios de cambio climático.

(2) La metodología utilizada, que consideramos innovadora, ha resultado muy eficiente al manejar simultáneamente técnicas convencionales de análisis de diversidad de hábitat, análisis multivariante y de agrupamiento, así como técnicas de análisis espacial, SIGs y modelos predictivos de idoneidad en diferentes escenarios climáticos.

(3) Los resultados se pueden aplicar en la domesticación del argán, en la búsqueda de nuevas áreas de cultivo o para la expansión forestal, tanto de las bojedas como de las dehesas de argán con calculada probabilidad de éxito.

(4) Ha resultado determinante para este estudio la posibilidad de manejar los datos georeferenciados de cientos de localidades en la distribución natural de ambas especies para la construcción de los modelos predictivos de esta tesis.

(5) Los resultados de este estudio corroboran el carácter polimórfico del argán y proporcionan información sobre las estrategias de adaptación específicas que ha permitido su cultivo en hábitats litorales, sublitorales, montañosos y

desérticos. La costa mediterránea del sureste de España representa la zona que mejor se asemejará a los requisitos ecológicos del argán en el futuro.

(6) La diversidad genética de la colección cultivada de argán estudiada en el sur de la península ibérica (Cortijo de Frías, T.M., Aguilar de la Frontera, Córdoba) es altamente representativa de la diversidad total estimada para las poblaciones silvestres de Marruecos, por lo que puede considerarse como una colección de diversidad *ex situ*.

(7) La presencia histórica y la introducción del argán en diversas regiones de la cuenca mediterránea permiten considerar estos materiales *ex situ* como una herramienta complementaria de gran interés para la conservación de la diversidad genética de la especie.

(8) Los modelos de idoneidad generados a partir de las localidades de distribución nativa del argán demuestran las posibilidades de su cultivo en una zona costera de anchura variable a lo largo de las áreas circunmediterráneas, especialmente en las regiones ibérica y norteafricana. Al ampliar los datos de ocurrencia con localidades *ex situ* donde se cultiva o conserva con éxito, se aumenta su rango ecológico y se demuestra la posibilidad de su cultivo en áreas más extensas.

(9) Bajo los escenarios climáticos previsibles según los modelos del IPCC, la distribución y/o área de cultivo del argán se desplaza hacia el N, desapareciendo gran parte del área actual de sus poblaciones silvestres en Marruecos, apareciendo nuevas áreas de cultivo en la vertiente N de la cuenca mediterránea.

(10) El estudio exhaustivo de las poblaciones de *Buxus balearica* en Andalucía demuestra su condición de especie olvidada e infrautilizada (NUS), integrante de formaciones vegetales que manifiestan un gran interés forestal por su función en la recarga de acuíferos en las montañas litorales mediterráneas.

(11) El análisis de la heterogeneidad del hábitat del boj de Baleares en Andalucía basado en estimaciones previas de diversidad genética revela información crítica sobre sus necesidades ambientales y manifiesta como sus poblaciones aparecen separadas en dos conjuntos de localidades: las del nivel termo-mediterráneo inferior, influidas por la compensación hídrica estival de las nieblas de advección, y las del nivel mesomediterráneo, dependientes de la humedad orográfica a mayor altitud.

(12) Los resultados también revelan una distribución bimodal en la frecuencia de la especie en relación con los modelos de temperatura, precipitación, altitud e insolación. Esta fragmentación de hábitat arroja luz sobre la interacción entre diversos factores ecológicos como la insolación, las temperaturas extremas y los patrones de precipitación en la configuración de la distribución de la especie.

(13) Esta tesis muestra la sensibilidad de *B. balearica* al estrés hídrico estival y su dependencia de fenómenos microclimáticos, lo que pone de relieve su vulnerabilidad al cambio climático que se está produciendo en la región.

(14) Los modelos de distribución potencial de *B. balearica*, desarrollados en esta tesis, indican un cambio significativo en el hábitat de la especie bajo futuros escenarios climáticos y muestran una preocupante tendencia de contracción y pérdida de hábitat, particularmente en los núcleos potenciales

occidental y oriental de la especie, con una marcada reducción en el área central de Málaga-Granada.

(15) Enfrentada a amenazas de extinción debidas a presiones antropogénicas, marginación y cambio climático, la adopción de nuevas medidas de conservación de *B. balearica* es urgente. Los esfuerzos de conservación *ex situ* de esta tesis se han materializado en el ingreso de 31 nuevas accesiones en el BGVA. A pesar ello esta tesis demuestra la necesidad de modificar los límites de las superficies protegidas y promover programas de reforestación de las bojedas en estos nuevos límites.

# APPENDICES

**Appendix 1** (Chapter II) Occurrence points.

| ID | Country | Province | Latitude | Longitude | Elevation (m) | Source |
|---|---|---|---|---|---|---|
| 2 | Algeria | Chlef | 36.17 | 1.33 |  | [72] |
| 4 | Algeria | Tindouf | 28.46 | -8.15 | 530 | [13] |
| 1 | Algeria | Alaimia | 35.73 | -0.26 |  |  |
| 3 | Algeria | Stidia | 35.80 | -0.07 |  |  |
| 5 | Morocco | El Aiuun | 27.16 | -13.23 |  | [72] |
| 8 | Morocco | Agadir Ida Outanane | 30.65 | -9.52 | 1100 | [28] |
| 13 | Morocco | Assa Zag | 28.43 | -9.42 | 336 |  |
| 14 | Morocco | Berkane | 34.85 | -2.57 | 195 |  |
| 18 | Morocco | ChtoukaAit Baha | 30.11 | -9.23 | 490 |  |
| 20 | Morocco | Essaouira | 31.79 | -9.40 | 360 |  |
| 21 | Morocco | Essaouira | 31.64 | -9.17 | 396 |  |
| 22 | Morocco | Essaouira | 31.54 | -9.48 | 269 |  |
| 23 | Morocco | Essaouira | 31.36 | -9.38 | 540 |  |
| 24 | Morocco | Essaouira | 31.32 | -9.57 | 242 |  |
| 25 | Morocco | Essaouira | 31.04 | -9.38 | 980 |  |
| 26 | Morocco | Essaouira | 31.01 | -9.62 | 576 |  |
| 27 | Morocco | Essaouira | 31.01 | -9.80 | 231 |  |
| 32 | Morocco | InezganeAit Melloul | 30.33 | -9.36 | 83 |  |
| 33 | Morocco | Khemisset | 33.46 | -6.37 | 403 |  |
| 37 | Morocco | Safi | 32.03 | -9.32 | 58 |  |
| 38 | Morocco | Safi | 31.94 | -9.40 | 120 |  |
| 39 | Morocco | Sidi Ifni | 29.40 | -9.73 | 954 |  |
| 40 | Morocco | Sidi Ifni | 29.11 | -10.11 | 349 |  |
| 42 | Morocco | Taroudant | 30.29 | -8.49 | 1247 |  |
| 43 | Morocco | Taroudant | 30.81 | -8.40 | 1178 |  |
| 44 | Morocco | Taroudant | 30.56 | -9.09 | 256 |  |
| 45 | Morocco | Taroudant | 30.62 | -8.11 | 782 |  |
| 46 | Morocco | Taroudant | 30.73 | -9.23 | 796 |  |
| 58 | Morocco | Tiznit | 29.71 | -9.06 | 900 |  |
| 9 | Morocco | Agadir Ida Outanane | 30.40 | -9.58 |  | [72] |
| 10 | Morocco | Agadir Ida Outanane | 30.40 | -9.51 |  |  |
| 11 | Morocco | Agadir Ida Outanane | 30.60 | -9.68 | 35 |  |
| 12 | Morocco | Agadir Ida Outanane | 30.60 | -9.65 | 32 |  |
| 15 | Morocco | Chichaoua | 31.09 | -9.01 |  |  |

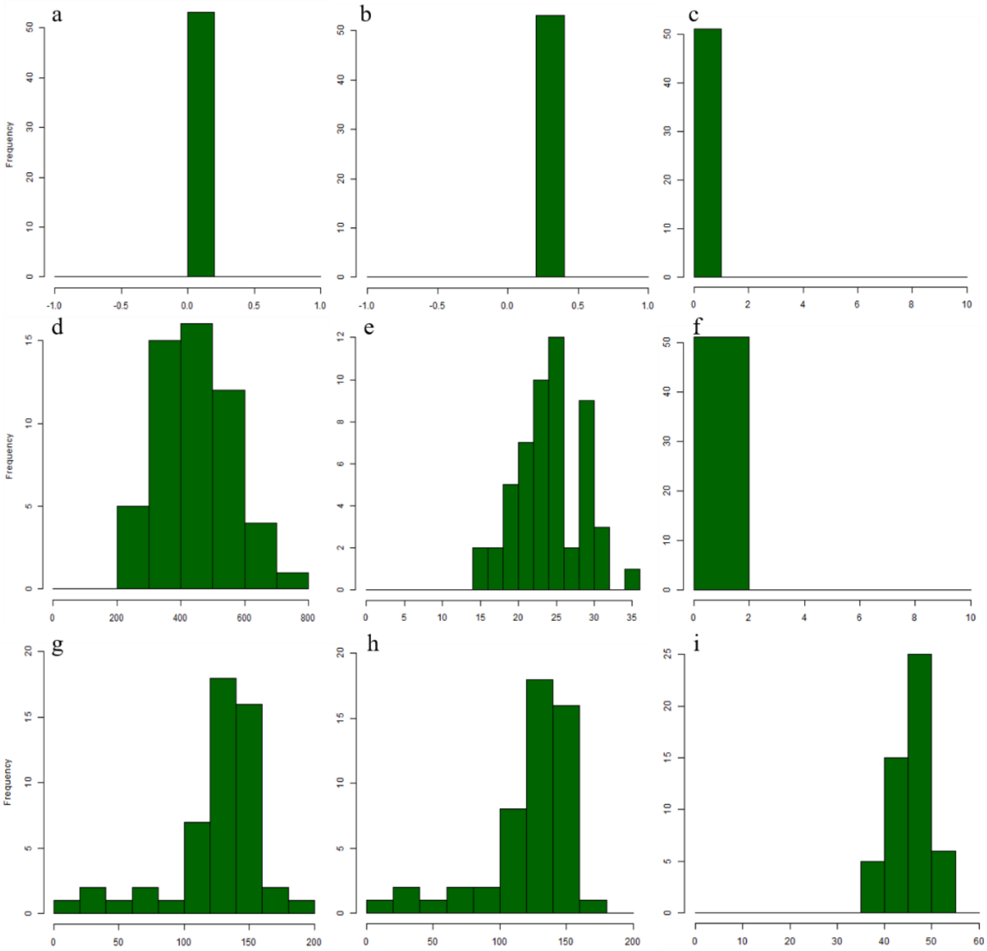
| ID | Country | Province | Latitude | Longitude | Elevation (m) | Source |
|---|---|---|---|---|---|---|
| 16 | Morocco | Chichaoua | 31.58 | -9.03 |  | [72] |
| 17 | Morocco | Chichaoua | 31.59 | -9.02 |  |  |
| 19 | Morocco | ChtoukaAit Baha | 30.06 | -9.69 |  |  |
| 28 | Morocco | Essaouira | 31.05 | -9.68 | 360 |  |
| 29 | Morocco | Essaouira | 31.51 | -9.64 |  |  |
| 30 | Morocco | Essaouira | 31.61 | -9.66 |  |  |
| 31 | Morocco | Guelmim | 28.97 | -10.08 | 300 |  |
| 41 | Morocco | Sidi Ifni | 29.24 | -10.08 | 200 |  |
| 47 | Morocco | Taroudant | 30.29 | -8.50 | 1240 |  |
| 48 | Morocco | Taroudant | 30.37 | -9.10 |  |  |
| 53 | Morocco | Taroudant | 30.50 | -9.16 | 550 |  |
| 49 | Morocco | Taroudant | 30.50 | -7.87 |  |  |
| 50 | Morocco | Taroudant | 30.53 | -7.89 |  |  |
| 54 | Morocco | Taroudant | 30.62 | -8.09 |  |  |
| 55 | Morocco | Taroudant | 30.75 | -9.13 | 620 |  |
| 56 | Morocco | Taroudant | 30.78 | -9.16 |  |  |
| 57 | Morocco | Taroudant | 30.79 | -8.38 |  |  |
| 51 | Morocco | Taroudant | 30.82 | -8.40 | 1300 |  |
| 59 | Morocco | Tiznit | 29.72 | -8.87 |  |  |
| 34 | Morocco | Nador | 35.09 | -2.80 | 40 | [9] |
| 35 | Morocco | Nador | 35.04 | -2.75 | 220 |  |
| 6 | Morocco | Agadir Ida Outanane | 30.42 | -9.38 |  |  |
| 7 | Morocco | Agadir Ida Outanane | 30.45 | -9.55 |  |  |
| 36 | Morocco | Berkane | 34.86 | -2.59 |  |  |
| 52 | Morocco | Taroudant | 30.68 | -9.25 |  |  |
| 60 | Spain | Alicante | 38.35 | -0.49 |  | [72] |
| 61 | Spain | Alicante | 38.35 | -0.51 |  |  |
| 62 | Spain | Alicante | 37.89 | -0.76 |  |  |
| 63 | Spain | Alicante | 38.42 | -0.42 |  |  |
| 64 | Spain | Alicante | 38.49 | -0.48 |  |  |
| 65 | Spain | Alicante | 38.38 | -0.45 |  |  |
| 67 | Spain | Murcia | 38.06 | -1.17 |  |  |
| 66 | Spain | Alicante | 38.39 | -0.46 |  | [41] |

**Appendix 2** (Chapter II) Climatic and environmental variables collected for this ecogeographic study.

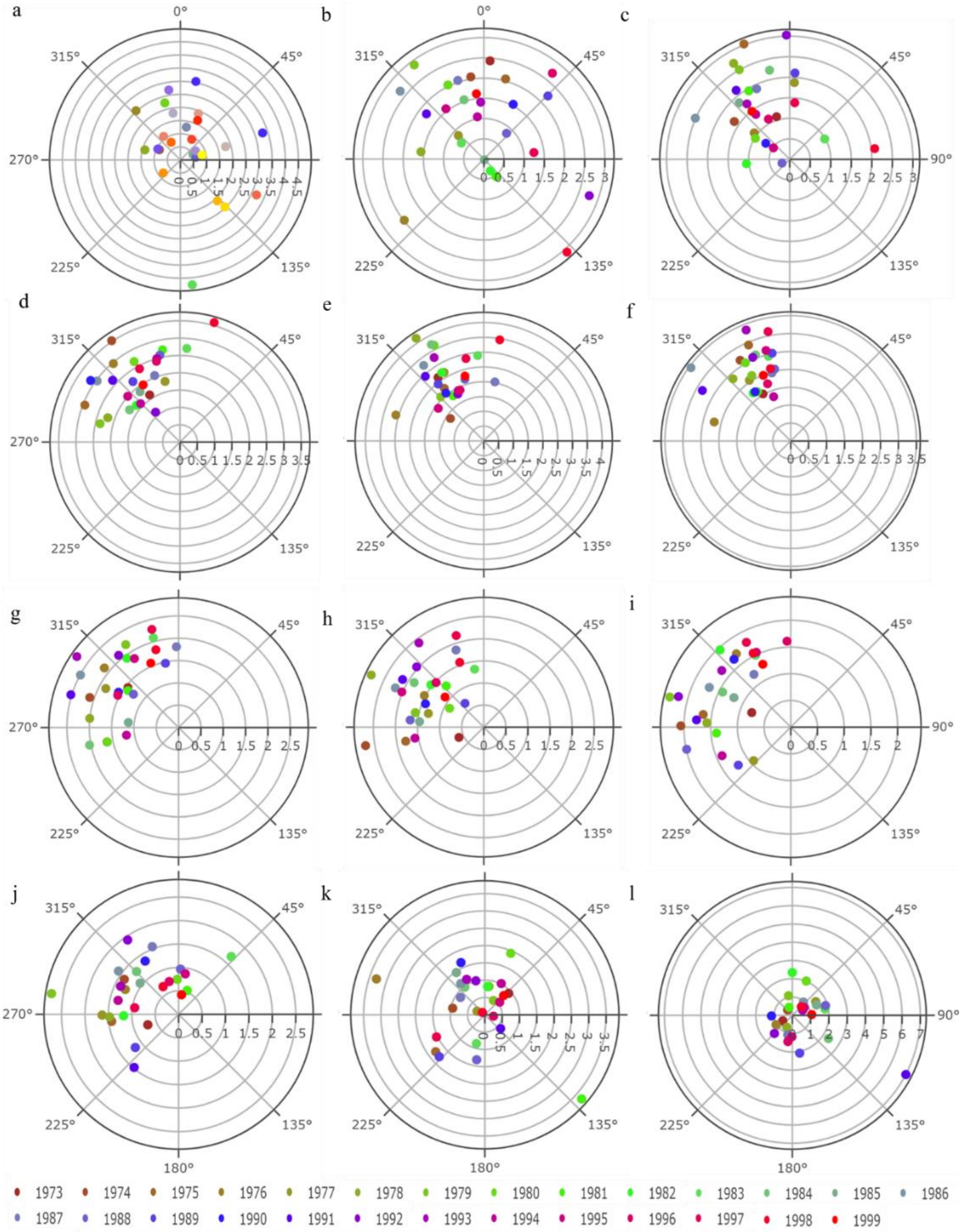
| Category | Code | Variables | Reference |
|---|---|---|---|
| Temperature | V1 | Annual Mean Temperature | [42] |
| | V2 | Mean Diurnal Range | |
| | V3 | Isothermality | |
| | V4 | Temperature Seasonality | |
| | V5 | Max Temperature of Warmest Month | |
| | V6 | Min Temperature of Coldest Month | |
| | V7 | Temperature Annual Range | |
| | V8 | Mean Temperature of Wettest Quarter | |
| | V9 | Mean Temperature of Driest Quarter | |
| | V10 | Mean Temperature of Warmest Quarter | |
| | V11 | Mean Temperature of Coldest Quarter | |
| Precipitation | V12 | Annual Precipitation | [42] |
| | V13 | Precipitation of Wettest Month | |
| | V14 | Precipitation of Driest Month | |
| | V15 | Precipitation Seasonality | |
| | V16 | Precipitation of Wettest Quarter | |
| | V17 | Precipitation of Driest Quarter | |
| | V18 | Precipitation of Warmest Quarter | |
| | V19 | Precipitation of Coldest Quarter | |
| Topographic | V20 | Elevation (SRTM) | [42] |
| | V21 | Hillshade | [73] |
| | V22 | Aspect | |
| | V23 | Slope | |
| | V24a | Land Surface Temperature April 2002 | [74] |
| | V24j | Land Surface Temperature July 2002 | |
| | V25a | Normalized Difference Vegetation Index April 2002 | |
| | V25j | Normalized Difference Vegetation Index July 2002 | |
| Dryness | V26 | Global Aridity Index | [43] |
| | V27 | Potential Evapotranspiration | |
| Wind | V28 | Wind speed | [44] |
| | V29 | Wind direction | |
| Restrictive | V30 | pH soil information at 0-5 cm Depth | [46] |

**Appendix 3** (Chapter II) Descriptive statistics of the variables used in the analysis.

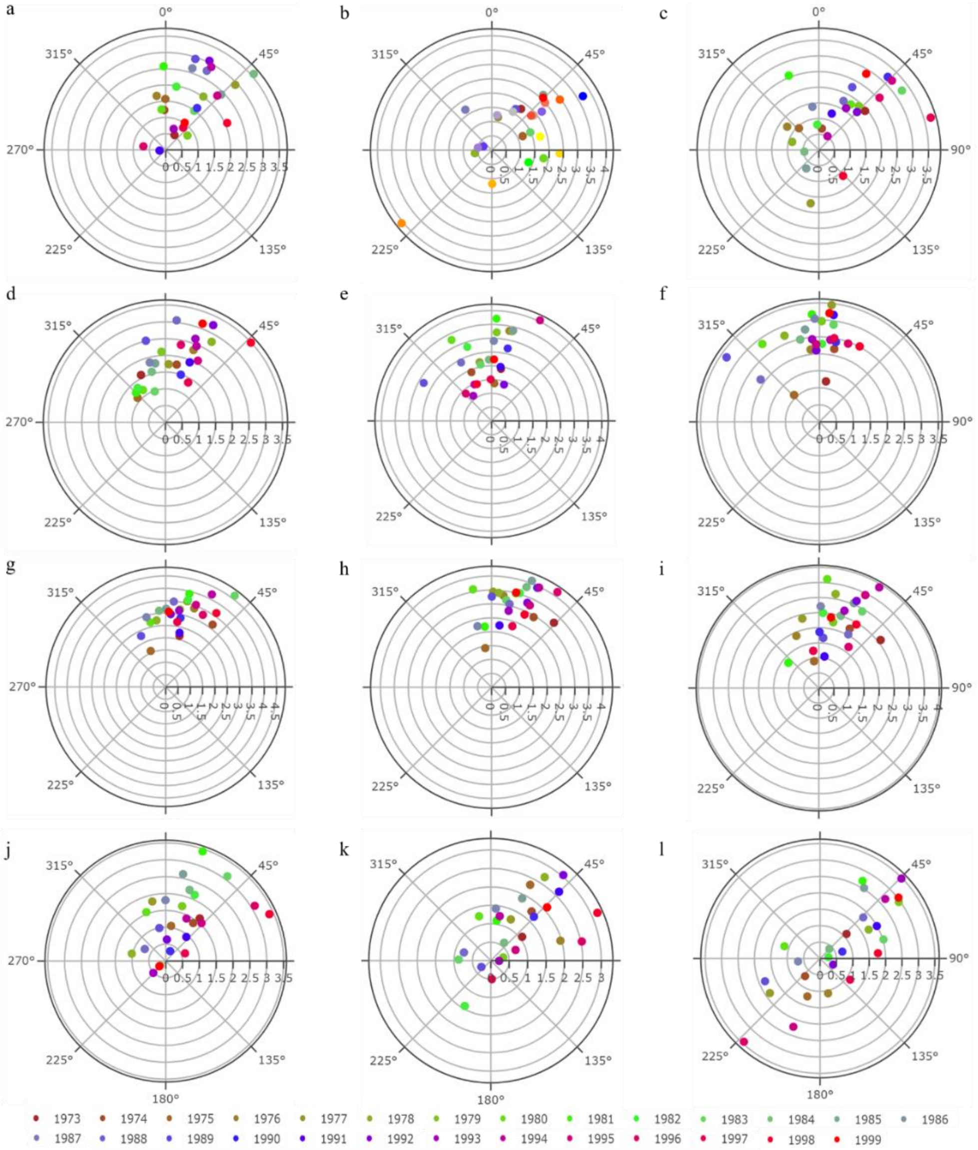
| Code | Variable | Mean | Max. | Min. | SD |
|---|---|---|---|---|---|
| V1 | Annual Mean Temperature | 17.87 | 22.24 | 14.00 | 1.65 |
| V2 | Mean Diurnal Range temperature | 10.97 | 13.58 | 7.86 | 1.14 |
| V5 | Max Temperature of Warmest Month | 30.25 | 39.60 | 25.00 | 2.57 |
| V6 | Min Temperature of Coldest Month | 5.88 | 12.00 | 1.30 | 2.42 |
| V7 | Temperature Annual Range | 24.38 | 34.30 | 15.90 | 4.00 |
| V12 | Annual Precipitation | 273.49 | 427.00 | 28.00 | 89.05 |
| V13 | Precipitation of Wettest Month | 48.53 | 69.00 | 8.00 | 12.76 |
| V14 | Precipitation of Driest Month | 0.78 | 2.00 | 0.00 | 0.58 |
| V17 | Precipitation of Driest Quarter | 7.04 | 16.00 | 1.00 | 3.83 |
| V20 | Elevation | 538.51 | 1413.00 | 26.00 | 420.62 |
| V26 | Global Aridity Index | 0.14 | 0.24 | 0.01 | 0.05 |
| V27 | Evapotranpiration | 1969.33 | 3224.00 | 153.00 | 296.83 |



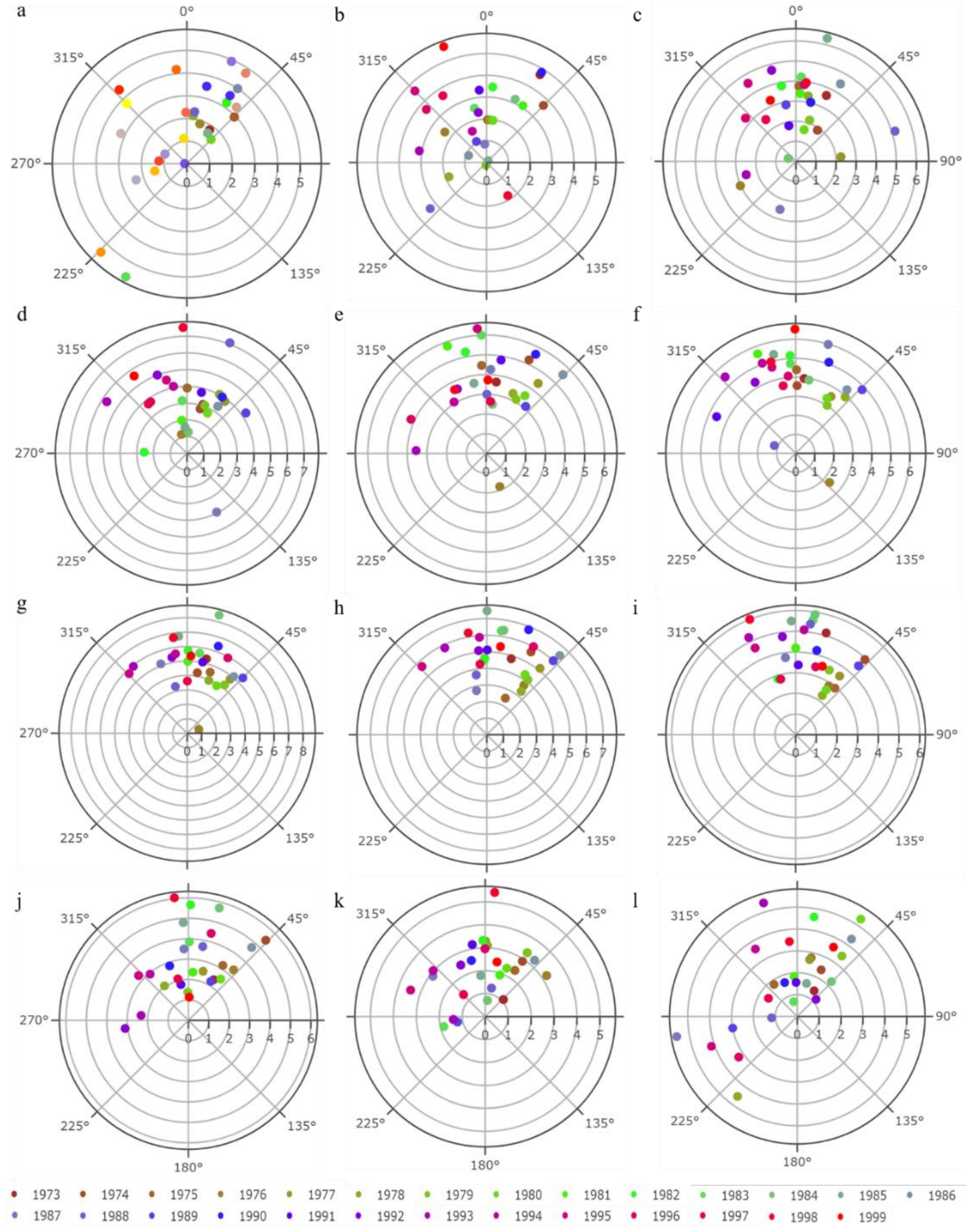
**Appendix 4** (Chapter II) Frequency distribution of rest of bioclimatic variables in North Africa (n = 51). **(a)** Normalized Difference Vegetation Index April 2002 (V25a). **(b)** Normalized Difference Vegetation Index April 2002 (V25j). **(c)** Isothermality (V2/V7) (V3). **(d)** Temperature Seasonality (V4). **(e)** Temperature Annual Range (V7). **(f)** Precipitation Seasonality (V15). **(g)** Precipitation of Wettest Quarter (V16). **(h)** Precipitation of Coldest Quarter (V19). **(i)** Land Surface Temperature April 2002 (V24a).



**Appendix 5** (Chapter II) Mean speed (m/s) and direction (degree) of the wind (period 1973-1999) at Sidi Ifni station. **(a)** January. **(b)** February. **(c)** March. **(d)** April. **(e)** May. **(f)** June. **(g)** July. **(h)** August. **(i)** September. **(j)** October. **(k)** November. **(l)** December.



**Appendix 6** (Chapter II) Mean speed (m/s) and direction (degree) of the wind (period 1973-1999) at Safi station. **(a)** January. **(b)** February. **(c)** March. (d) April. **(e)** May. **(f)** June. **(g)** July. **(h)** August. **(i)** September. **(j)** October. **(k)** November. **(l)** December.

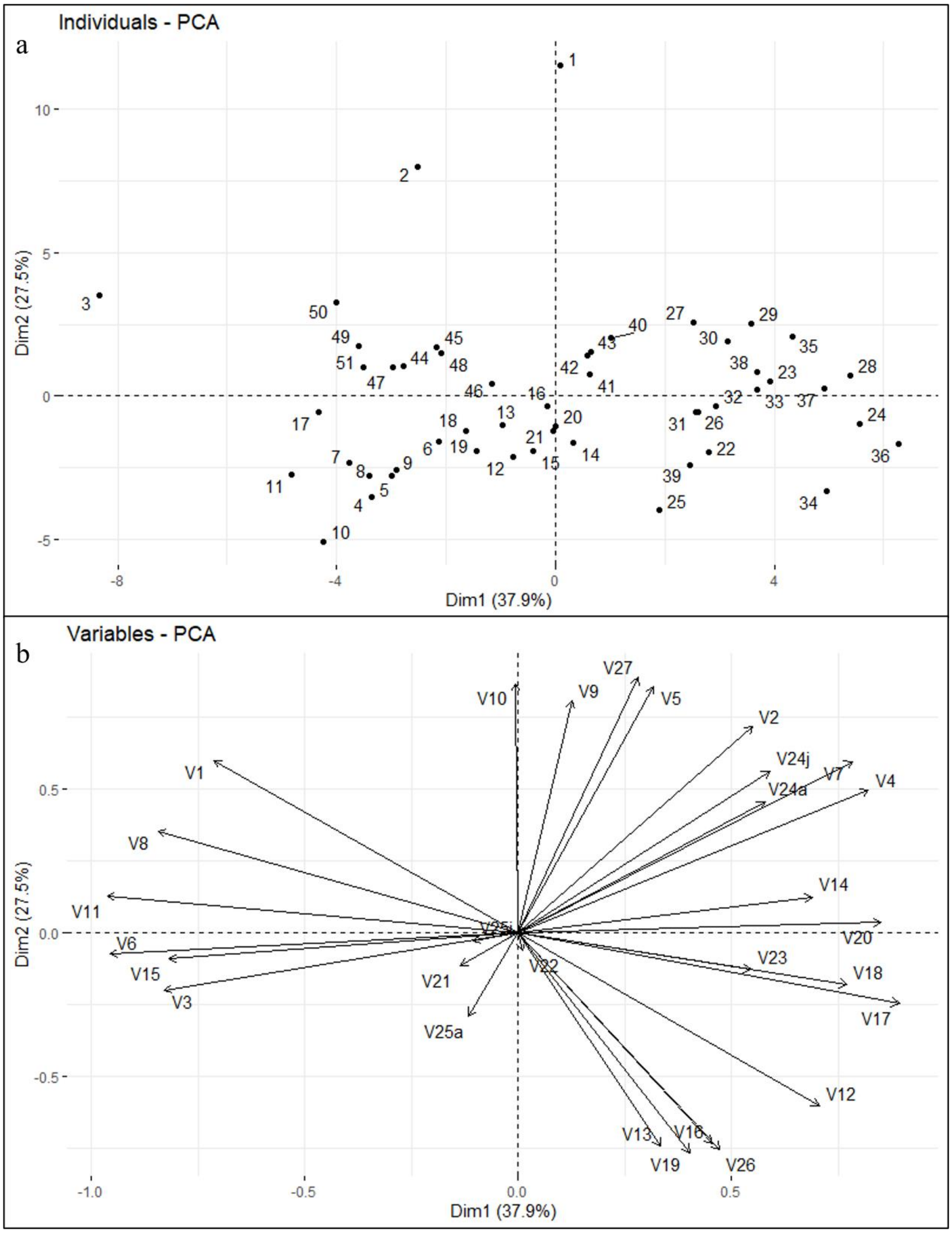


**Appendix 7** (Chapter II) Mean speed (m/s) and direction (degree) of the wind (period 1973-1999) at Essaouira station. **(a)** January. **(b)** February. **(c)** March. **(d)** April. **(e)** May. **(f)** June. **(g)** July. **(h)** August. **(i)** September. **(j)** October. **(k)** November. **(l)** December.

**Appendix 8** (Chapter II) Clustering groups using 29 bioclimatic variables (Euclidean distance, and clustering algorithm using Kmeans).

**Appendix 9** Loadings and the accumulated variance explained by PCs.

| Code | Variable | Principal Component (PC) | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| V1 | Annual Mean Temperature | -0.21 | -0.21 | -0.20 |
| V2 | Mean Diurnal Range | 0.17 | -0.25 | 0.00 |
| V3 | Isothermality | -0.25 | 0.07 | 0.17 |
| V4 | Temperature Seasonality | 0.25 | -0.18 | -0.13 |
| V5 | Max Temperature of Warmest Month | 0.10 | -0.30 | -0.23 |
| V6 | Min Temperature of Coldest Month | -0.29 | 0.03 | -0.10 |
| V7 | Temperature Annual Range | 0.24 | -0.21 | -0.09 |
| V8 | Mean Temperature of Wettest Quarter | -0.25 | -0.12 | -0.06 |
| V9 | Mean Temperature of Driest Quarter | 0.04 | -0.29 | -0.32 |
| V10 | Mean Temperature of Warmest Quarter | 0.00 | -0.31 | -0.29 |
| V11 | Mean Temperature of Coldest Quarter | -0.29 | -0.04 | -0.09 |
| V12 | Annual Precipitation | 0.21 | 0.21 | -0.20 |
| V13 | Precipitation of Wettest Month | 0.10 | 0.26 | -0.27 |
| V14 | Precipitation of Driest Month | 0.21 | -0.04 | 0.17 |
| V15 | Precipitation Seasonality | -0.25 | 0.03 | -0.04 |
| V16 | Precipitation of Wettest Quarter | 0.14 | 0.26 | -0.24 |
| V17 | Precipitation of Driest Quarter | 0.27 | 0.09 | 0.04 |
| V18 | Precipitation of Warmest Quarter | 0.23 | 0.06 | -0.09 |
| V19 | Precipitation of Coldest Quarter | 0.12 | 0.27 | -0.24 |
| V20 | Elevation | 0.26 | -0.01 | 0.25 |
| V21 | Hillshade | -0.04 | 0.04 | -0.15 |
| V22 | Aspect | 0.00 | 0.02 | -0.13 |
| V23 | Slope | 0.16 | 0.05 | 0.34 |
| V24a | Land Surface Temperature April | 0.17 | -0.16 | 0.02 |
| V24j | Land Surface Temperature July | 0.18 | -0.20 | -0.02 |
| V25a | Normalized Difference Vegetation Index April | -0.03 | 0.10 | -0.14 |
| V25j | Normalized Difference Vegetation Index July | -0.03 | 0.01 | -0.28 |
| V26 | Global Aridity Index | 0.14 | 0.27 | -0.24 |
| V27 | Evapotranspiration | 0.08 | -0.31 | 0.07 |
| | **Proportion of Variance (PV)** | **38.52** | **26.57** | **9.10** |
| | **Cumulative Proportion (CP)** | **38.52** | **65.09** | **74.19** |

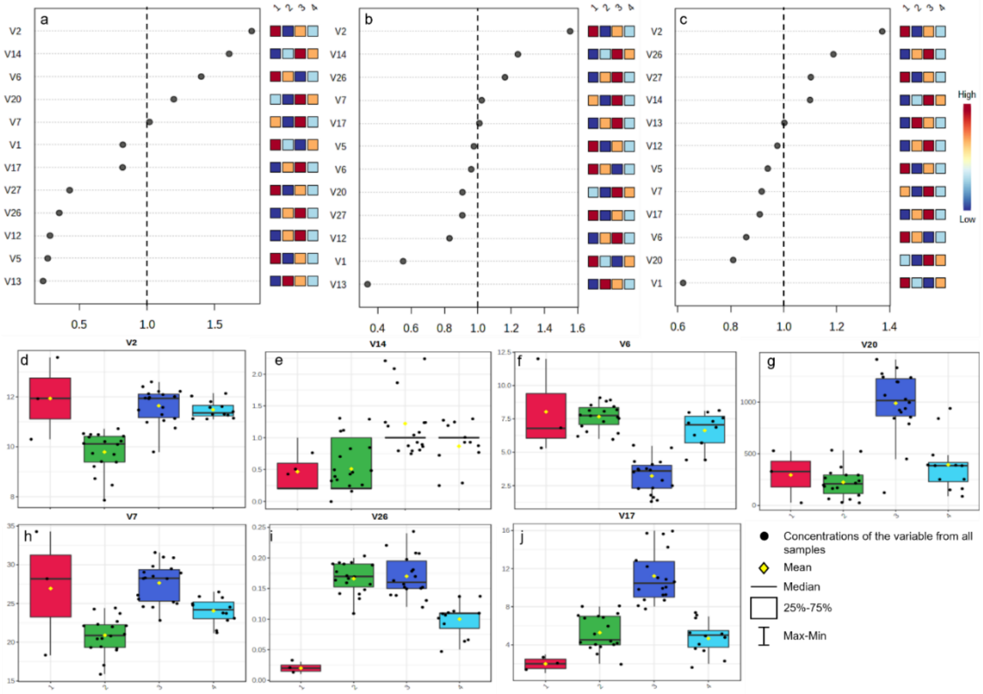


**Appendix 10** (Chapter II) Principal Components Analysis using 29 bioclimatic variables. **(a)** Individuals between dimension 1 and dimension. **(b)** Variables between dimension 1 (Dim1) and dimension 2 (Dim2).

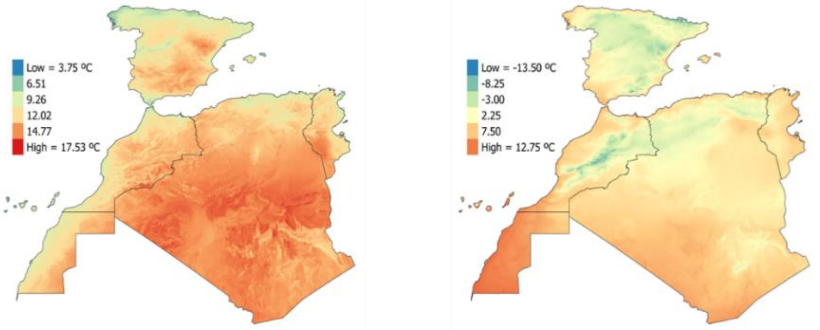
**Appendix 11** (Chapter II) Clustering results using *K*means.

| Class | Group | Provinces (samples in each cluster) |
|---|---|---|
| 1 | Desert ($n = 2$) | Assa-Zag (02). Tindouf (01)* |
| 2 | Littoral ($n = 18$) | Agadir (17, 7, 8, 11). Essaouira (6, 9, 10, 12, 13, 14, 15, 16, 18). Nador (19, 20). Safi (4, 5). Berkane Taourirt (21). |
| 3 | Mountain ($n = 18$) | Agadir (25). Chichaoua (34). Essaouira (22). Taroudant (23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 36, 38). Tiznit (37). Khemisset (39). Chlef (35)* |
| 4 | Sublittoral ($n = 13$) | Chichaoua (42, 43). Chtouka-AitBaha (46). Guelmim (50). Inezgane-Ait Melloul (44). Laayoune** (3). Taroudant (45, 47, 48). Tiznit (40, 41, 49, 51). |

*Localities in Algeria. **Locality in Western Sahara. The rest of the localities belong to Morocco.

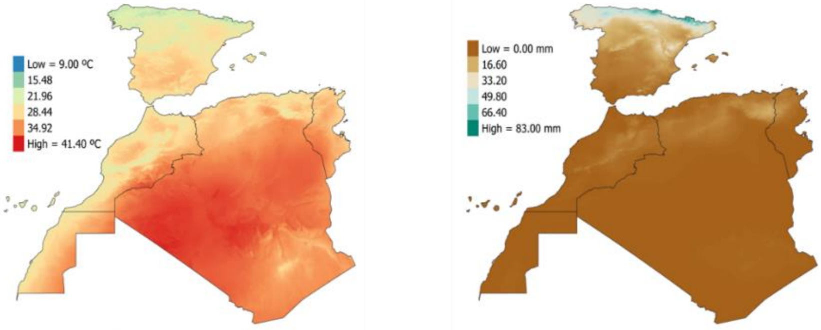


**Appendix 12** (Chapter II) Important features identified by PLS-DA. **(a)** VIP score of Component 1. **(b)** VIP score of Component 2. **(c)** VIP score of Component 3. The colored boxes on the right indicate the relative concentrations of the variable in each group: Desert (1), Littoral (2), Mountain (3) and Sublittoral (4). Boxplots (The black dots represent the concentrations of the selected variable from all samples. The notch indicates the 95% confidence interval around the median of each group, defined as +/- 1.58 IQR/sqrt(n). The notch can be used to evaluate differences between groups; if the notches do not overlap, the medians are likely different. Meanwhile, the mean concentration of each group is indicated with a yellow diamond). **(d)** Mean Diurnal Range Temperature (V2) boxplots. **(e)** Precipitation of the Driest Month (V14) boxplots. **(f)** Temperature of the Coldest Month (V6) boxplots. **(g)**Elevation (V20) boxplots. **(h)** Temperature Annual Range (V7). **(i)** Global Aridity Index (V26) boxplots. **(j)** Precipitation of the Driest Quarter (V17) boxplots.



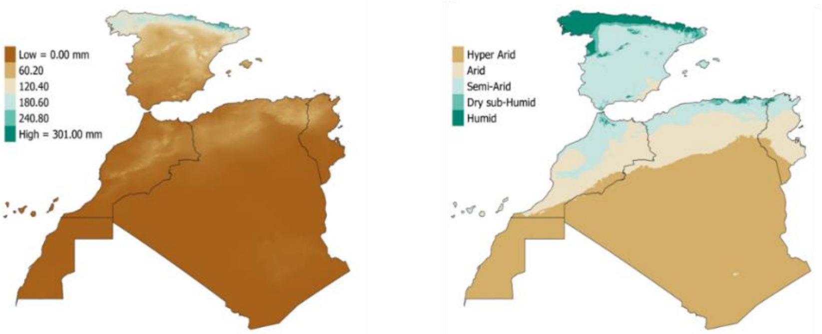
Mean diurnal range temperature °C (V2)

Min. temp. coldest month °C (V6)



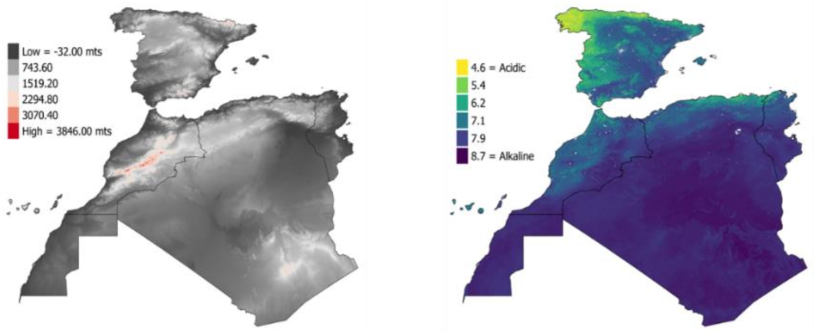
Temperature annual range °C (V7)

Prec. driest month (V14)



Prec. driest quarter (V17)

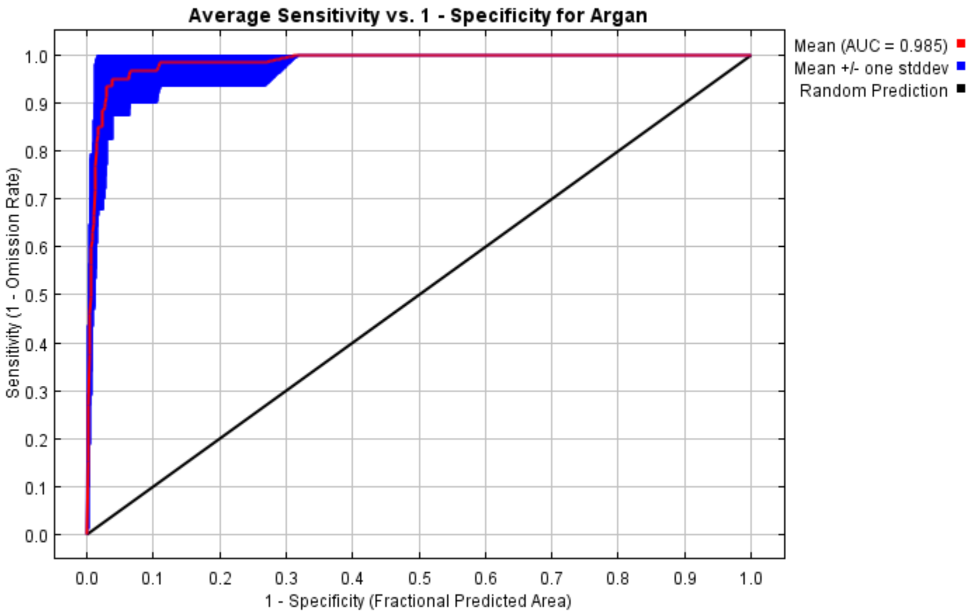
Global Aridity Index (V26)



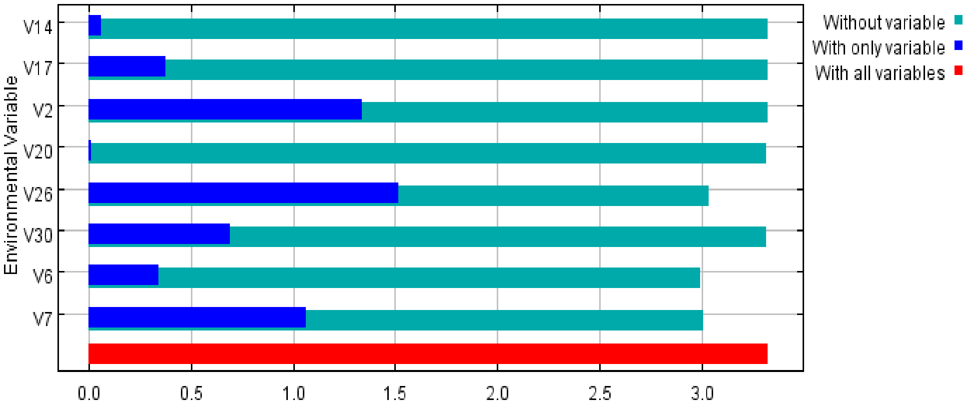
Elevation (V20)

Mean Soil pH in H2O (V30)

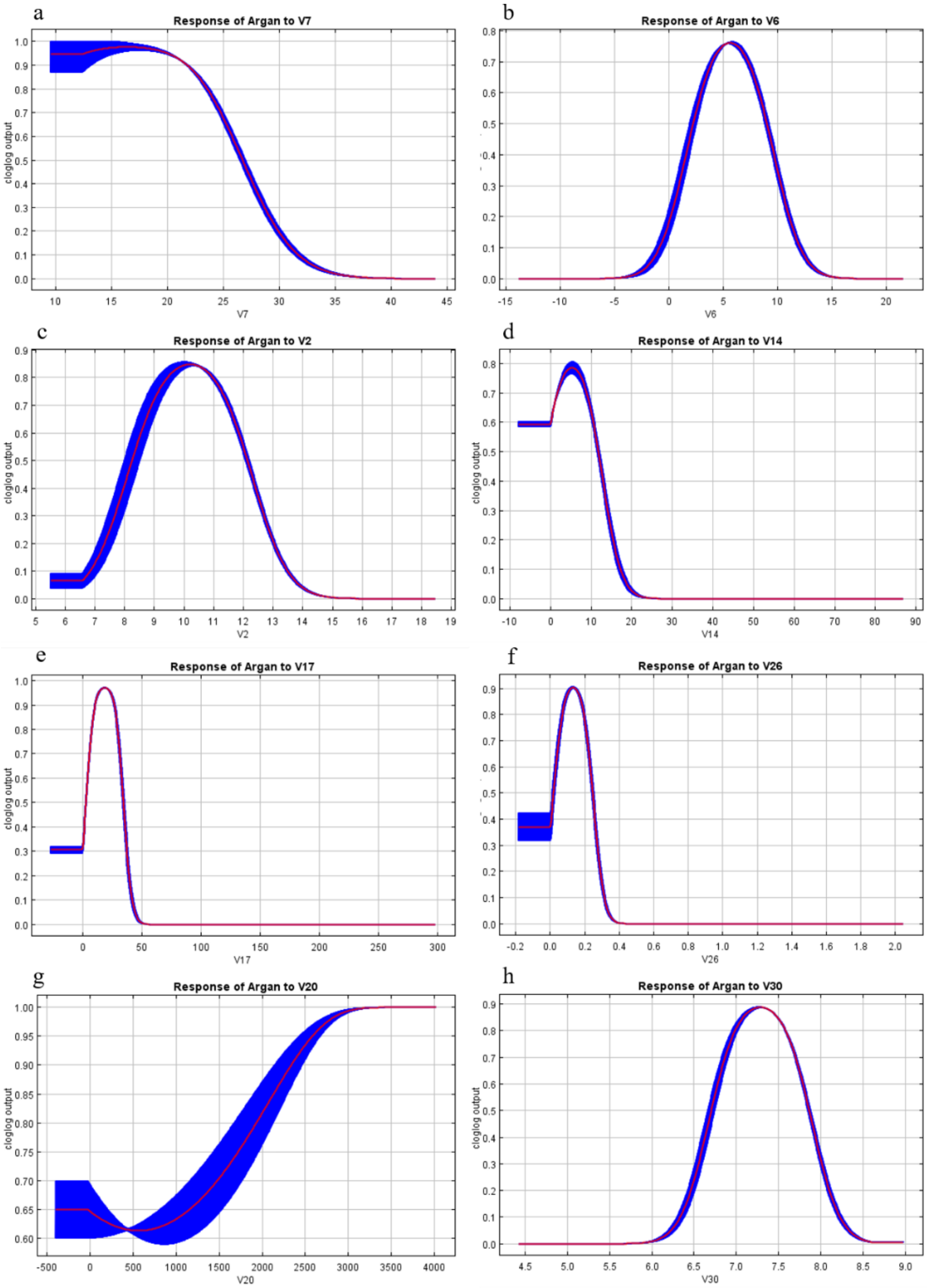
**Appendix 13** (Chapter II) Environmental and climatic variables used to build the *A. spinosa* habitat suitability model.



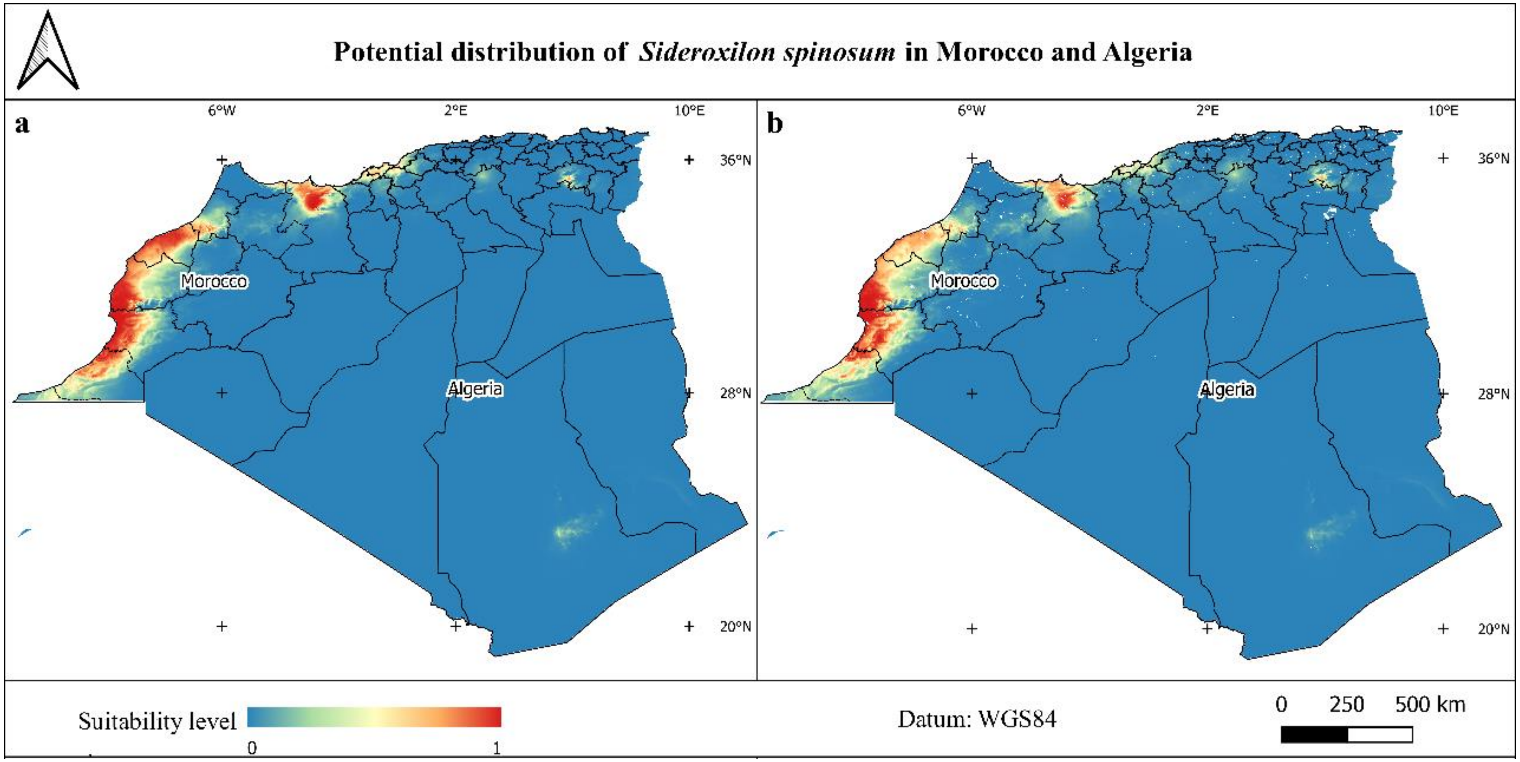
**Appendix 14** (Chapter II) The average area under the curve (AUC) for 10 MaxEnt runs. The red line is the mean value. and the blue line represents ±1 standard deviation. The mean AUC value of 0.99 indicates an excellent model.



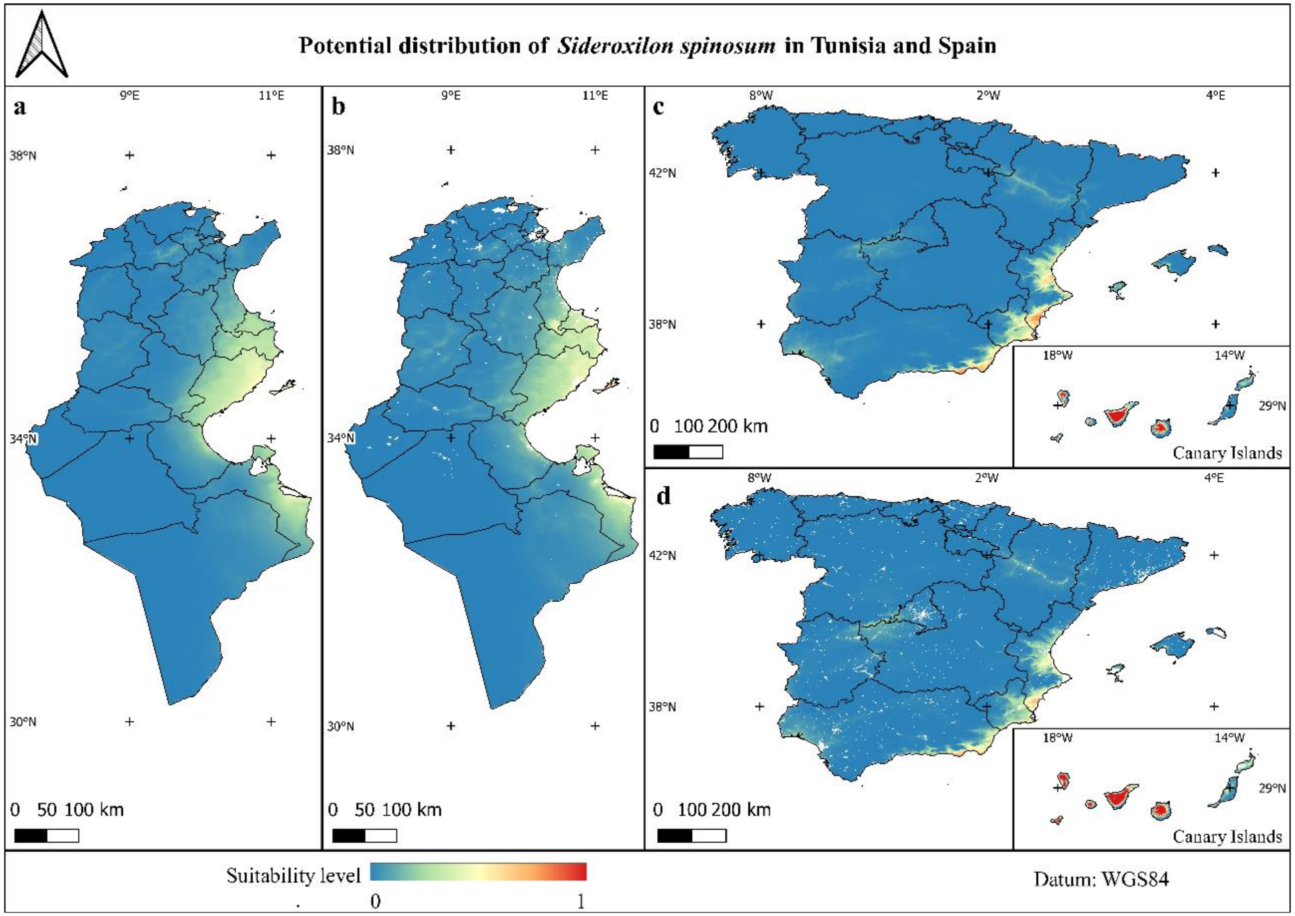
**Appendix 15** (Chapter II) Jackknife test of the importance of individual climatic and environmental variables (blue bars) in the development of the MaxEnt model relative to all climatic and environmental variables (red bar). Aridity index (V26) is the most effective single variable for predicting the distribution of the occurrence data.



**Appendix 16** (Chapter II) Response curves showing the relationships between the probability of presence and climatic and environmental predictors of *A. spinosa*.



**Appendix 17** (Chapter II) Potential distribution of *A. spinosa* in Morocco and Algeria. **(a)** Seven environmental variables (Excluding pH). **(b)** Eight environmental variables (Including pH). Mean diurnal range (V2), minimum temperature of the coldest month (V6), temperature annual range (V7), precipitation of the driest month and quarter (V14, V17), elevation (V20), aridity index (V26) and Soil pH in H2O (V30).



**Appendix 18** (Chapter II) Potential distribution of *A. spinosa* in Tunisia. **(a)** 7 environmental variables (Excluding pH). **(b)** 8 environmental variables (Including pH). Potential distribution of *A. spinosa* in Spain. **(c)** 7 environmental variables (Excluding pH). **d)** 8 environmental variables (Including pH).

Used variables: Mean diurnal range (V2), minimum temperature of the coldest month (V6), temperature annual range (V7), precipitation of the driest month and quarter (V14, V17), elevation (V20), aridity index (V26) and Soil pH in H2O (V30).