Departamento de Producción Vegetal

Programa de Doctorado en Recursos y Tecnologías Agrícolas

Tesis Doctoral

Mejora de la germinación para la propagación sexual de la alcaparra (*Capparis spinosa* L.)

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Resumen

La alcaparra (*Capparis spinosa* L.) es un arbusto característico de la región mediterránea, del que se aprovechan sus botones florales y frutos. Uno de los inconvenientes más importantes para su cultivo es la dificultad en su propagación.

El fruto es una baya con muchas semillas, que presentan un porcentaje de germinación que no supera el 5%. Las semillas son de color marrón oscuro, de 2 a 3 mm y la cubierta tiene una capa lignificada con paredes radiales engrosadas. La baja germinación se relaciona con distintos tipos de latencia, que no ha sido científicamente atribuida a ningún tipo concreto.

El objetivo de esta tesis es estudiar las distintas fases del proceso de germinación para determinar el tipo de latencia, cómo superarla, y establecer pautas para una propagación viable mediante semillas.

Los ensayos se realizaron en el Departamento de Producción Vegetal de la Universitat Politècnica de València, utilizando semillas de producción propia y de lotes comerciales. Para determinar el tipo de latencia, se estudió el proceso de imbibición durante la germinación, la resistencia mecánica de la cubierta seminal y el endospermo frente a la protusión de la radícula, y el efecto de la aplicación de giberelinas.

La absorción de agua sigue las dos primeras fases del modelo trifásico típico de absorción de agua en la germinación y la hidratación comienza por la región hilo-micropilar. La imbibición no es un factor limitante para su germinación, alcanzando un contenido de humedad del 32%, con el que se obtienen porcentajes de germinación superiores al 90%. Por tanto, no tienen una cubierta impermeable al agua, es decir, no muestran latencia física. Las semillas comerciales presentan menor viabilidad y germinación que las de producción propia, lo que se ha evidenciado con el deterioro en la cubierta seminal que se relaciona con los procesos de extracción, limpieza y almacenamiento.

Para obtener altos porcentajes de germinación se requiere el uso de giberelinas, lo que indica la presencia de latencia fisiológica. Se recomienda el uso de 500 mg L^{-1} de ácido giberélico (AG) para humedecer el sustrato de germinación. El AG desencadena el incremento del contenido de giberelinas activas endógenas y la disminución del ácido abscísico.

La germinación consta de dos eventos separados temporalmente, el agrietamiento de la cubierta, en el área hilo-micropilar y luego la perforación del endospermo micropilar, por donde emerge la radícula. Las giberelinas activas no disminuyen la fuerza de punción para perforar el endospermo, sino que aumentan el potencial de crecimiento del embrión, logrando una mayor germinación y en menor tiempo. La viabilidad y germinación de semillas recién extraídas de frutos recolectados en su dehiscencia alcanzan valores muy elevados. Si la recolección de los frutos se retrasa y la pulpa se seca, la viabilidad y la germinación disminuyen, por lo que, como regla general, se aconseja recolectar los frutos al menos una vez por semana, e inmediatamente extraer las semillas y ponerlas a germinar.

En el ensayo de flotación, las semillas que flotan y que presentan buena germinación son muy pocas (0.24%), por lo que se recomienda desecharlas.

La germinación no es afectada por la iluminación con diferentes longitudes de onda, por lo que puede realizarse en oscuridad, lo que puede suponer un ahorro económico para semilleros comerciales. La irradiación con láser He-Ne durante tiempos cortos de exposición mejora el porcentaje de germinación en semillas previamente humedecidas, pero no sustituye a la adición de AG al sustrato, complementando su efecto.

Con la ultrasonicación se escarifica la testa sin afectar al tegmen, y se acelera la imbibición inicial, aunque la humedad alcanzada en las semillas es la misma que en el control. Existe una correlación lineal y negativa, entre la germinación y la temperatura alcanzada con estos tratamientos.

Abstract

The caper (*Capparis spinosa* L.) is a shrub characteristic of the Mediterranean region. It is use for its flower buds and fruits. One of the main drawbacks of its cultivation, is the difficulty presented by its propagation, both vegetative and through seeds.

The fruit is a berry containing many seeds, these have a very low germination percentage, which usually is at most 5%. The seeds are dark brown colour, measure 2 - 3 mm, and the cover has a lignified layer with thickened radial walls. Low germination power has been linked to different types of dormancies, but, it has not been scientifically attributed to any specific type.

This thesis aims to study the different phases of the germination process to determine the type of dormancy, how to overcome it, and to establish guidelines for viable propagation through their seeds.

The tests have been carried out in the Universitat Politècnica de València Plant Production Department, mainly using own-produced seeds, and seeds from commercial lots. To determine the type of dormancy, the imbibition process that occurs during germination has been studied, as well as the mechanical resistance exerted by the seed coat and the endosperm against the protrusion of the radicle and the effect of the external gibberellins application.

Water uptake follows the first two phases of the typical three-phase water uptake pattern in seed germination, and seed hydration begins in the hilum-micropillar region. Imbibition is not a limiting factor for germination, reaching a moisture content of 32%, which allows germination percentages higher than 90%. Therefore, caper seeds do not have a waterproof coat; that is, they do not show physical dormancy. Commercial seeds showed lower viability and germination than those of own production, which has been related to seed deterioration. This deterioration, which has been evident in the seed coat, is related to their extraction, cleaning, and storage processes.

Obtaining high percentages of germination requires gibberellins, which indicates the presence of physiological dormancy. The use of 500 mg L^{-1} of gibberellic acid (GA) is recommended to moisten the germination substrate. GA triggers an increase in endogenous active gibberellins content and a decrease in abscisic acid.

The germination consists of two temporarily separated events: the cracking of the coat in the hilum-micropillar area, and then the perforation of the micropillar endosperm, where the radicle emerges. Active gibberellins do not decrease the puncture force needed to pierce the endosperm but increase the growth potential of the embryo, achieving higher germination percentages in less time. The viability and germination of seeds freshly extracted from fruits harvested in their dehiscence reach very high values. If the harvest of the fruits is delayed and the pulp dries, the viability and the percentage of germination decrease so, as a rule, for the production of commercial seed, it is advisable to collect the fruits at least once a week and immediately extract the seeds and put them to germinate.

In the flotation test, floating seeds with germination capacity are very few (0.24%), so it is recommended to discard them.

The germination is not affected by different wavelengths lights, so it can be carried out in darkness, which can mean economic savings in commercial nurseries. He-Ne laser irradiation during short exposure times improves the germination of previously moistened seeds but does not replace the addition of GA to the substrate but complements its effect.

With the ultrasonication treatment, the testa is scarified without affecting the tegmen, and the initial imbibition is accelerated, although the seed moisture is the same as in the control. There is a linear and negative correlation between germination and the temperature reached with these treatments.

Resum

La tàpera (*Capparis spinosa* L.) és un arbust característic de la regió mediterrània, que s'aprofita pels seus botons florals i fruits. Un dels principals inconvenients que presenta el seu cultiu, es la dificultat que presenta la seua propagació.

El fruit és una baia que té moltes llavors, que presenten una germinació que no sol superar el 5%. Les llavors són de color marró fosc, mesuren 2 - 3 mm, i la coberta té una capa lignificada amb parets radials engrossides. La baixa germinació s'ha relacionat amb diferents tipus de latència, però fins ara no ha estat científicament atribuïda a cap tipus concret.

L'objectiu d'aquesta tesi és estudiar les diferents fases de la germinació per determinar el tipus de latència, com superar-la, i establir les pautes per a una propagació viable mitjançant les seves llavors.

Els assajos s'han realitzat en el Dep. Producció Vegetal de la Universitat Politècnica de València, utilitzant llavors de producció pròpia, i de lots comercials. Per determinar el tipus de latència, s'ha estudiat el procés d' imbibició que es produeix durant la germinació, la resistència mecànica que exerceixen la coberta seminal i l'endosperma enfront de la protrusió de la radícula, i l' efecte de l'aplicació de gibberel·lines.

L'absorció d'aigua segueix les dues primeres fases del model trifàsic típic d'absorció d'aigua en la germinació, i la hidratació comença per la regió micropilar. La imbibició no és un factor limitant per a la seua germinació, aconseguint un contingut d'humitat del 32%, permet percentatges de germinació superiors al 90%. Per tant, les llavors no tenen una coberta impermeable a l'aigua, és a dir, no mostren latència física. Les llavors comercials presenten menor viabilitat i germinació que les de producció pròpia, relacionat amb el seu deteriorament, que ha estat evident a la coberta; conseqüència dels processos d'extracció, neteja i emmagatzematge.

Per obtenir alts percentatges de germinació es requereix l'ús de gibberel·lines, la qual cosa indica la presència de latència fisiològica. Es recomana l'ús d'una solució de 500 mg L^{-1} d'àcid gibberèl·lic (AG). L'AG desencadena l'augment del contingut de gibberel·lines actives endògenes i una disminució d'àcid abscísic.

La germinació consta de dos esdeveniments separats temporalment, l'esquerdament de la coberta, que comença a l'àrea micropilar i a continuació es produeix la perforació de l'endosperma micropilar, per on emergeix la radícula. Les gibberel·lines actives no disminueixen la força de punció necessària per perforar l'endosperma, sinó que augmenten el potencial de creixement de l'embrió, aconseguint major germinació i en menor temps. La viabilitat i el percentatge de germinació de les llavors obtinguts de fruits recol·lectats en la seva dehiscència assoleixen valors molt elevats. Si la recol·lecció dels fruits es retarda i la polpa s'asseca, la viabilitat i la germinació disminueixen, per la qual cosa, com a regla general, per a la producció de llavor comercial s'aconsella recol·lectar els fruits almenys una vegada per setmana, i immediatament extreure les llavors i posar-les a germinar.

En l 'assaig de flotació, les llavors que floten i que presenten capacitat germinativa són molt poques (0,24%), recomanant-se rebutjar les llavors flotants.

La germinació no és afectada per la il·luminació amb llums de diferents longituds d'ona, per la qual cosa la germinació pot realitzar-se en foscor, i, pot suposar un estalvi econòmic en planters comercials. La irradiació amb làser He-Ne durant temps curts d'exposició millora el percentatge de germinació de les llavors prèviament humitejades, però no substitueix l' addició d'AG al substrat, sinó que complementa el seu efecte.

Amb la ultrasonicació, s'escarifica la testa sense afectar el tegmen i s'accelera la imbibició inicial, però la humitat assolida en les llavors és la mateixa que el control. Hi ha una correlació lineal i negativa, entre la germinació i la temperatura assolida amb estos tractaments.

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Capítulo 1. Introducción General

1.1. Generalidades

La alcaparra (*Capparis spinosa* L.) es una planta que puede encontrarse tanto en estado silvestre como cultivada en la cuenca mediterránea. Es un arbusto xerófilo, perenne, de hojas caducas que se adapta y puede crecer en suelos pobres, calcáreos y en climas áridos. Destaca por sus características agronómicas, nutricionales y farmacológicas. Es una especie importante para la preservación del suelo, al evitar su erosión, y del agua, por ser resistente a la sequía; también tiene valor ornamental, particularmente por sus vistosas flores. Se aprovechan como alimento, principalmente los botones florales, los frutos inmaduros y, en menor medida, los brotes tiernos, todos ellos procesados como encurtidos. En los últimos años se han estudiado las propiedades nutricionales y nutracéuticas por la actividad antioxidante que presentan sus compuestos bioactivos. Por tanto, es un cultivo promisorio para la protección del medio ambiente, la alimentación y la salud.

Aunque es una especie cada vez más demandada a nivel comercial, en los últimos años ha disminuido la superficie cultivada en los países desarrollados, debido mayoritariamente a la elevada mano de obra que exige, además de los problemas que presenta su propagación, tanto sexual como asexual (Chedraoui et al., 2017; Sottile et al., 2021), por lo que para facilitar y aumentar su cultivo comercial deberían mejorarse las técnicas de su propagación. La propagación sexual es problemática al presentar índices muy bajos de germinación de las semillas y de establecimiento de las plantas, por lo que es necesario profundizar y aumentar las investigaciones sobre esta temática.

1.2. Origen y taxonomía

El término *Capparis*, acuñado por Teofrasto (siglo IV a. C.) y respaldado por Dioscórides (siglo I), procede del griego "kapparis" (Juan, 2017) y fue ampliamente utilizado en la Edad Media por la cultura árabe (Sozzi, 2001). El vocablo *spinosa* proviene del latín, haciendo referencia a la presencia de espinas.

El género *Capparis* incluye numerosas especies de origen tropical o subtropical, muchas de ellas distribuidas en las regiones mediterráneas (Chedraoui et al., 2017).

Descubrimientos arqueológicos del Paleolítico tardío sugieren que la alcaparra ya se consumía en el Alto Egipto (entre 16.000 y 15.000 años a. C.; Hillman, 1989; Chedraoui et al., 2017). Se han encontrado restos de frutos silvestres datados en 6000 a. C. en el sudoeste de Irán y en Iraq (Tigris) (Sozzi, 2001), también se encontraron restos de semillas de alcaparras en diferentes sitios arqueológicos que datan de 9000 a 8000 a. C. (Sozzi et al., 2012).

Se han encontrado semillas de *C. spinosa* en Tell es-Sawwan (Iraq), datadas en 5800 a. C. y en las tumbas de Yanghai, del distrito de Turpan en China, 2800 a. C. En Tell es-Sweyhat (Siria) se han hallado botones florares de alcaparra, carbonizados, en jarras de la Edad de Bronce (1700 a 1100 años a. C.), por lo que se deduce que el consumo de alcaparras encurtidas se remonta a dicha época (Rivera et al., 2002). En este mismo emplazamiento, la planta fue utilizada por griegos, hebreos y romanos (Chedraoui et al., 2017).

Aunque *Capparis* es el principal género de la familia Capparaceae, su taxonomía ha sido infra estudiada durante mucho tiempo (Jacobs, 1965). Zohary, (1960), estudió el género *Capparis* y basándose en los caracteres morfológicos estableció su diversidad en torno a dos especies principales, *C. spinosa* y *C. ovata* (además de las especies *C. orientalis*, *C. sícula* y *C. aegyptia*). Realizó un enfoque sintético de esta sección debido a la presencia de individuos intermedios en los especímenes de herbario, que dificultaban la diferenciación entre especies. La mayoría de los taxones que anteriormente estaban subordinados a una u otra de estas dos especies principales con el rango de variedad o subespecie, fueron reconocidos posteriormente como especies o subespecies por Inocencio et al. (2006).

Estudios realizados por Inocencio et al. (2005), basados en la técnica *Amplified Fragment Length Polymorph* (AFLP), apoyaron la diferenciación de cuatro de los cinco taxones involucrados. Estos autores consideraron que el grupo de plantas reconocidas como *C. spinosa* sobre la base de caracteres morfológicos, incluía varios cultivares y aparecía en una posición intermedia entre *C. orientalis* y *C. sicula*, superponiéndose con *C. orientalis*. Las otras dos especies *C. aegyptia* y *C. ovata* estaban separadas del resto. *C. spinosa* presentaba un bajo número de bandas únicas en

comparación con las otras especies. En base a este estudio, Inocencio et al. (2005) hipotetizaron el origen híbrido de *C. spinosa*, y que las especies parentales más probables eran *C. orientalis* y *C. sicula*.

Posteriormente, Fici (2014) realizó una revisión del grupo *C. spinosa* en el sur de Europa, el norte de África, y Asia occidental y central, con el fin de proporcionar un tratamiento taxonómico uniforme de sus representantes. Reconoció una sola especie, *C. spinosa*, representada en el área de estudio por dos subespecies: (i) *C. spinosa* subsp. *spinosa*, que presenta un elevado polimorfismo y un amplio rango de distribución, desde el Mediterráneo hasta China y Nepal; (ii) *C. spinosa* subsp. *rupestris*, con menor variabilidad y con caracteres fenotípicos más cercanos al tronco tropical del grupo, presente en la Región Mediterránea y el Sahara. En base a investigaciones realizadas tanto en herbarios como en campo, analizó la variabilidad y distribución de las dos subespecies, proponiendo cuatro nuevas combinaciones: (i) *C. spinosa* subsp. *spinosa* var. *herbacea*; (ii) *C. spinosa* subsp. spinosa var. *atlantica*; (iii) *C. spinosa* subsp. *rupestri*s var. *ovat*a; (iv) *C. spinosa* subsp. *rupestri*s var. *myrtifolia*.

Esta alta variabilidad puede explicarse por diferentes factores como la plasticidad fenotípica, los procesos de hibridación, la selección de formas cultivadas y la diferenciación ecogeográfica (Fici, 2014), que ha dado lugar a una serie de variedades cultivadas, la mayoría de ellas dentro de *C. spinosa* (Rivera et al., 2002), que a menudo, se encuentran como cultivo asociado a almendros, olivos, higueras, o trigo, y también en los márgenes de parcelas dedicadas a estos cultivos, incluyendo también a los cítricos (Juan, 2017), dedicándose en estos últimos casos al consumo familiar.

Con estudios posteriores utilizando marcadores moleculares se ha identificado una alta diversidad genética en las muestras de *C. spinosa* procedentes del oeste de Irán (Mahmudi et al., 2022), así como la influencia de los eventos geológicos y climatológicos en la estructura filogeográfica y la diversidad genética de *C. spinosa* en las zonas áridas de Asia Central Oriental, que respondieron de manera gradual a los eventos geológicos y climáticos del Pleistoceno (Wang et al., 2016). Recientemente, Wang et al. (2022), estudiaron el genoma de *C. spinosa* var. *herbacea* como genoma de referencia para la familia Capparaceae. Esto podría servir para proporcionar un sistema para estudiar la diversidad, la especiación y la evolución de esta familia y servir como un recurso importante para comprender el mecanismo de resistencia a la sequía y a las altas temperaturas que presenta la especie.

La revisión IV del sistema de clasificación taxonómica APG (APG IV, 2016), desarrollado por el Grupo de Filogenia de las Angiospermas (APG por sus siglas en inglés, *Angiosperm Phylogeny Group*) y basado mayoritariamente en estudios moleculares, ordena las familias de angiospermas en un árbol filogenético estructurado en clados y órdenes, en el cual se clasifica a la alcaparra dentro de la familia Capparaceae de la siguiente manera:

Reino: Plantae
Clado: Angiospermae
Clado: Eudicotiledoneae
Clado: Superrosidae
Clado: Rosidae
Clado: Malvidae
Orden: Brassicales
Familia: Capparaceae
Géreno: *Capparis*
Especie: *spinosa*

La familia Capparaceae, a la que pertenece la alcaparra, está conformada actualmente por 15 géneros aceptados entre los que se encuentra el género *Capparis* Tourn. ex L. que incluye 144 especies descritas y aceptadas. Dentro de *C. spinosa* L. se incluyen 12 taxones infraespecíficos entre variedades y subespecies, entre las que se encuentra *Capparis spinosa* L. subsp. *rupestris* (Sm.) Nyman (Royal Botanic Gardens, 2022), que es la subespecie utilizada en el presente estudio.

1.3. Morfología y descripción botánica

La alcaparra es un arbusto rastrero, con hábito de crecimiento decumbente, es perenne, caducifolio de ciclo estival, con una altura de 0,5 a 1 m. Las ramas adultas pueden alcanzar una longitud de 3 a 4 m de largo, son rectas y colgantes (Figura 1), glabrescentes y de color verde hasta el agostamiento. con entrenudos distanciados 1,5 a 3,5 cm. Es una planta típica de la flora mediterránea, xerófila, que vegeta en climas áridos y semiáridos, es extremadamente resistente a la sequía, a las altas temperaturas y tolerante a la salinidad (Abbas y El-Oqlah, 1992; Yazdani Biouki et al., 2020). El agostamiento de las ramas se produce en otoño, momento a partir del cual también pierden las hojas. La parte aérea se renueva anualmente a partir de las yemas que están presentes en la base de las ramas (que se podan en el invierno), o en la cepa, de aproximadamente 25 cm de altura (Inocencio et al., 2006; Juan, 2017).

La brotación se produce en primavera – verano y comienza lentamente el crecimiento de los tallos. La yema apical origina el crecimiento vegetativo de las ramas, mientras que las yemas laterales pueden dar origen a flores o ramificaciones (yema terminal vegetativa y yemas axilares mixtas); la yema apical tiene una fuerte dominancia por lo que para favorecer la ramificación lateral es necesario realizar un despunte y de esta forma aumentar la productividad. La floración comienza en verano luego de iniciada la brotación. Posee estípulas que nacen en la base del pecíolo de las hojas, son rectas, setáceas y débiles, no decurrentes en la base, de color amarillo pálido a rojizo y con una longitud de hasta 2,5 mm (Figura 2). La forma, color, dirección de la curvatura y la decurrencia en la base de las estípulas son caracteres que permiten diferenciar a las especies y las subespecies dentro de la especie *C. spinosa* (Inocencio et al., 2006; Fici, 2014).

Las hojas son orbiculares, ovadas u obovadas (4-5 x 2,5-3,5 cm), de color verde y de consistencia coriácea, crasa, poco gruesas; alternas y enteras, con indumento muy laxo, las nervaduras de las hojas no son prominentes; base redondeada o algo ahusada, ápices agudos; pecíolos cortos de 0,4 a 2 cm de longitud. Los botones florales pueden tener ápices agudos o redondeados con pedicelos florales de 5-6,5 cm de largo (Luna y Perez, 1985; Inocencio et al., 2006; Fici, 2014). Las flores son tetrámeras, hermafroditas, solitarias, axilares, de polinización cruzada (originando heterogeneidad genética), ligeramente zigomorfas, con un diámetro de 2 a 8 cm. El cáliz está formado por cuatro sépalos oblongos u ovados, de 1 a 2 cm de largo. Los pétalos son blancos o blanco-rosados, aovados o redondeados-ovados, (1,7-2,8 cm × 1,4-2,3 cm). Los estambres son numerosos (de 50 a 250), con filamentos alargados de color violáceo (2,3-3,7 cm), y anteras de 1,3-2,5 mm de largo. El ovario tiene forma elipsoidal mide de 3 a 6 mm de largo, se encuentra en el extremo del largo ginóforo (2,5 a 4,7 cm de longitud) el cual es una prolongación del eje floral en forma de columna que tiene en lo alto el gineceo que luego da lugar al fruto (Figura 3) (Font Quer, 2001; Legua et al., 2013; Fici, 2014; Juan, 2017). Es un arbusto de floración nocturna produciéndose la antesis aproximadamente desde 18:00 hasta 10:00 h de la mañana siguiente (Petanidou et al., 1996).

Debido a la proximidad de sus hábitats y a la polinización entomófila, el flujo genético entre las plantas cultivadas y las poblaciones silvestres puede ser elevado, dando origen a biotipos más heterogéneos cuando los agricultores llevan a cabo la propagación por semillas, mientras que las formas cultivadas son más homogéneas cuando la propagación se realiza por esquejes (Fici y Gianguzzi, 1997).

Figura 1. Plantas de *C. spinosa* subsp. *rupestris* en los márgenes de parcelas de cultivos comerciales.

Figura 2. Estípulas rectas de *C. spinosa* subsp. *rupestris*.

Figura 3. Diferentes partes aéreas de la planta de alcaparra. a. Rama con hojas, flores y botones florales; b. Flor con el ovario sostenido sobre el ginóforo.

El fruto es una baya ovalada, de 3 a 5 cm de largo, mayormente con el extremo apical agudo o apiculado; es de color verde cuando está inmaduro con estrías de color blanquecino y algo rojizo en la madurez, en la que se produce la dehiscencia permitiendo la dispersión de sus semillas. La pulpa es carnosa, blanquecina cuando está inmadura y amarillo-verdosa al madurar (Melgarejo, 2000; Fici, 2014) (Figura 4).

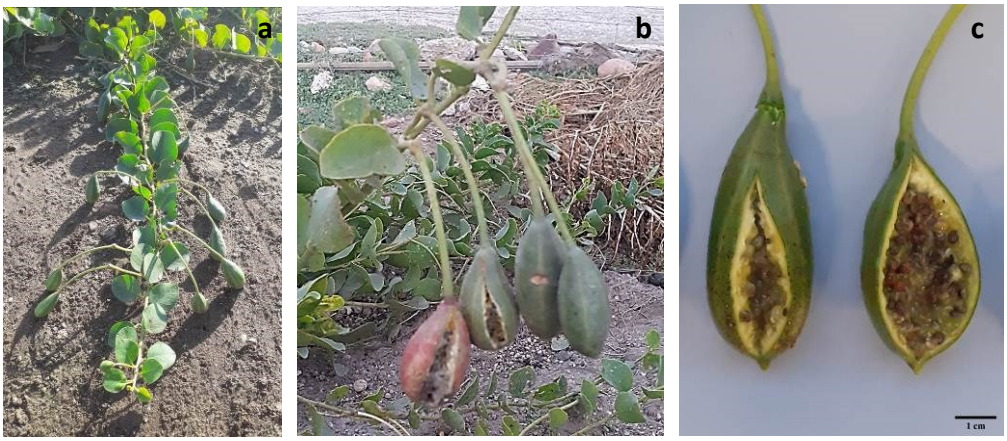

Figura 4. Frutos de la alcaparra. a y b. Ramas con frutos maduros de distinto grado de madurez, antes y después de la dehiscencia; c. Frutos maduros tras la dehiscencia, conteniendo las semillas.

Las semillas del género *Capparis* proceden de óvulos campilótropos, quedando la chalaza, el funículo y el micrópilo casi en un mismo plano (Dimitri y Orfila, 2000). La cubierta es bitégmica, formada por testa y tegmen, siendo impermeable y dura al tacto, con un espesor de 0,2 a 0,3 mm, presenta dos estructuras anatómicas casi inapreciables, hilo y micropilo (Corner, 1976). En la Figura 5, se muestra en detalle un corte longitudinal de la cubierta seminal, con una tinción específica para lignina con floroglucina que da un color rojo-granate (D`Ambrogio de Argüeso, 1986; Santamarina et al., 2004), en la que se pueden observar, en la parte superficial de la cubierta la epidermis o cutícula, la exotesta y endotesta con células engrosadas, el exotegmen, formado por esclereidas engrosadas y lignificadas, y el endotegmen, formado por una capa fina de células fibrosas. También se observa la cutícula del endospermo y el tejido de reserva que rodea al embrión. En análisis realizados en las semillas se determinó la presencia de celulosa 17% (m.s.), hemicelulosa (10%) y lignina 30% (Juan, 2017).

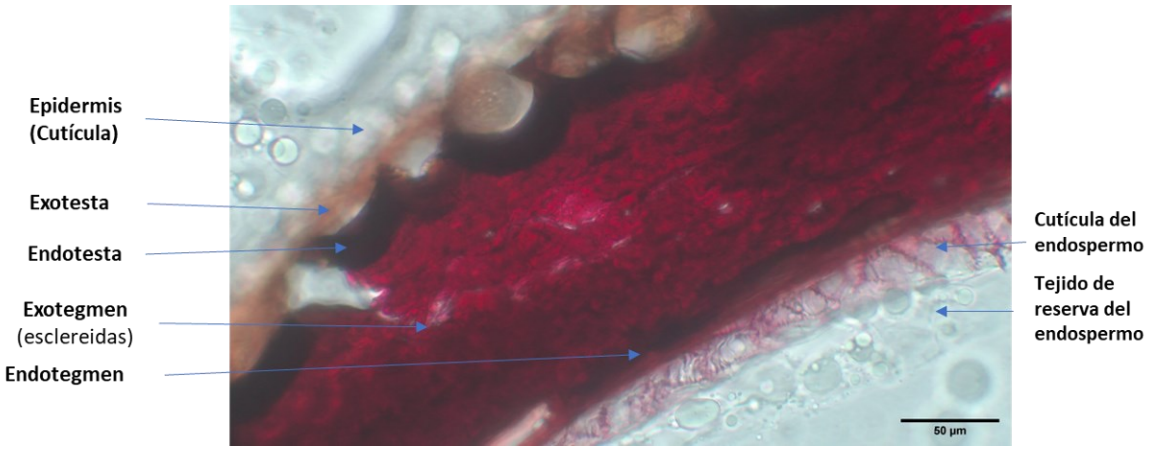

Figura 5. Sección longitudinal de la cubierta seminal de *C. spinosa*. Tinción para lignina con floroglucina, aumeto de 400x. Fuente: elaboración propia.

Las semillas son reniformes (2,2-3,6 × 2-3 mm), de color marrón oscuro rojizo, y se encuentran incrustadas en la pulpa de los frutos (Figura 6). El peso de mil semillas oscila entre 6 y 9 g (Juan, 2017; Marković et al., 2019). El embrión se encuentra curvado en espiral, y presenta cotiledones acumbentes plegados entre sí (Figura 6) (Kubitzki y Bayer, 2003). La nascencia de las plántulas es epígea (Jacobs, 1965), como se muestra en la Figura 7.

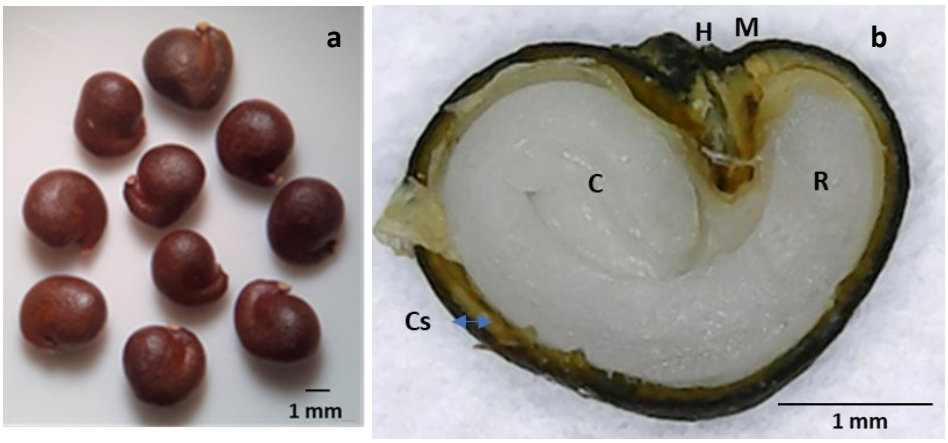

Figura 6. a. Semillas maduras de *C. spinosa* subsp. *rupestris* (aumento 10x); b. Corte longitudinal de una semilla (aumento 25x): hilo (H), micrópilo (M), cubierta seminal (Cs), radícula (R) y cotiledones (C).

Figura 7. Nascencia epígea de plántulas de *C. spinosa* subsp. *rupestris*.

La raíz es pivotante, no obstante, el sistema radical es más superficial cuando la planta se ha obtenido mediante estaquillado (Melgarejo, 2000). Las raíces son medianamente ramificadas, gruesas, fuertes y pueden alcanzar hasta diez metros de profundidad, lo que facilita el aprovechamiento de toda la humedad que puede haber en el suelo, adaptándose a climas áridos y semiáridos y por ello dándole resistencia a la sequía (Reche, 1967; Luna y Perez, 1985; Melgarejo, 2000; Juan, 2017). También es importante señalar la presencia de micorrizas en las raíces, que permiten maximizar la captación de minerales de suelos pobres (Juan, 2017; Bouskout et al., 2022), y en la rizosfera también se han aislado bacterias fijadoras de nitrógeno, tales como *Pseudomonas stutzeri* var. *mendocina*, *Comamonas* sp., *Agrobacterium tumefaciens* biovar. 2 y *Sphingobacterium* sp. que permiten el aprovechamiento del nitrógeno atmosférico para un mejor desarrollo vegetal (Andrade et al., 1997). Es un cultivo de potencial interés para adaptarse en el contexto de cambio climático y al manejo ecológico (Ashraf et al., 2018; Ikromjonovich y Turgunovich, 2022; Kdimy et al., 2022). Por otra parte cabe destacar que a pesar de que podemos encontrar a esta especie tanto como planta cultivada como en condiciones naturales, se considera en riesgo de erosión genética, estando incluida entre las especies de plantas desatendidas e infrautilizadas (Rhizopoulou y Psaras, 2003; Rhizopoulou y Kapolas, 2015; Chedraoui et al., 2017; Sottile et al., 2021), y ha sido incluida en la Lista Roja de Especies Amenazadas de la Unión Internacional para la Conservación de la Naturaleza (UICN), que es un inventario a nivel mundial del estado de conservación de especies, tanto de animales como de plantas (Rankou et al., 2020) .

1.4. Órganos de consumo y composición

Para el consumo en alimentación se aprovechan las diferentes partes de la planta entre ellas principalmente los botones florales, llamados alcaparras, el fruto inmaduro, llamado alcaparrón, y los tallos tiernos que se consumen como ensalada, todos ellos encurtidos en salmuera y vinagre (Figura 8). Las alcaparras y alcaparrones se clasifican a nivel comercial según el calibre (determinado con cribas de orificios circulares para las alcaparras, y en función del diámetro para los alcaparrones), como se indicaba en las normas de calidad en 1984 en el Boletín Oficial del Estado (actualmente disposición derogada), pero que con el objetivo de clasificación por tamaño se siguen utilizando las siguientes denominaciones (Legua et al., 2013):

- Alcaparras: Non Pareilles < 7 mm; Surfines 7-8 mm; Capucines 8-9 mm; Capotes 9-11 mm; Finas 11-13 mm y Gruesas > 13 mm
- Alcaparrones: Finos (F) < 13 mm; Medianos (M) 13-20 mm; Gruesos (G) > 20 mm

Para la recolección, de manera general, se recomiendan intervalos de 3 a 5 días entre pases sucesivos, para obtener la producción de la mayor calidad posible, tal y como sucede en España (Melgarejo, 2000; Legua et al., 2013).

En los últimos años se han realizado varias investigaciones sobre los usos, la importancia medicinal y nutraceútica de las plantas de alcaparra (Lansky et al., 2014; Akbar, 2020; Annaz et al., 2022; Sun et al., 2023), principalmente por poseer importantes propiedades antioxidantes (Mollica et al., 2019; Petrosyan, 2019; Tir et al., 2019; Yahia et al., 2020; Grimalt et al., 2022) y anticancerígenas (Yu et al., 2010; Mohammed et al., 2018; Moghadamnia et al., 2019), lo que implica que las alcaparras son una buena fuente de antioxidantes naturales. También se destaca su valor como diurético y su efecto hepatoprotector (Ali et al., 2018; Khavasi et al., 2018), sus efectos anti-reumáticos y protectores del sistema cardiovascular (Zeggwagh et al., 2007) y otros que están siendo investigados como los efectos anti-diabéticos (Fallah et al., 2013; Taghavi et al., 2014; Eddouks et al., 2017). Estas propiedades benéficas para la salud se deben a diversos componentes químicos y compuestos bioactivos que son extraídos de las diferentes partes comestibles de la planta tales como: las alcaparras (El Amri et al., 2019; Grimalt et al., 2019; Mollica et al., 2019; Wojdyło et al., 2019), los alcaparrones (Zeggwagh et al., 2007; Taghavi et al., 2014; Grimalt et al., 2018; Jiménez-López et al., 2018; Ziadi et al., 2019), las hojas y brotes tiernos (Alzergy y Elgharbawy, 2015; Moghadamnia et al., 2019; Samari et al., 2019; Baradaran Rahimi et al., 2020; Benachour et al., 2020; Kianersi et al., 2020; Grimalt et al., 2022), las raíces (Al-Tamimi et al., 2019; Meddour et al., 2019; Khadem et al., 2020), y las semillas maduras (Haciseferoğullari et al., 2011; Duman y Özcan, 2014; Badr y El-Waseif, 2017; El Gharbaoui, 2017; Tir et al., 2019). Se han realizado varios estudios sobre el uso de semillas de alcaparras como una nueva fuente potencial de aceite, tocoferoles (como vitamina E) y carotenoides (Tlili et al., 2009), como también se ha propuesto en varias investigaciones, el uso de aceites no comestibles de semillas de alcaparra para obtener biodiesel, siendo una fuente de energía alternativa y renovable, cuya utilización produciría un bajo impacto en el medio ambiente, y podría disminuir los efectos del cambio climático y reducir el uso de combustibles fósiles (Liu et al., 2021; Munir et al., 2021).

Los compuestos bioactivos más importantes que se han encontrado en las plantas de alcaparra son los polifenoles, flavonoides (quercetina, rutina y kaempferol, entre otros) (Rodrigo et al., 1992; Rahnavard y Razavi, 2016; Gull et al., 2019; Lo Bosco et al., 2019; Mollica et al., 2019; Kianersi et al., 2020; Redford y Abbott, 2020; Shahrajabian et al., 2021), terpenos, alcaloides (estaquidrina, caparisinas) (Khatib et al., 2016; Jasiem, 2018), compuestos azufrados, principalmente glucosinolatos (Annaz et al., 2022), vitaminas (Tlili et al., 2010), aceites esenciales y ácidos grasos (Brevard et al., 1992; Ramezani-Gask et al., 2008; El-Haci et al., 2010; Argentieri et al., 2012; Bakr y El Bishbishy, 2016; Mottese et al., 2018; Jasiem, 2018; Mollica et al., 2019; Wojdyło et al., 2019; Gull et al., 2019; Lo Bosco et al., 2019; Yahia et al., 2020; Kianersi et al., 2020; Aksay et al., 2021; Kdimy et al., 2022).

Figura 8. Órganos de consumo de C. *spinosa* encurtidos en salmuera y vinagre. a. Botones florales; b. Frutos inmaduros; c. Tallos tiernos.

La composición química de las alcaparras, tallos, frutos y semillas se ve afectada por el genotipo, el tamaño, la fecha de recolección, las condiciones ambientales y los tipos de conservación (Özcan y Akgül, 1998; Özcan, 1999). Estudios sobre compuestos benéficos se han realizado también comparando diferentes cultivares y estados de desarrollo, tanto de los botones florales como de los frutos (Grimalt et al., 2018, 2019), y han puesto de manifiesto el potencial antioxidante que presentan las alcaparras y que cuanto más pequeñas son, más antioxidantes contienen, mientras que con los frutos ocurre lo contrario ya que contienen más compuestos bioactivos cuanto más grandes son.

Tanto las alcaparras como los alcaparrones y los brotes jóvenes son una fuente rica en minerales como el K, Ca, S, Mg, Zn y P (Özcan, 2005). Los procedimientos de conservación disminuyen el contenido de proteínas, fibras, minerales (Mg, K, Mn) y vitaminas (tiamina, riboflavina, ácido ascórbico), mientras que el contenido de cenizas reflejado en los análisis aumenta en los preparados, debido a la adición de NaCl (Sozzi et al., 2012). En la Tabla 1 se muestra un resumen de los diferentes componentes químicos y minerales presentes en *C. spinosa.* Se destaca el contenido de aceites en las semillas, alrededor del 35%, siendo el ácido linoleico el principal ácido graso, cuyo contenido varía entre el 25 y el 63% del total de los lípidos, seguido del ácido oleico (15 a 49%) y el palmítico (11-13,2%) (Matthäus y Özcan, 2005; Sozzi et al., 2012; Liu et al., 2021). En la Tabla 2 se indica la información nutricional de las alcaparras, siendo el agua su principal componente (88,6%), seguido de los hidratos de carbono, fibras y proteínas, estando recomendado el consumo de alcaparras para dietas bajas en calorías.

Tabla 1. Composición química aproximada de las alcaparras, alcaparrones y de las semillas maduras de *C. spinosa* (% m.s.). Elaboración propia, basada en diversos estudios recientes (Haciseferoğullari et al., 2011; Sozzi et al., 2012; Duman y Özcan, 2014; Badr y El-Waseif, 2017; Chedraoui et al., 2017; Gull et al., 2019; Tir et al., 2019).

Constituyente	**Botones florales (alcaparras)**	**Frutos inmaduros (alcaparrones)**	**Semillas maduras**
Agua (%)	76,8 - 80,3	79,6 - 82,7	5,7 - 6,9
Proteínas (%)	4,6 - 6,8	3,3 - 4,6	18 - 22
Lípidos (%)	0,5 - 1,8	3,6	26,6 - 32,5
Hidratos de Carbono (%)	-	3,2	2 -34
Fibras (%)	2,0 - 5,9	7,2	13,7 - 33,6
Cenizas (%)	1,3 - 1,8	1,8	1,9 - 3
Contenido de minerales (mg/kg)			
Ca	1830 - 4190	280	419 - 738,4
Fe	13,7 - 21	5 - 9	63,4 - 68
K	5024 - 5983	3269 - 3830	1570 - 2421,3
Mg	469 - 811	390	2113 - 4812,1
Mn	2.9	7,2	33
Na	59 - 285	121 - 180	74,3 - 6520
P	264 - 1036	1168	4217,8 - 6790
Zn	-	-	32,4 - 55
Vitaminas (mg/100 g)			
Ácido ascórbico	26	23	4
Tiamina	0,7	0,7	-
Riboflavina	0,22	-	-
Antioxidantes naturales (mg/g)			
Flavonoides	0,06 - 0,28	0,155	1,7
Compuestos fenólicos	0,02	0,125	1,2 - 6,2

- Sin datos

Tabla 2. Información nutricional (por 100 g de alcaparras; Valero et al., 2018).

Energía (kcal)	44
Agua (g)	88,6
Hidratos de carbono (g)	4,9
Fibra (g)	3,2
Proteínas (g)	2,4
Lípidos totales (g)	0,9
Colesterol (mg/1000 kcal)	0
Sodio (mg)	2964
Hierro (mg)	1,67
Calcio (mg)	40
Potasio (mg)	40
Tiamina (mg)	0,02
Riboflavina (mg)	0,14
Equivalentes niacina (mg)	0,65
Vitamina B6 (mg)	0,02
Vitamina C (mg)	4,3
Vitamina A: Eq. Retinol (μg)	14
Vitamina E (mg)	0,9

1.5. Importancia económica del cultivo

La comercialización de las alcaparras, se puede considerar un mercado importante a nivel internacional, tanto para países productores, como exportadores e importadores de este producto a granel o procesado, y la calidad depende del tamaño, de manera que las alcaparras más pequeñas y uniformes son las de mayor precio y por lo tanto de mayor valor agregado al producto. La producción a nivel mundial de alcaparras entre los años 1980 y 2000 aumentó progresivamente, con una tasa de crecimiento anual del 6%, posiblemente debido a que la demanda y el consumo crecieron a medida que el estilo de vida fue más saludable, basado en alimentos sanos, sostenibles y orientados a consumidores más selectivos y comercios de *gourmet*, que demandan productos especiales utilizados como delicatesen en la gastronomía (Sozzi et al., 2012). Es por ello que la calidad y la presentación son reconocidas por los consumidores y se pagan precios mayores, por ejemplo en el caso de productos italianos (en algunos casos con denominacción geográfica), españoles, franceses y griegos, donde las alcaparras son generalmente producidas en muy pequeñas cantidades para ser vendidas a los interesados y de esta manera se generan nichos de demanda de productos diferenciados (Costa y Rossi, 2005).

En países como Italia y España el aumento de la producción hasta la década de los 80 fue debido a la recesión de cultivos tradicionales como la viña y el olivo y a la creciente demanda interna de alcaparras, a la mejora de las técnicas culturales y de procedimientos de conservación, a la creación de asociaciones y a la mejora en los niveles socioeconómicos. Posteriormente y hasta la actualidad, la superficie cultivada y el volumen de ventas han disminuido progresivamente al sustituir las alcaparras por otros cultivos de mayor rendimiento económico y con menos necesidad de mano de obra, que en este caso es muy costosa e intensiva en la época de recolección, ya que la cosecha es manual. La misma representa dos tercios de la mano de obra total en el proceso de manejo del

cultivo, ya que se requiere mucho tiempo en la recolección, que es temporal y concentrada en los meses de verano, lo que dificulta su disponibilidad y aumenta los costes del cultivo. El comercio mundial de alcaparras involucra alrededor de 160 países comercializadores y la producción anual promedio se estima en alrededor de 10 000 t (Figura 9). Se considera a los EE.UU. como el consumidor más importante, donde el precio alcanza los 25 USD kg^{-1} de alcaparras listas para el consumo (Chedraoui et al., 2017; Juan, 2017).

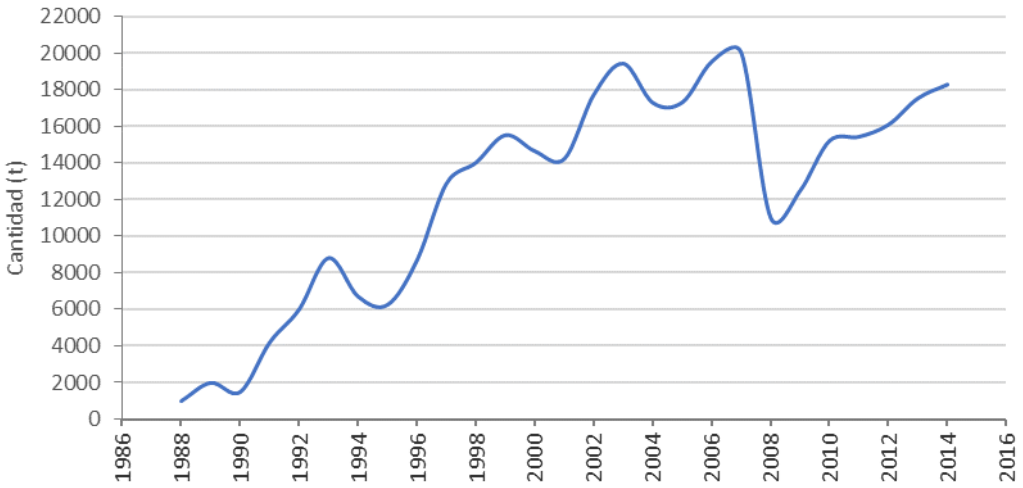

Figura 9. Evolución del comercio mundial de alcaparras (hasta 2008 t exportadas por los 10 principales países exportadores; a partir de 2008 t exportadas por Marruecos). Elaboración propia a partir de datos de UN-DATA, 2023.

Entre los años 1980 y 1990, España lideraba el comercio mundial de alcaparras, produciendo unas 4000 t, en una superficie cultivada de 4000 ha; estos valores representaban el 44% del volumen total comercializado, seguido por Turquía (33%) y en tercer lugar se encontraba Marruecos con un 17% (Costa y Rossi, 2005). Al aumentar los costos de la mano de obra en los principales países productores y exportadores, como España e Italia, comenzaron a abandonar la producción local, trasladando su tecnología y experiencia a Marruecos, país que duplicó y luego triplicó los volúmenes exportados, consolidando su liderazgo a nivel mundial. Estos países son ejemplo de importadores de materia prima, que luego envasan el producto para el consumidor y lo reexportan con valor añadido. En Turquía hacen esta misma logística, aparte de su producción nacional, compran materia prima a países de su entorno, que son buenos productores como Siria, Irán y Uzbequistán, entrando en la rueda de importación-reexportación (Eguilior, 2016).

Debido a estos cambios en el mercado, los principales exportadores de alcaparras en los últimos 20 años han pasado a ser en primer lugar Marruecos con aproximadamente un volumen de exportación de 12 000 t anuales (que implica un comercio de casi 24 millones de dólares) seguido de Siria con 4000 t y Turquía con 3000 t, en cuarto y quinto lugar se encuentran Kyrgyzstan y España con un promedio de 1200 t cada uno (lo que representa una comercialización de aproximadamente 2 millones de dólares) (Tabla 3) (UN-DATA, 2023). Cabe destacar que para la comercialización de sus productos Marruecos depende de España, con quien comercializa gran parte del 75% que vende a la Unión Europea. Turquía también concentra el 79% en la Unión Europea, exportando también a América del Norte y América del Sur. En cuanto a las importaciones, los principales países según el volumen son: la UE-27 (destacándose Italia y España), seguidos de Turquía, Venezuela y Alemania (Tabla 3). Se debe resaltar la situación de España que, aunque ha reducido sus plantaciones, es uno de los principales importadores de alcaparras a granel, en salmuera u otros conservantes, provenientes de Marruecos en mayor medida, seguido de Turquía, Argelia y Túnez que abastecen a España de materia prima procesada. Algunas importantes empresas españolas tienen sus plantas de

procesado en Marruecos y luego realizan el envasado y etiquetado final del producto en España, y lo exportan a otros países y del continente americano como USA, Méjico, Venezuela, entre otros (Costa y Rossi, 2005; Kdimy et al., 2022).

Tabla 3. Comercio internacional en el periodo 2000 – 2018. Valores promedio anuales de la cantidad y de su valor por países exportadores e importadores. Elaboración propia a partir de datos de las estadísticas de comercio de productos básicos de las Naciones Unidas (UN-DATA, 2023).

Exportadores	Millones de USD	Cantidad (t)	Importadores	Millones de USD	Cantidad (t)
Marruecos	23,6	12 138,8	UE	21,7	11 668,2
Siria	13,3	3939,7	Italia	9,0	4804,7
Turquía	5,8	2935,7	España	8,7	4775,3
Kyrgyzstan	0,5	1195,5	Turquía	3,1	2709,0
España	2,3	1142,5	Venezuela	1,6	1093,3
UE-28	1,5	800,3	Alemania	1,8	968,9
Irán	0,5	575,1	Francia	1,2	613,9
USA	0,3	121,0	Brasil	0,7	436,9
Italia	0,4	101,9	Bélgica	0,8	337,2
Alemania	0,1	41,0	Canadá y Marruecos	0,55	316,8

Otros países productores de alcaparras son los de alrededor de la cuenca mediterránea, tales como Francia, Grecia y Chipre en los que se llevan a cabo recolecciones ocasionales de plantas silvestres. En Argentina el cultivo de esta especie se adapta a diferentes tipos de climas y suelos; se realiza bajo riego y no necesita maquinarias especiales; existe una variedad propia, denominada AR1, que es una variedad sin espinas, resistente a enfermedades y a la sequía, la producción se destina principalmente al mercado local (Rico, 2005).

A nivel nacional, en España según las estadísticas anuales del Ministerio de Agricultura, Pesca y Alimentación (MAPA, 2023), el cultivo de alcaparra ocupa el segundo lugar según la superficie cultivada dentro de los cultivos leñosos minoritarios, con una superficie total de 505 ha, después del algarrobo del cual se encuentran aproximadamente 40 000 ha. De la superficie total de alcaparras se aprovechan o encuentra en producción el 17,6% (89 ha), de las cuales se cultivan en secano 57 ha, y en regadío 32 ha, con un rendimiento promedio de 0,7 y 3,4 t ha^{-1} respectivamente (Figura 10).

En España, la superficie cultivada que se encuentra en producción disminuyó significativamente a partir de los años 90, pasando de unas 4000 ha a solo 89 ha en 2021 en todo el territorio español, las que se encuentran fundamentalmente en Región de Murcia (31 ha), Andalucía (54 ha), y una pequeña superficie en Baleares (4 ha). Como se ha indicado anteriormente, en España e Italia las plantaciones de alcaparra han sido sustituidas por otros cultivos que requieren menos mano de obra, y han quedado en su mayoría como un cultivo dentro de la agricultura familiar y de pequeños productores, que coexiste con plantaciones silvestres, por lo que esta situación impide el incremento de la superficie dedicada al cultivo de alcaparras. Además, actualmente estas plantas se encuentran amenazadas por la recolección indiscriminada y poco cuidadosa de las alcaparras silvestres, ya que es una especie muy sensible a una mala manipulación. También es difícil competir con los precios que impone el mercado marroquí debido a su mano de obra más barata (Juan, 2017).

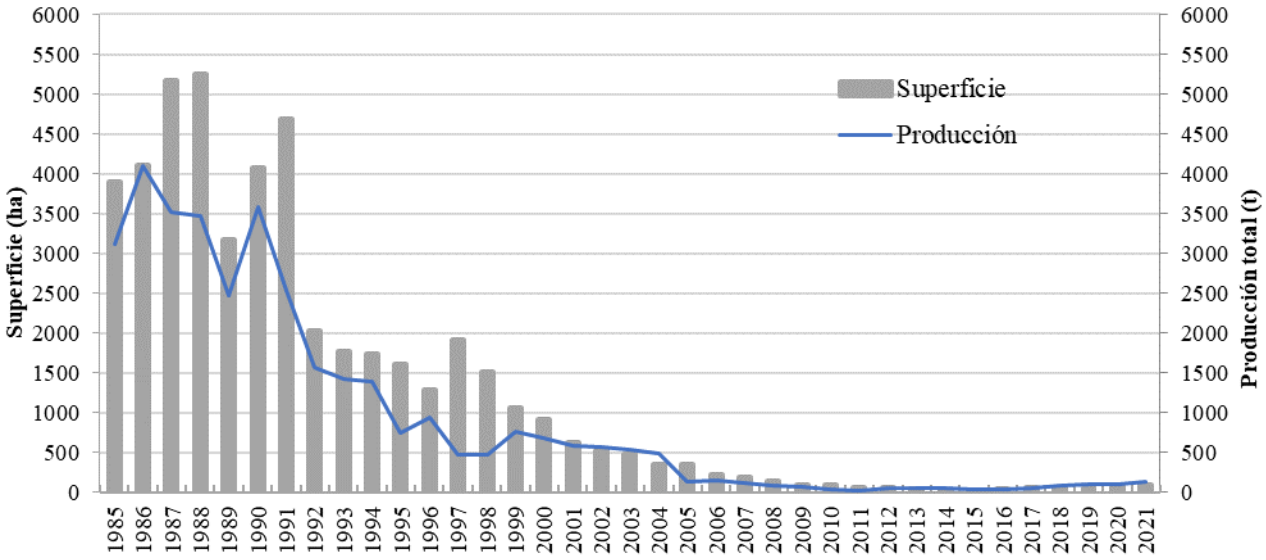

Figura 10. Evolución de la superficie cultivada (ha) y la producción total (t) de alcaparras en España durante el periodo 1985-2021 (MAPA, 2023).

1.6. Propagación

La alcaparra, puede reproducirse de manera sexual por semillas, asexualmente por estaquillas o mediante micropropagación o cultivo *in vitro*. Los métodos tradicionales de multiplicación ya sean sexual o asexual resultan problemáticos y por ello han sido objeto de estudio en varias investigaciones en diferentes países desde hace más de 50 años hasta la actualidad (Reche, 1967; Orphanos, 1983; Sozzi y Chiesa, 1995; Pascual et al., 2009; Juan, 2017; Koufan et al., 2022; Nowruzian y Aalami, 2022). Esta dificultad para lograr una sencilla propagación podría ser uno de los principales problemas que limitan la expansión de su cultivo a mayor escala y una de las causas por las que puede verse disminuida la superficie cultivada (Pascual et al., 2008a, 2008b), ya sea como plantaciones para cultivo comercial de productos alimenticios o farmacéuticos, así como su utilización como planta ornamental. Para poder cambiar esta tendencia, es importante mejorar las técnicas actuales de propagación, principalmente las convencionales con el uso de semillas al presentar muy baja germinación y también mejorar el enraizamiento de estaquillas leñosas o semileñosas. Al ser difícil de propagar por los métodos convencionales, se han desarrollado otras técnicas como la micropropagación de meristemos o microestaquillas que ofrecen una alternativa para obtener a partir de un explante, material vegetal uniforme y libre de patógenos. Al mismo tiempo, es decisiva la inversión en investigación en programas de mejora para la obtención de cultivares de alcaparras valiosos genéticamente, productivos y fáciles de propagar (Sottile et al., 2021).

1.6.1. Propagación vegetativa

El uso de esquejes de tallo evita una alta variabilidad en términos de producción y calidad. El arbusto de alcaparras es una especie leñosa difícil de enraizar y la propagación exitosa requiere una cuidadosa consideración de los biotipos y los parámetros estacionales y ambientales. Son posibles porcentajes de enraizamiento de hasta 55% cuando se utiliza madera de un año, dependiendo de la época de realización y del sustrato utilizado. Cada estaquilla debe tener al menos dos nudos y una longitud entre seis y diez centímetros (Caglar et al., 2005; Mohammad et al., 2012). Las estaquillas pueden obtenerse de segmentos de madera dura, semidura o de madera blanda (herbáceas) (Güleryüz et al., 2009). Trabajos llevados a cabo por Juan (2017) y Pascual et al. (2008a) indicaron que para la multiplicación a través de estaquillas el mejor estado fenológico es de estaquillas lignificadas o parcialmente lignificadas y yema hinchada, alcanzando valores de prendimiento cercanos al 100%, y que las estaquillas semiduras procedentes de la zona central de las ramas pueden enraizar, pero no pueden brotar si sus yemas ya han dado lugar a flores o brotes y no queda ninguna yema, debido a

que los nudos poseen una única yema y ésta puede dar lugar a una única flor o a un brote mixto (hojas y flores). El prendimiento de las estaquillas duras y semiduras mejora al aumentar el diámetro de las mismas, de manera que las estaquillas con diámetros superiores a 6 mm presentan los mejores resultados, mientras que por debajo de 4 mm apenas prenden. Con estaquillas de 2 cm de longitud obtenidas a partir de la sección apical y subapical de las ramas se consiguieron porcentajes de enraizamiento superiores al 70%, pero la brotación de las yemas en ningún caso alcanzó el 50%.

Las estaquillas de madera dura se obtienen principalmente en la época de reposo vegetativo (se pueden trasplantar inmediatamente o conservar a bajas temperaturas hasta el momento del trasplante), las de madera semidura se obtienen desde mediados de primavera hasta principios de otoño, y las de madera suave se obtienen tras la brotación en primavera (abril) (Melgarejo, 2000). En cuanto a las estaquillas de madera suave se recomienda un diámetro ≥ 3mm y una longitud de 6 a 9 cm y su enraizamiento en invernaderos utilizando sistemas de calefacción de fondo y niebla intermitente (Luna y Perez, 1985). Para estaquillas de diámetro menor de 3 mm, procedentes de brotes suaves, Juan (2017) recomienda la aplicación de auxinas, concretamente ácido indolbutírico (IBA) para mejorar el enraizamiento, indicando que este efecto no es evidente en otros tipos de estaquillas. Esquejes tratados con ácido naftalenacético (ANA) durante 7 minutos dieron como resultado un 51,6% de enraizamiento en perlita (Yildrim y Bayram, 2001). Los resultados de la rizogénesis también han variado según la presencia o no de hojas, en estaquillas de madera semidura, de 15 cm de longitud, de modo que estaquillas con hojas tratados con 6000 y 9000 mg L^{-1} de IBA mostraron porcentajes de enraizamiento más altos (67%) que las sin hojas (61%) (Ramezani-Gask et al., 2008). El tratamiento de las estaquillas con bajas temperaturas antes de su plantación mejoró el prendimiento, adelantando la época de brotación, así como el número y la longitud de los brotes por estaquilla; también se indicó un efecto positivo en el enraizamiento con del sellado de la parte superior e inferior de las estaquillas con masilla para injertos (parafina), así como del lesionado de la base, para mejorar el prendimiento, indistintamente de la lesión realizada (Juan, 2017).

La utilización del injerto podría ser interesante para evitar la heterogeneidad en las plantas obtenidas de semillas, o para cambiar de variedad en un cultivo ya establecido. Se puede realizar en estaquillas, en plantas de vivero o en el campo, con tasas de prendimiento cercanas al 70%. No obstante, es el método menos utilizado como técnica de propagación en la alcaparra, al ser demasiado costoso (Luna y Perez, 1985; Melgarejo, 2000; Sottile et al., 2021).

1.6.2. Micropropagación *in vitro*

La micropropagación *in vitro* puede utilizarse en la multiplicación a gran escala para la obtención de materiales genéticamente uniformes y libre de patógenos (Sottile et al., 2021; Koufan et al., 2022). Con estas técnicas se pueden utilizar diferentes tipos de explantes en medios de cultivo como el de Murashige y Skoog (MS; Murashige y Skoog, 1962), líquido o sólido suplementado generalmente con sacarosa y reguladores de crecimiento. El crecimiento y el desarrollo *in vitro* son promovidos por reguladores de crecimiento que son los responsables de la división y el crecimiento celular, entre ellos las auxinas y citoquininas que regulan el desarrollo de diferentes órganos de la planta durante el crecimiento *in vitro* (Gianguzzi et al., 2020; Sottile et al., 2021; Koufan et al., 2022). La germinación *in vitro* de semillas y posterior desarrollo de plántulas es una técnica que ha sido utilizada por varios autores; así por ejemplo un alto porcentaje de germinación de semillas (78,6%) se obtuvo del tratamiento con 500 ppm de ácido giberélico (AG) durante 24 horas (Yildrim y Bayram, 2001); mientras que Awatef et al. (2017), obtuvieron mejores porcentajes de germinación (75%) con el uso de agua como medio de cultivo y el pretratamiento de las semillas con 2000 mg L^{-1} de AG durante 48 horas, que con el uso de MS *in vitro* (37,5%). Chalak et al (2003) lograron mayores porcentajes de germinación (71%), en medio MS desprovisto de hormonas que con en agua estéril (64%), realizando en ambos casos un tratamiento de ruptura de latencia, por escarificación de la cubierta con bisturí. El porcentaje de germinación *in vitro* de semillas de alcaparras se incrementó significativamente por escarificación mecánica y por una hora de pretratamiento a 40 °C, antes del cultivo *in vitro* (Germanà y Chiancone, 2009).

La propagación *in vitro* por microesquejes, también conocida como propagación por segmentos nodales de brotes, se basa en la multiplicación de brotes axilares derivados de microesquejes (segmentos de tallos tiernos con 1 a 3 yemas que miden de 1 cm de longitud) (Al-Safadi y Rana, 2011; Gianguzzi et al., 2020). También se pueden utilizar brotes de ápices provenientes de yemas terminales o laterales (extraídos bajo microscopio) con un tamaño aproximado de 1 mm (Kereša et al., 2019). Luego, las plántulas se transfieren a un invernadero para su aclimatación, permitiendo la producción de plantas fieles al tipo (Koufan et al., 2022). Utilizando esquejes de segmentos de brotes con 2-3 nudos, se obtuvieron brotes múltiples de yemas nodales en medio MS y MS modificado suplementado con 6-bencilaminopurina (BAP) (1,5 mg L^{-1}), IBA (0,05 mg L^{-1}), AG (0,1 mg L^{-1}), a intervalos de 4 semanas (Chalak et al., 2003). Sottile et al (2020), evaluaron un protocolo de micropropagación para mejorar la multiplicación de genotipos seleccionados en Salina (Islas Eolias), Sicilia (Italia); la mayor cantidad de brotes se observó con la concentración máxima de BAP y la concentración más baja de IBA, los brotes jóvenes se recolectaron en la primavera y se le quitaron las hojas, luego se cortaron en segmentos nodales, de 1 cm de largo.

También se han realizado estudios mediante el cultivo de diferentes órganos, basándose en la totipotencia de las células, lo que implica el desarrollo de brotes y raíces, ya sea directamente en el explante o después de la formación de callos en condiciones de cultivo *in vitro* que luego conducen a la formación de plantas completas (Koufan et al., 2022). Elmaghrabi (2017) utilizaron explantes de hojas cultivadas en medio MS conteniendo 1,2 mg L^{-1} de ácido 2,4-diclorofenoxiacético (2,4-D) para inducir callogénesis. Para la formación de brotes y raíces, sugirieron transferir los callos en medio MS suplementado con 2 mg L^{-1} de BAP. Las plántulas luego se aclimataron con éxito a las condiciones del invernadero. Fahmideh et al (2019) sugirieron utilizar 2 mg L^{-1} de kinetina y 2 mg L^{-1} de ANA para la inducción de brotes a partir del cultivo de callos, y 1 mg L^{-1} de ANA para el enraizamiento de brotes. Carra et al (2012) intentaron la regeneración de plantas *in vitro* utilizando estigmas, anteras y óvulos no fecundados de flores sin abrir recolectadas en el campo; consiguieron resultados del 100% de explantes enraizados cuando fueron sometidos durante 10 min en 50 mg L^{-1} de IBA. Las nuevas plantas fueron vigorosas, de buena calidad y presentaron caracteres fenotípicos similares a plantas madre.

Ajustando los protocolos de micropropagación, estos podrían ser métodos útiles y eficientes para producir plántulas deseables para la multiplicación de la alcaparra (Chedraoui et al., 2017). Para mejorar y modernizar el cultivo de alcaparras sería también importante que se realizasen programas de mejora genética, para obtener materiales de alta productividad con gran cantidad de botones florales, de color verde oscuro, redondeados y fáciles de recoger; bayas de alcaparra blandas, de color verde claro, de forma ovalada; buena resistencia a plagas y enfermedades; sin espinas o con un número escaso de espinas; entre otros (Sottile et al., 2021).

1.6.3. Propagación sexual por semillas

La propagación sexual es la forma convencional, más común sencilla y económica de propagar la alcaparra (Chedraoui et al., 2017), aunque con este tipo de reproducción se obtiene una mayor variabilidad genética de la descendencia y por lo tanto en las poblaciones, es de gran interés por la cantidad de semillas que poseen los frutos y porque aunque existe una cierta variabilidad, es limitada, de modo que las plantas obtenidas se consideran suficientemente homogéneas (Ramezani-Gask et al., 2008; Davies et al., 2018; Sottile et al., 2021). Los frutos contienen entre 100 a 400 semillas cada uno, según su tamaño y peso, de manera que cuanto mayor es el tamaño y el peso de los frutos, más semillas maduras contienen. El peso de 1000 semillas es aproximadamente de 6 a 9 g, lo que puede variar con el material vegetal utilizado (Melgarejo, 2000; Pascual et al., 2008b; Chedraoui et al., 2017; Juan, 2017; Marković et al., 2019; Sottile et al., 2021).

Aunque las plantas de alcaparra pueden producir muchos frutos y semillas, el porcentaje de germinación de éstas es muy bajo, en la mayoría de los casos inferior al 5-15%, y también presentan una germinación lenta, que puede deberse a la presencia de latencia, a las estructuras de la semilla y/o

a una baja viabilidad y vigor intrínseco de las semillas, lo cual es un problema para la multiplicación de la especie (Sozzi y Chiesa, 1995; Pascual et al., 2004; Juan, 2017; Nowruzian y Aalami, 2022). En el apartado 1.7 se detallan los tipos de latencia y posibles tratamientos realizados para romperla y mejorar la germinación.

La dispersión de las semillas de alcaparra en hábitats naturales puede llevarse a cabo por diversos animales que se alimentan de la pulpa fresca de los frutos, entre otros lagartijas, hormigas, avispas, pájaros, ratas y conejos, entre otros (Sozzi et al., 2012; Sottile et al., 2021). Yang et al. (2021) observaron que, tras pasar por el tracto digestivo de lagartijas, en las semillas de *C. spinosa* se incrementó la permeabilidad de las cubiertas de las semillas, lo que promovió una rápida absorción de agua y aumentó la tasa de germinación de las semillas.

La longevidad de las semillas (tiempo en el que las semillas pierden el 50% de su viabilidad inicial) es muy variable, dependiendo de las condiciones de almacenamiento y del tipo de semilla (Walters et al., 2005; Bewley et al., 2013a; Solberg et al., 2020). Según Solberg et al. (2020), bajo condiciones ambientales, la vida media de las semillas ortodoxas es corta, de 5 a 10 años, mientras que, en condiciones óptimas controladas de baja humedad y temperatura, la vida media puede ser entre 40 a 60 años, con una gran variación entre especies. La longevidad de un lote de semillas además de estar determinada por la humedad de las semillas y la temperatura de almacenamiento también depende de factores genéticos de las semillas y de las interacciones ambientales durante la maduración y cosecha de las mismas (Walters et al., 2010).

En general las semillas ortodoxas presentan la mayor longevidad con bajos contenidos de agua (4 a 10%) y bajas temperaturas de almacenamiento (0 a -18 ºC), ya que las reacciones que conducen a la pérdida de viabilidad tienden a disminuir cuando estas semillas se secan o enfrían. Con temperaturas entre 0 y 50 ºC cada disminución de 5 ºC duplica la vida en almacenamiento (Walters, 2015; Davies et al., 2018). En el almacenamiento de las semillas se establece un equilibrio entre la humedad ambiental y el contenido de humedad de las semillas, por lo que conviene almacenar las semillas secas en recipientes herméticos, sellados a prueba de agua (Davies et al., 2018). La longevidad de las semillas de alcaparra, almacenadas a 7 ºC está en torno a 4 años. En lotes diferentes, Pascual et al. (2006) obtuvieron una longevidad de 3,85 años, y Juan (2017) de 4,4 años. Sin embargo, ambos estudios recomendaron un período de almacenamiento no mayor a dos años, ya que durante este período no disminuyó la viabilidad y se obtuvieron altos porcentajes de germinación. Por este motivo, tras la recolección de las semillas, el equipo de investigación las seca a un nivel del 8-9% y las conserva en recipientes herméticos de vidrio en frigorífico a una temperatura de 7 ºC.

La recolección de las semillas de alcaparra se realiza en verano y puede extenderse hasta principios de otoño. Las semillas se extraen de los frutos, separándolas de la pulpa por frotamiento del fruto, seguido de lavado con agua corriente con la ayuda de un colador y se seleccionan mediante el método de flotación, que consiste en separar las semillas viables de las no viables, colocando las semillas en un recipiente con agua para que las semillas maduras y más densas se hundan y las semillas inmaduras, livianas y las vanas o vacías floten quedando en la parte superior del recipiente, de donde serán descartadas (Juan, 2017; Davies et al., 2018). Tras la extracción se realiza el secado de las semillas a la sombra y a temperatura ambiente durante unos 15 días.

La siembra se debe realizar a principios de primavera, en macetas, bandejas de germinación (*speedling*) o directamente en semilleros, colocando 1,5-2 g de semillas m^{-1} a 1,5 cm de profundidad aproximadamente, en hileras con una separación de 30 o 40 cm. Como se ha indicado anteriormente, la alcaparra tiene una germinación epigea, propia de plantas que germinan en primavera-verano con temperaturas templadas. Pero las semillas tardan entre 2 a 3 meses en germinar y como se mencionó anteriormente el porcentaje de germinación final es muy bajo y la germinación lenta, lo que dificulta la propagación por semillas, motivo por el cual se han realizado en los últimos años varios trabajos de investigación en diferentes países sobre este tema para poder mejorar la germinación de las semillas de esta especie, como en España los llevados a cabo en el Departamento de Producción Vegetal de la Universitat Politècnica de València (Pascual et al., 2004; Juan, 2017; Juan et al., 2020),

Italia (Barbera y Di Lorenzo, 1984; Sottile et al., 2021), Irán (Makkizadeh Tafti et al., 2012; Vojodi Mehrabani y Valizadeh Kamran, 2020; Nowruzian y Aalami, 2022), Argentina, India (Sozzi y Chiesa, 1995; Sozzi et al., 2012), Marruecos (Saifi et al., 2013; Bourhim et al., 2021), Turquía (Duman y Özcan, 2014; Çelik y Özcan, 2016), entre otros.

En este apartado se destacan, por su interés, varios estudios realizados con el objetivo de mejorar la germinación y tratar de romper la posible latencia con diferentes tipos de escarificación, ya sean mecánicas, químicas, térmicas o biológicas y con la adición de AG (Pascual et al., 2004, 2006; Juan, 2017; Labbafi et al., 2018; Abadi et al., 2021; Nowruzian y Aalami, 2022). También se ha ensayado, el uso de campos magnéticos, y el efecto del envejecimiento acelerado en las semillas (Pascual-Seva et al., 2011; Juan, 2017; Juan et al., 2020).

Los resultados positivos obtenidos en estos estudios podrían indicar una posible latencia física en las semillas de alcaparra impuesta por la impermeabilidad de la cubierta y una latencia fisiológica causada por el embrión. El vigor de la semilla reflejado en el porcentaje y la velocidad de germinación de las semillas también se ve afectado por la madurez de las semillas, el peso y el grado de madurez del fruto (Pascual et al., 2003).

1.7. La germinación y latencia de las semillas

El proceso de germinación se inicia con la imbibición, que es la absorción de agua por la semilla seca, y termina con la emergencia de la radícula a través de las estructuras envolventes que las rodean, de modo que la emergencia de la radícula se toma como un indicador de que se ha producido la germinación. La germinación de las semillas está afectada por factores internos como la latencia, la viabilidad, el vigor, la cantidad de reservas, el espesor de la cubierta de la semilla, la existencia de un equilibrio hormonal entre sustancias que favorecen o estimulan la germinación, como las giberelinas y sustancias inhibidoras, como el ácido abscísico (ABA), o la presencia de embriones poco desarrollados, y por factores externos como la humedad del sustrato, la temperatura, la disponibilidad de oxígeno o la presencia de luz (Azcón-Bieto y Talón, 2013). Por ejemplo, en semillas de las especies modelo *Nicotiana sp* y *Brassica napus*, el ABA inhibe la ruptura del endospermo, pero no la ruptura de la testa. El AG, el etileno y los brasinoesteroides (BR) promueven la ruptura del endospermo y contrarrestan los efectos inhibitorios del ABA (Finch-Savage y Leubner-Metzger, 2006).

La latencia o dormición de las semillas, hace referencia a que existen semillas que no tienen la capacidad de germinar durante un período de tiempo, aunque se encuentren en condiciones ambientales favorables para su germinación (Baskin y Baskin, 2004).

La latencia de las semillas se denomina primaria si la capacidad germinativa de las semillas está bloqueada antes de su dispersión y es producida por factores internos; se denomina secundaria si el bloqueo se produce después de su dispersión, y es debido a condiciones ambientales desfavorables. La latencia primaria se produce durante la maduración de los órganos de dispersión en la planta madre y la hormona responsable en semillas ortodoxas es el ABA. La latencia primaria se puede eliminar mediante tratamientos de frío (estratificación), iluminación, AG, etileno, karrikinas (derivados de la butenolida, presentes en el humo producido en la quema de material vegetal) u óxido nítrico, entre otros. Así, durante la estratificación de semillas de algunas especies, y concretamente en semillas de alcaparrra (Fernández, 2016) se produce un descenso en la forma fisiológicamente activa del ABA (ABA-libre), incrementando la capacidad germinativa. Durante la imbibición de semillas de *Arabidopsis thaliana*, las karrikinas mejoran la germinación mediante la expresión de los genes para la síntesis de AG (Flematti et al., 2007; Nelson et al., 2009). En cambio, la ruptura de la latencia secundaria suele iniciarse cuando las condiciones medioambientales naturales son las adecuadas para germinar. La latencia secundaria es inherente a muchas semillas del banco de semillas del suelo (Azcón-Bieto y Talón, 2013).

La germinación se logra principalmente mediante la regulación del metabolismo y la señalización de las giberelinas y del ABA, dos fitohormonas con funciones antagónicas. Su equilibrio espacio-temporal juega un papel fundamental en la biología de las semillas, favoreciendo la latencia sobre la germinación cuando la relación [ABA]/[Giberelinas] es alta, y lo contrario cuando es baja (Bewley et al., 2013b; Carrera-Castaño et al., 2020). Una vez que se desencadena la germinación, también se produce una interacción de fuerzas mecánicas a medida que el embrión empuja contra los tejidos circundantes. La expresión génica de las giberelinas se induce muy temprano, tras la imbibición, en el ápice de la radícula. La expansión celular a lo largo del eje embrionario ayuda a la ruptura de la testa y las células del endospermo se alargan adaptándose al crecimiento del embrión, hasta que se produce su perforación (Carrera-Castaño et al., 2020).

Existen diferentes tipos o sistemas de clasificación de la latencia de las semillas (Harper, 1957; Nikolaeva, 1977, 2001; Lang et al., 1987; Baskin y Baskin, 1998, 2004, 2014), pero según lo propuesto por Baskin y Baskin (2014), algunos de estos esquemas no explican de manera suficiente los diferentes tipos de dormición; por ejemplo, el sistema de clasificación de Harper (dormancia innata, forzada e inducida) es restringido y no cubre los diferentes tipos de latencia, o la clasificación de Lang en endodormancia, paradormancia y ecodormancia, se basa sólo en la fisiología y no tiene aplicación en las semillas con embriones subdesarrollados o en semillas con cubiertas impermeables. Sin embargo, el sistema de clasificación de Nikolaeva (1977), es más completo y adecuado para diferenciar los tipos de latencia en las semillas. En el mismo se distinguen dos tipos generales de latencia en las semillas: endógena y exógena. En la latencia endógena, alguna característica del embrión puede impedir la germinación y dentro de la latencia endógena distinguió diferentes tipos de latencia, fisiológica (no profunda, intermedia y profunda) y morfofisiológica (simple intermedia, simple profunda, epicotilo simple profunda, doble simple profunda, compleja no profunda, compleja intermedia y compleja profunda). La latencia exógena se produce por alguna sustancia química o por las características de las estructuras (como el endospermo, las cubiertas seminales o alguna parte de los frutos), que rodean al embrión e impiden la germinación. Este esquema de Nikolaeva, (1977) fue modificado por Baskin y Baskin (1998, 2004), siendo el sistema de clasificación de latencia de las semillas más completo y en el cual se contemplan los diferentes tipos de latencia que se describen a continuación:

Latencia fisiológica (LFg): reconocieron tres niveles: profunda, intermedia y no profunda, cuyas características son las siguientes:

- Profunda: el embrión extirpado produce una plántula anormal; el AG no promueve la germinación y las semillas requieren de 3 a 4 meses de estratificación refrigerada para superar la latencia.
- Intermedia: el embrión extirpado produce una plántula normal; el AG promueve la germinación en algunas especies (no en todas); las semillas requieren de 2 a 3 meses de estratificación refrigerada para interrumpir la latencia y el almacenamiento en seco puede acortar el periodo de estratificación.
- No profunda: el embrión extirpado produce una plántula normal. El AG promueve la germinación; dependiendo de la especie, la estratificación refrigerada (0-10 ºC) o cálida (≥15 ºC) supera la latencia; las semillas pueden post-madurar en el almacenamiento en seco y la escarificación puede promover la germinación.

Latencia morfológica (LM): en las semillas con latencia morfológica (LM), el embrión es pequeño (subdesarrollado) y diferenciado, es decir, cotiledón(es) e hipocótilo-radícula pueden ser distinguidos. Los embriones no están fisiológicamente latentes y no requieren un pretratamiento para romper la latencia para germinar; simplemente necesitan tiempo para crecer hasta su tamaño completo y luego germinar (protrusión de la radícula).

Latencia morfofisiológica (LMFg): las semillas con este tipo de latencia tienen un embrión subdesarrollado con un componente fisiológico de latencia. Por lo tanto, para germinar requieren un pretratamiento que rompa la latencia. En este tipo de semillas el crecimiento del embrión/emergencia de la radícula requiere un período de tiempo considerablemente más largo que en semillas con LM.

Latencia física (LF): es causada por una o más capas de células en empalizada que son impermeables al agua y se encuentran en la cubierta de la semilla o el fruto (Baskin et al., 2000).

Latencia combinada: en semillas con (LFg + LF), la cubierta de la semilla (o fruto) es impermeable al agua y, además, el embrión está fisiológicamente inactivo.

Esta clasificación posteriormente fue ampliada y mejorada por Baskin y Baskin (2014), quienes propusieron una clave dicotómica para distinguir las diferentes clases de latencia. Dichos autores denominan latencia del epicotilo o latencia fisiológica del epicotilo, a los casos en que existe un largo retraso entre la emergencia de la radícula y de la plúmula; la clasificación se muestra en la Tabla 4. La clave dicotómica presentada en dicha tabla utiliza información sobre la morfología del embrión: si el embrión tiene órganos diferenciados o solo una masa de células; si el embrión crece o no antes de la germinación; la permeabilidad al agua de las semillas; la capacidad (o no) de las semillas para germinar dentro de aproximadamente 4 semanas, y si la radícula y el brote emergen aproximadamente al mismo tiempo, o la emergencia de los brotes se retrasa varias semanas después de la emergencia de la radícula.

Tabla 4. Clave dicotómica para diferenciar los tipos de latencia: morfológica, morfofisiológica, física, combinada (física + fisiológica) y las dos subclases (epicotilo y regular) de la latencia fisiológica. Deben utilizarse semillas recién maduradas, incubadas a temperaturas adecuadas para su germinación (Baskin y Baskin, 2014).

1. Embrión diferenciado y desarrollado completamente.	2
2. La semilla embebe agua.	3
3. La emergencia de la radícula ocurre antes de 4 semanas (normalmente pocos días).	4
4. Tras la emergencia de la radícula, la plúmula emerge en pocos días.	No Latente
4. Tras la emergencia de la radícula, la plúmula necesita 3-4 (o más) semanas para emerger.	Latencia Fisiológica del Epicotilo
3. La emergencia de la radícula necesita más de 4 semanas.	5
5. Tras la emergencia de la radícula, la plúmula emerge en pocos días.	Latencia Fisiológica Regular
5. Tras la emergencia de la radícula, la plúmula necesita 3-4 (o más) semanas para emerger.	Latencia Fisiológica del Epicotilo
2. La semilla no embebe agua.	6
6. Las semillas escarificadas se embeben completamente de agua (normalmente en 1 día) y germinan antes de 4 semanas.	Latencia Física
6. Las semillas escarificadas se embeben completamente de agua (normalmente en menos de 1 día) pero no germinan antes de 4 semanas.	Latencia Combinada
1. Embrión no desarrollado o no desarrollado completamente.	7
7. Embrión no diferenciado.	8
8. Tras la dispersión de las semillas, el embrión se diferencia y crece en la semilla embebida.	9
9. La semilla germina antes de 4 semanas.	Latencia Morfológica
9. La semilla no germina antes de 4 semanas.	Latencia Morfofisiológica
8. Tras la dispersión de las semillas, el embrión nunca se diferencia en un eje radícula-plúmula.	10
10. La semilla germina antes de 4 semanas.	Latencia Morfológica Especializada
10. La semilla no germina antes de 4 semanas.	Latencia Morfofisiológica Especializada
7. Embrión diferenciado, pero no desarrollado completamente.	11
11. Tras poner las semillas en un sustrato húmedo, el embrión crece, y la semilla germina antes de 4 semanas.	Latencia Morfológica
11. Tras poner las semillas en un sustrato húmedo, el embrión no crece, y la semilla no germina antes de 4 semanas.	Latencia Morfofisiológica

1.8. Estado del arte de la germinación y de la latencia de las semillas de alcaparra

Las semillas de alcaparra presentan en condiciones naturales porcentajes muy bajos de germinación, los cuales se encuentran en la mayoría de los casos entre el 5 y el 15 %, y germinan dentro de los 2–3 meses posteriores a la siembra (Luna y Perez, 1985; Sozzi y Chiesa, 1995; Pascual et al., 2003; Bahrani et al., 2008; Sozzi et al., 2012; Juan, 2017).

Según la clave presentada en la Tabla 4, las semillas de alcaparra podrían presentar latencia física (LF), producida por la cubierta de la semilla impermeable, generalmente causada por una o más capas de células en empalizada en la cubierta seminal (Baskin et al., 2000; Baskin y Baskin, 2004), como se argumenta en varios estudios (Sozzi y Chiesa, 1995; Piotto, 2003). También podría presentarse latencia fisiológica (LFg) ya que las semillas embebidas y con el embrión completamente desarrollado no germinan debido al bajo potencial de crecimiento que presentan (Orozco-Segovia et al., 2007). Otros autores reportan la presencia de latencia combinada (LF + LFg; Orphanos, 1983; Ölmez et al., 2004; Pascual et al., 2004; Soyler y Khawar, 2007; Bahrani et al., 2008; Khaninejad et al., 2012; Marković et al., 2019; Nowruzian y Aalami, 2022) tras realizar diferentes tratamientos para romper la latencia fisiológica, como la utilización de nitrato potásico, de reguladores de crecimiento como el AG, o la estratificación fría.

Entre los tratamientos realizados por este grupo de investigación para eliminar la supuesta latencia física impuesta por la dureza de la cubierta seminal destacan la escarificación ácida (con ácido sulfúrico), mecánica (con papel de lija, realizando cortes en la cubierta de las semillas, siempre teniendo la precaución de no dañar al embrión), térmica (con agua caliente), física (ultrasonidos), o enzimática (con Driselasa) (Pascual et al., 2004). Posteriormente también se ensayó la incubación enzimática con el complejo *Cellulase from Trichoderma reseei* (CTR); con esta escarificación se logró una disminución del contenido en celulosa, hemicelulosa y lignina de las semillas de *Capparis spinosa* L. subsp. *rupestris*, lo que podría haber disminuido la resistencia que la cubierta y el endospermo ofrecen a la protrusión de la radícula, sin dañar el embrión, mejorando la germinación de las semillas (Juan, 2017).

Otros autores (Abadi et al., 2021) utilizaron *priming* biológico con bacterias de la especie *Bacillus megaterium* mejorando la germinación de las semillas de alcaparra. También se ha estudiado el efecto de otros tratamientos en los que se han modificado determinados factores ambientales que afectan a la germinación, por ejemplo, a través de los fitocromos como respuesta a la iluminación con diferentes longitudes de onda y/o la oscuridad (Takaki, 2001; Casal, 2013). En esta tesis doctoral se estudia el efecto de la iluminación, de la irradiación con luz láser, y de la ultrasonicación de las semillas que ha sido poco estudiado en alcaparra.

En la actualidad los ensayos de germinación suelen realizarse en cámaras de cultivo, y generalmente siguiendo las recomendaciones de la Asociación Internacional de Análisis de Semillas (*International Seed Testing Association*; ISTA, 2018), con iluminación y determinados fotoperiodos; concretamente los ensayos de germinación de las semillas de alcaparra realizados por este equipo de investigación se han utilizado tubos fluorescentes de color blanco frío (Philips TL-D 36W/54), que proporcionan una densidad de flujo de fotones fotosintéticos de 81,1 ± 1,7 μmol m^{-2} s^{-1}, con fotoperiodo de 12 h.

La luz es una de las señales ambientales para las plantas (Takano et al., 2009), siendo un factor importante para superar la latencia de algunas semillas (Bewley et al., 2013b). Para responder a las señales ambientales, las plantas han desarrollado varias familias de fotorreceptores, que son pigmentos fotosensibles capaces de ser activados por fotones de longitudes de onda específicas y, a su vez, activar vías de transducción de señales, permitiendo a las plantas responder a estímulos lumínicos (Meisel et al., 2011). Estos fotorreceptores incluyen a fitocromos [fotorreceptores de luz roja (600–700 nm) y roja lejana (700–800 nm)], criptocromos [fotorreceptores de luz azul (400–500 nm) y ultravioleta A (320–400 nm)] y fototropinas [fotorreceptores de luz azul y UV-A] (Takano et al., 2009; Casal, 2013). Las luces LED (por sus siglas en inglés, *Light Emitting Diode*) son una alternativa a las lámparas incandescentes y a los tubos fluorescentes en el cultivo de plantas. Este tipo

de luz, que es principalmente monocromática, tiene ventajas, como el pequeño tamaño de la lámpara, su larga vida útil, la baja temperatura de emisión, la alta eficiencia en la conversión de energía y la posibilidad de seleccionar longitudes de onda específicas (He et al., 2019; Lejeune et al., 2022).

Recientemente se han realizado varios estudios que han demostrado que las luces LED de diferentes longitudes de onda pueden modificar la germinación, el crecimiento y el desarrollo de plántulas en diversas especies (Cho et al., 2008; Tang et al., 2012; Bewley et al., 2013b; Paniagua et al., 2015; Enache y Livadariu, 2016; Aguado y Álvarez, 2021; Paradiso y Proietti, 2022).

La irradiación de las semillas con luz láser (por sus siglas en inglés, *Light Amplification by Stimulated Emission of Radiation*) también es una alternativa para mejorar la germinación de las semillas de diversas especies, debido a su efecto bioestimulante (Hernandez et al., 2010; Krawiec et al., 2018; Swathy et al., 2021). Los efectos de la irradiación láser dependen tanto de parámetros del láser [como la longitud de onda, la duración de la irradiación, la potencia, la dosis y el método (constante/pulso); Hernandez et al., 2010], como de las semillas [características genéticas y propiedades fisiológicas (sanidad y calidad de las semillas)], e incluso de su orientación durante la irradiación (Krawiec et al., 2018). El láser de helio-neón (He-Ne) es el más utilizado en agricultura debido a que su longitud de onda de 632,4 nm corresponde a la luz roja, responsable de la activación de los fitocromos (Krawiec et al., 2018). La mejora de la germinación de las semillas se ha relacionado con la inducción de actividades enzimáticas, un cambio de los parámetros termodinámicos y la aceleración de los metabolismos fisiológico y bioquímico de las semillas, aumentando (en algunos casos) los niveles de AG y disminuyendo los de ABA (Chen et al., 2005b, 2005a; Soliman y Harith, 2009; Perveen et al., 2010; Podleśny et al., 2012; Jamil et al., 2013).

El término ultrasonido describe a las ondas sonoras cuyas frecuencias están por encima del límite audible del oído humano en adultos jóvenes sanos (20 kHz). Se denomina sonicación al acto de aplicar energía sonora para agitar partículas en una muestra; cuando se utilizan frecuencias ultrasónicas, el proceso se conoce como ultrasonicación. Los efectos de la ultrasonicación de líquidos son causados por la cavitación. Cuando se introducen ultrasonidos de alta potencia en un medio líquido, las ondas se transmiten y crean ciclos alternos de alta presión (compresión) y baja presión (rarefacción). Las ondas ultrasónicas de alta intensidad crean pequeñas burbujas en el líquido durante el ciclo de baja presión. Cuando estas burbujas alcanzan un volumen en el que ya no pueden absorber energía, colapsan violentamente durante un ciclo de alta presión; es el fenómeno denominado cavitación (Ranade et al., 2013). En las aplicaciones ultrasónicas se pueden usar sondas o baños. La sonicación en sonda ultrasónica es más efectiva que la del baño ultrasónico; mientras el baño ultrasónico produce una ultrasonicación débil y no uniforme, la sonda ultrasónica proporciona una ultrasonicación intensa y uniforme (Asadi et al., 2019). La zona de sonicación intensa está justo debajo de la sonda (sonotrodo), por lo que la distancia de irradiación ultrasónica se limita a un área cercana la punta del sonotrodo. El colapso de la burbuja provoca un incremento de la temperatura del líquido, mientras que las diferencias de presión pueden dañar mecánicamente las estructuras celulares y tisulares.

Cuando las burbujas colapsan cerca de la cubierta de la semilla, su superficie puede dañarse y generarse poros. El aumento de la porosidad de la cubierta de la semilla podría incrementar la imbibición y, en consecuencia, la germinación de las semillas cuya cubierta representa una barrera física a la entrada de agua (Debeaujon et al., 2000; López-Ribera y Vicient, 2017), es decir, las semillas duras. Dado que acciones como la eliminación o el rajado de las cubiertas aceleran el proceso de germinación en las semillas de alcaparra, es previsible obtener un resultado similar con la hipotética apertura de poros, tal y como se ha obtenido en estudios de germinación de semillas tanto de plantas cultivadas (Yaldagard et al., 2008; López-Ribera y Vicient, 2017; Xia et al., 2020) como de vegetación arvense (Babaei-Ghaghelestany et al., 2020), siendo, por otra parte, una de las metodologías menos estudiada en los ensayos de germinación de las semillas de alcaparra.

1.9. Objetivos de la tesis

1.9.1. Antecedentes

Esta tesis doctoral se enmarca en la línea de investigación de mejora de la propagación de la alcaparra, iniciada a principios de este siglo, en el seno del departamento de Producción Vegetal de la Universitat Politècnica de València. En este marco se han realizado diversos estudios sobre la propagación, sexual y vegetativa, de esta planta. Entre los estudios iniciales merecen destacarse los dedicados a analizar la influencia que tienen en la germinación de las semillas: la madurez del fruto, su peso y la posición del mismo en la planta madre (Pascual et al., 2003); diferentes tratamientos para superar su latencia (Pascual et al., 2004); la posibilidad de utilizar como órgano de propagación los frutos enteros (Pascual et al., 2008b); la longevidad de sus semillas (Pascual et al., 2006); el remojo (Pascual et al., 2009), y el envejecimiento acelerado (Pascual-Seva et al., 2011).

Posteriormente se realizó una tesis doctoral titulada "Estudio para la mejora de las técnicas de propagación de la alcaparra (*Capparis spinosa* L.)" (Juan, 2017), en la que se estudió tanto la propagación sexual como vegetativa mediante las técnicas utilizadas habitualmente en los viveros, dejando aparte las técnicas de micropropagación. En primer lugar, se estudió la histología y la anatomía de los órganos reproductivos y vegetativos y a continuación se abordaron aspectos poco estudiados hasta entonces, como la imbibición, la influencia de la edad de las semillas, de la escarificación enzimática, del tratamiento con campos magnéticos (Juan et al., 2020) y de la estratificación, refrigerada y cálida, en la germinación de sus semillas. En lo referente a la propagación vegetativa, se estudió la influencia del estado fenológico de las plantas madre, de la posición en la rama de donde se obtienen las estaquillas y del grado de lignificación de las estaquillas, en el prendimiento de éstas, así como la utilización de diversas técnicas con el objetivo de mejorar el prendimiento.

1.9.2. Objetivos

Con estos antecedentes se planteó esta tesis doctoral con el objetivo general de estudiar las distintas fases del proceso de germinación de las semillas para determinar el tipo de latencia, cómo superarla, y establecer las pautas para su propagación viable mediante la utilización de sus semillas.

Para lograr este objetivo, se han planteado los siguientes objetivos específicos:

- Estudiar el proceso de la imbibición que se produce durante la germinación de las semillas para comprobar si la cubierta es, o no, impermeable.
- Estudiar la influencia de la cubierta y del endospermo en la germinación de las semillas de alcaparra, así como el efecto de la aplicación de AG en la superación de la latencia.
- En base a los resultados en estos apartados, determinar el tipo de latencia que presentan estas semillas.
- Proponer pautas de recolección y selección para lograr una propagación comercial viable de la alcaparra a través de sus semillas.
- Estudiar la influencia de determinados tratamientos poco o no utilizados hasta la fecha en las semillas de alcaparra, como la iluminación con luces de diferentes longitudes de onda, la irradiación con rayos láser He-Ne y la ultrasonicación.

1.10. Estructura de la tesis

Esta tesis doctoral se presenta en forma de "compendio de artículos". Tras la Introducción general se presentan seis artículos publicados en revistas de nivel alto (primer cuartil) incluidas en *JCR Science Edition*, y dos artículos presentados en el XXX IHC (ISHS) y publicados en *Acta Horticulturae*, ordenados en tres capítulos correspondientes a los objetivos planteados, seguidos de una Discusión general y de las Conclusiones. A continuación, se presentan los capítulos con los títulos de los artículos y sus respectivas referencias:

Capítulo 2: Germinación de las semillas de alcaparra. Influencia de la cubierta, del endospermo y de las giberelinas para la determinación del tipo de latencia

- Water uptake and germination of caper (*Capparis spinosa* L.) seeds. Agronomy 2020, 10, 838; doi:10.3390/agronomy10060838
- Influence of seed-covering layers on caper seed germination. Plants 2023, 12, 439. https://doi.org/10.3390/plants12030439
- Gibberellins improve caper seeds germination: guidelines for their application. Acta Horticulturae 1365. ISHS 2023. DOI 10.17660/ActaHortic.2023.1365.7

Capítulo 3: Criterios de recolección, selección y período de almacenamiento de las semillas de alcaparra

- Collection guidelines to achieve a viable caper commercial propagation. Agronomy 2022, 12, 74. https://doi.org/10.3390/agronomy12010074
- Criteria for the caper seeds collection and selection for commercial use. Acta Horticulturae 1365 ISHS 2023. DOI 10.17660/ActaHortic.2023.1365.8
- The imbibition, viability, and germination of caper seeds (*Capparis spinosa* L.) in the first year of storage. Plants 2022, 11, 202. https://doi.org/10.3390/plants11020202

Capítulo 4: Tratamientos infrautilizados en los ensayos de germinación de las semillas de alcaparra

- Influence of lighting and laser irradiation on the germination of caper seeds. Agriculture 2022, 12, 1612. https://doi.org/10.3390/agriculture12101612
- Effects of high intensity ultrasound stimulation on the germination performance of caper seeds. Plants 2023, 12, 2379. https://doi.org/10.3390/plants12122379

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Capítulo 2. Germinación de las semillas de alcaparra. Influencia de la cubierta, del endospermo y de las giberelinas para la determinación del tipo de latencia

2.1. Water uptake and germination of caper (*Capparis spinosa* L.) seeds

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2.1.1. Abstract

Caper is a perennial deciduous sub-shrub that grows in almost all circum-Mediterranean countries. The specialized literature presents three possible dormancy types that can cause low germination of caper seeds: Physiological dormancy (PD), physical dormancy (PY), and combinational dormancy (PY + PD). We conducted three experiments to analyze the imbibition, viability, and germination of seeds of different ages, provenances, and the level of deterioration of the seed cover. None of the commercialized lots of standard seeds tested exceeded 6% germination, nor 35% viability, while the owned seeds reached 90% in both parameters, indicating that all viable seeds germinated. The seed moisture content along the soaking period followed the first two phases of the typical triphasic model of water uptake in seed germination: The imbibition and lag phases (phase I and II of germination, respectively). Seed hydration began through the hilar region. The fact that all viable owned seeds germinated, together with their moisture content being lower than that of standard seeds, indicated that caper seeds do not have a water-impermeable coat sensu stricto, i.e., they do not show PY; nevertheless, the need to use gibberellic acid to obtain high germination percentages, demonstrated the presence of PD.

Keywords: gibberellic acid; imbibition; physical and physiological seed dormancy; seed coat; viability

2.1.2. Introduction

Caper (*Capparis spinosa* L.) is a perennial deciduous sub-shrub that grows in almost all circum-Mediterranean countries. At maturity, it is woody and reaches up to 1 m high, with up to 3 m long branches and deep roots. We know, given archaeological evidence, that it was already consumed 18,000 years ago in Ancient Egypt [1]. It is mainly cultivated for the floral buds, which are called capers, however, their fruits, and, to a lesser extent, their vegetative shoots are also consumed. Caper floral buds, fruits, and shoots are usually consumed pickled. Furthermore, the flowers have a high ornamental value, thus, caper plants are included in gardening, particularly in xeriscape.

Although it is considered as an underutilized crop, recent studies showed that there is increased interest in the use of the different organs of the plant in medicinal and food technology applications, as they may provide an additional value to their organoleptic properties. Although it is not intended to be an exhaustive list, some of the latest published references for the different parts of the plant are: Flower buds [2,3], leaves [4], fruits [5], aerial parts [6], and roots [7], not forgetting studies on cultivation, particularly irrigation [8].

Global demand for these products has increased; however, the poor field emergence of caper seeds greatly restricts the expansion of this crop. The specialized literature presents the low germinative power of caper seeds [9–18], related to their dormancy [19]. A dormant seed is a viable seed that does not have the capacity to germinate in favorable conditions of humidity, temperature, oxygen concentration, etc. [20], after the dispersion of the mother plant. Physical dormancy (PY) is the dormancy caused by a water-impermeable seed coat [21], and is generally caused by one or more water-impermeable layers of palisade cells in the seed (or fruit) coat [20]. The major reason why imbibed seeds with fully developed embryos fail to germinate is the low growth potential, and these seeds have physiological dormancy (PD) [22]. Both PY [10,23] and PD [19], as well as combinational dormancy (PY + PD) [9,24,25], have been reported for caper seeds.

Scarification (mechanical, chemical, enzymatic, etc.) may promote germination in seeds with non-deep PD, thus it is possible to report PY for a particular taxon, when this is, in fact, not the case [20]. In those studies, the lack of water uptake was not documented by imbibition tests both in scarified and non-scarified seeds [20]. The only way to determine if seed coats are water-permeable is to conduct imbibition studies [22]. The main goal of this study is to determine if caper seeds present PY due to a water-impermeable seed coat that restricts the water uptake and limits their germination.

With this objective, three experiments were done; in the first, we conducted viability and germination tests for four seed lots, with different ages from three different enterprises. In view of the low germination obtained in the four lots, the second experiment analyzed the imbibition, viability, and germination of the youngest seed lot. The third experiment was conducted to compare the water uptake, viability, and germination of these seeds with seeds harvested by the research team and that presumably would present an acceptable germination.

2.1.3. Materials and Methods

2.1.3.1. Experiment I

Viability and germination tests were performed with seeds of standard category [26] from four lots of different ages: 0.5, 2, 4, and 5 years, corresponding to three different enterprises, all of them within the period guaranteed by the producer. Two of these lots were purchased in 0.5 g packets, and, therefore, the number of seeds available was limited. Both viability and germination tests started on March 2019, lasting 48 h and 100 days, respectively.

The seed viability was determined by the tetrazolium test [27]. According to the Tetrazolium Test Manual [28], four categories of tissue indemnity were established: Healthy (H); weak, but viable (WV); weak, not viable (WNV); and dead (D). Samples consisted of 200 seeds (four replications of 50 seeds each). As far as we know, there has been no topographic map published for caper seeds which could be used to establish the seed viability, thus, both the H and H + WV sets were analyzed.

Germination tests followed the International Rules for Seed Testing [29], particularly the Between Paper method (BP) as described in [13,18]. The substrate was moistened with pure water (Wasserlab G.R. Type II analytical grade water system; herein referred to as water), or 500 mg L^{-1} of gibberellic acid solution (Berelex L.; herein referred to as GA). In both cases, 2 g L^{-1} captan (CAPTAN 50 BAYER) was added to prevent fungal problems. The seeds were considered germinated when the radicle protruded from the seed coat [29]. Four replicates were used, consisting of 100 seeds. The assays were only considered satisfactory when the difference between the maximum and minimum germination percentage of the four replicates was lower than the International Seed Testing Association (ISTA) tolerance levels [29]. In the present study, it was not necessary to repeat any test. For each replicate, the germination data were fitted to the logistic function [12,30]:

$$G = A/1 + e\ (\beta - kt) \quad (1)$$

This is defined as a special case of Richards' function [31], where G is the cumulative germination (%); t is the germination time (days); A represents the final germination percentage; and β and k are function parameters. These parameters were used to determine the parameters with biological significance, as the time (in days) required to reach 50% of G ($Gt_{50} = \beta/k$) and the mean relative cumulative germination rate ($k/2$, $days^{-1}$).

2.1.3.2. Experiment II

Seeds of the 0.5-year old lot used in Experiment I were used in this experiment, as this lot was the only one with enough seeds available. The seeds were classified into four groups with the aid of a stereomicroscope (Leica MZ APO): (i) Intact seeds (IS); (ii) scrapped seeds (SS; seeds with the cuticle scrapped); (iii) cracked seeds (CS; the minute cracks passes through the cuticle and reaches the testa); and (iv) broken seeds (BS; the fractured coat exposes the perisperm). Next, the relative importance of each group in the sample was determined following the ISTA rules [29] for seed sampling, i.e., determining the composition of four replicates of 5 g each, and obtaining the average. These seed groups were subjected to imbibition, viability, and germination tests, starting on April 2019, and lasting 8, 2, and 100 days, respectively. In these tests, assays were considered satisfactory

only when the difference between the maximum and the minimum values of the four replications was not higher than the tolerance level indicated in the ISTA rules [29].

For the imbibition test, four treatments were assayed. The imbibition was performed in the same conditions as the germination test (at room temperature, 23–25 ºC, 20–50% relative humidity) indicated in Experiment I, using both water and GA to saturate the substrate. Seeds were also soaked in a 10 cm deep column of water (EC = 0.0 µS cm^{-1}; osmotic potential (Ψo) = −0.13 MPa) or GA (EC = 0.1 µS cm^{-1}; Ψo = −0.65 MPa) at room temperature. Prior to the start of the test, the seed moisture content was determined for each group, by drying samples of 50 seeds in quadruplicate for 24 h at 103 ºC [29] in a forced-air oven (Selecta 297; Selecta, Barcelona, Spain). The seed moisture content [29] was determined daily, following that reported by [17], who reported a linear water uptake during the first 24 h, stabilizing afterwards. Seeds were taken out from the Petri dish or from the solution column, blotted with a paper towel, immediately weighed, and returned to the Petri dish [22] or to the solution column. The seed dry mass was determined as previously mentioned. The seed moisture content was calculated on a fresh mass basis [29]:

$$\text{Seed moisture (\%)} = \frac{(\text{Fresh mass} - \text{Dry mass})}{\text{Fresh mass}} \times 100 \quad (2)$$

This test lasted eight days. This is when the maximum water imbibition was stabilized [17]. As water is colorless, the imbibition process cannot be visually detected, so in parallel to this test, an imbibition test was conducted with a dye commonly used in plant histology, methylene blue. Thirty seeds of each category were immersed for eight days in methylene blue for microscopy with a concentrated aqueous solution (Sigma-Aldrich, Steinheim, Germany). Every day, three seeds of each category were extracted, blotted with a paper towel, cut longitudinally, and observed with a photomicroscope (U500X Digital Microscope; Cooling Tech, Guangdong, China) as reported in [22]. The viability and germination tests were carried out as reported for Experiment I.

2.1.3.3. Experiment III

The imbibition, viability, and germination tests were performed as explained in Experiments I and II, using IS seeds selected from the lot of standard seeds used in Experiment II (herein referred to as standard seeds) and the seeds obtained by the research team (herein referred to as owned seeds). These owned seeds were extracted from ripe fruits that we collected before dehiscence, in September 2018, from four adult plants of *Capparis spinosa* L. subsp. *rupestris*, as it was done in previous studies [12,18]. The seeds were extracted from the fruits, rinsed in tap water, and dried at room temperature (23–25 ◦C, 20–50% relative humidity) for two weeks. Mature, dark-brown seeds were selected, and we rejected both the light brown seeds by flotation in tap water and the small ones (<2 mm). Afterward, they were stored in glass containers at room temperature until use in the study.

2.1.3.4. Statistical Analysis

The results were subjected to analyses of variance (ANOVA; Statgraphics Centurion for Windows, Statistical Graphics Corp. [32]). The percentage data were arcsin transformed before analysis. ANOVA for the viability test studied one factor, the type of seeds (these depended on the experiment). A two-way ANOVA was applied for germination, considering the type of seeds and the solution used to saturate (distilled water or GA solution) the substrate as factors. Finally, a three-way ANOVA was applied for the imbibition test, determining the effect of the type of seeds, saturation solution, and imbibition medium (filter paper or liquid column) at one, four, and eight days of soaking. A probability of ≤0.05% was considered significant. Mean separations were performed when appropriate, using the Fisher's least significance difference (LSD test) at $p \leq 0.05$.

2.1.4. Results and Discussion

2.1.4.1. Experiment I

The viability of the four seed lots was very low, ranging from 5 to 12.5% (without significant differences (at $p \leq 0.05$; Table 1)) when healthy seeds (category H) were considered, and between 10% and 35% (corresponding with the lowest value to 2-year old seeds ($p \leq 0.05$)) in seeds with viable tissues (including weak ones; category H + category WV). These results are in line with those obtained by [33], who reported, for a commercialized seed lot of standard category, a viability of 19.5% considering H, and 29.3% considering H + WV.

Table 1. Effects of the seed lot age (years) on the viability (%) of the seeds, considering only the healthy seeds (H) and considering both the healthy and viable but with weak tissues (H + weak, but viable (WV)). Mean values.

Seed Lot Age (Y)	Viability (H)	Viability (H + WV)
0.5	12.5	32.5 a
2	5.0	10.0 b
4	7.5	22.5 ab
5	12.5	35.0 a
Analysis of variance		
Source (degrees of freedom)	% Sum of squares	
Y (3)	34.2 NS	59.62 *
Residual (12)	65.8	40.38
Standard deviation	6.1	9.4

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. * Indicates significant differences at $p \leq 0.05$. NS indicates not significant differences.

Seeds cannot retain their viability indefinitely and, after a period of time, they deteriorate. In addition to the species characteristics, seed longevity also depends on the individual seed characteristics and on the storage conditions. The researchers in [9] reported that caper seed germination declined when the seeds were kept at room temperature for more than 12 months. However, the authors in [34] found that the seed viability (harvested, cleaned, dried, and stored by themselves) was maintained over 84% after 3-years of storage, with germination percentages (without scarification or GA addition) similar to those of the recently-harvested seeds (around 30%). These authors also determined that the caper seed longevity (considered as the time taken for 50% of the seeds to die [33–35]) stored at 7 ºC was 3.85 years [34]. Therefore, the low viability of the caper seeds used in this study (particularly the 0.5 and 2 year old seeds) may not be due to a natural deterioration during storage, but rather to intrinsic seed characteristics.

Germination data was fitted to the logistic function ($p \leq 0.01$), presenting coefficients of determination (R^2) for the 32 curves (four replicates from eight combinations of variation sources) greater than 0.91. This allows the utilization of the variable A (instead of G), as well as other variables, such as β and k, and then β/k and $k/2$, as done in previous studies of caper seed germination [18,34]. Figure 1 presents the cumulative germination curves fitted to the logistic model obtained for the average values of each seed age and saturation solution combination. In all cases, germination was very low, and the GA increased the A value in relation to those saturated with water. The highest A value ($p \leq 0.05$; Table 2) was obtained for 5-year old seeds using GA (6%), not differing from that for 0.5-year old seeds. The lowest A value was obtained for 2-year old seeds, not differing from that for 4-year old seeds. GA increased A ($p \leq 0.01$). The seed age x saturation solution (water or GA) interaction was significant ($p \leq 0.05$) given that GA significantly ($p \leq 0.05$) increased the value of A in the 5-year old seeds and did not significantly change the other lots.

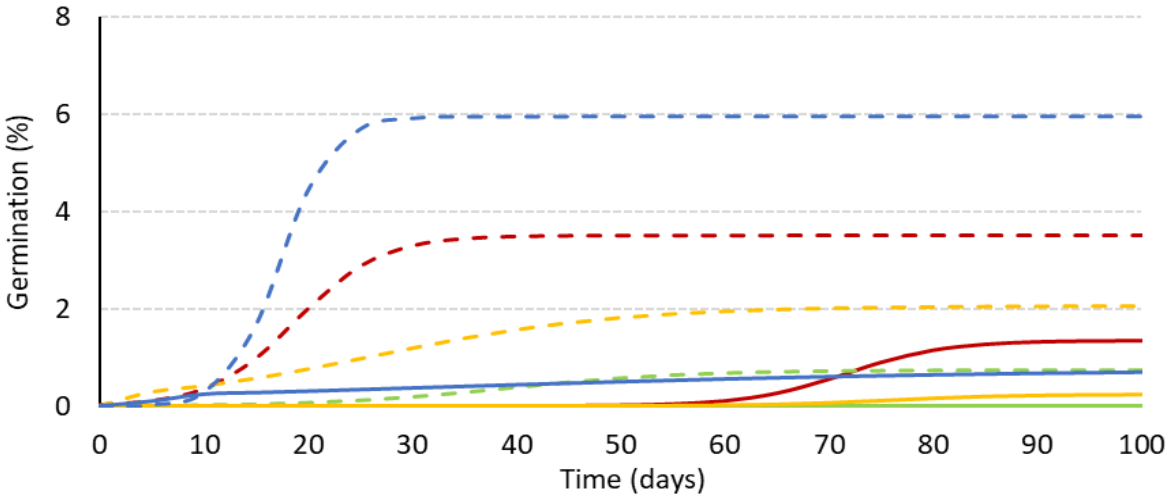

Figure 1. A logistic model fitted to the cumulative germination curves of caper seeds. Mean values for the combination of seed age (0.5-, 2-, 4-, and 5-year old seeds in red, green, yellow, and blue, respectively) and substrate saturation solution (water in continuous lines and gibberellic acid (GA) solution in dashed lines).

No differences (at $p \leq 0.05$) were detected for Gt_{50} or $k/2$. Although Gt_{50} can seem long, it is short in relation to that obtained in similar studies using the owned seeds, but in which much higher germination percentages were obtained (A = 90.5%, Gt_{50} = 50 days; [13]). The $k/2$ values of the seeds with greater germination percentages (0.5- and 5-year old) were comparable to those of the aforementioned study (0.13 d^{-1}). These results were in agreement with those obtained by [33], who reported germination percentages lower than 0.5% in the standard seeds moistened both with water and GA. They were also consistent with the low viability of these seeds, particularly considering only the healthy ones.

The germination percentage values were lower than those of viability, as expected, taking into account that, on one hand, germination loss precedes the viability loss, as stated by [36], and, on the other hand, that if seeds had imbibed the substrate solution, the main reason preventing germination in many H, and probably all WV seeds, was the low embryo growth potential ("push power"), i.e., these seeds presented PD, according to [22]. This theory is also supported by the fact that the GA application improved germination. The GA application to saturate the two filter papers or to soak the seeds, are treatments, alone or in combination with scarification or NO3K utilization, that succeeded in increasing the germination of caper seeds, probably due to breaking their PD [13,16], increasing the embryo vigor, or weakening the seed coat.

Table 2. Effects of the seed lot age (years) and the saturation solution on the germination parameters: Final germination percentage (*A*, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate (*k/2*; d^{-1}). Mean values.

	A	***Gt_{50}***	***k/2***
Seed lot age (Y)			
0.5	2.44 ab	27.41	0.11
2	0.37 c	9.79	0.02
4	1.15 bc	20.71	0.05
5	3.37 a	13.22	0.11
Saturation solution (S)			
Water	0.59 b	17.37	0.05
GA	3.07 a	21.45	0.10
Analysis of variance			
Source (degrees of freedom)	% Sum of squares		
Y (3)	25.1 **	10.8 NS	9.9 NS
S (1)	29.1 **	0.9 NS	4.7 NS
Y × S (3)	13.0 *	9.8 NS	12.3 NS
Residuals (24)	32.8	71.3	73.2
Standard deviation	1.5	21.3	0.1

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** (*) Indicates significant differences at p 0.01 ($p \leq 0.05$). NS indicates not significant differences.

2.1.4.2. Experiment II

The distribution of the different categories established in the 0.5-year old seed lot was: IS, 40.1%; SS, 13.0%; CS, 30.7%; and BS, 16.2%. Initially, the seed moisture content of each category was: IS, 8.8%; SS, 7.7%; CS, 7.1%; and BS, 6.9%. Although it might be expected that an increase in seed coat damage results in a decrease of the initial moisture content of seeds, the difference in moisture contents were not significant ($p \leq 0.05$; ANOVA not shown). The seed moisture content (Figure 2) increased quickly during the first 24 h of soaking, stabilizing in the most deteriorated coat seeds (BS), while the water uptake continued slowly until the fourth day in intact seeds (IS). Although in previous studies our research team reported a linear water uptake during the first 24 h [17], in view of the herein presented seed water content after one day of imbibition, and given that water uptake speed could influence the subsequent germination process, it would be interesting to determine the water uptake rate during the first 24 h, analyzing the seed water content every hour, which is intended to be addressed in a future study. As the researchers in [37] stated, imbibition is a physical process and is a consequence of the matric forces that occur within dry seeds with water permeable seed coats, independently of whetherthey are alive or dead, or dormant or non-dormant.

In Figure 2, the seed moisture content along the soaking period followed the first two phases of the typical triphasic model of water uptake in seed germination. First (phase I of germination; imbibition itself), the water uptake was initially rapid, followed by a slower linear wetting step. At the end of phase I (from the first to the fourth day, depending on the category of the seed), the water uptake stopped as the seed entered the lag phase of germination (phase II) in which there was a limited water uptake, while, according to the literature [37], the metabolism was supposedly active. Radicle protrusion would result from a second period of fresh weight gain driven by additional water uptake(phase III; not shown in Figure 2).

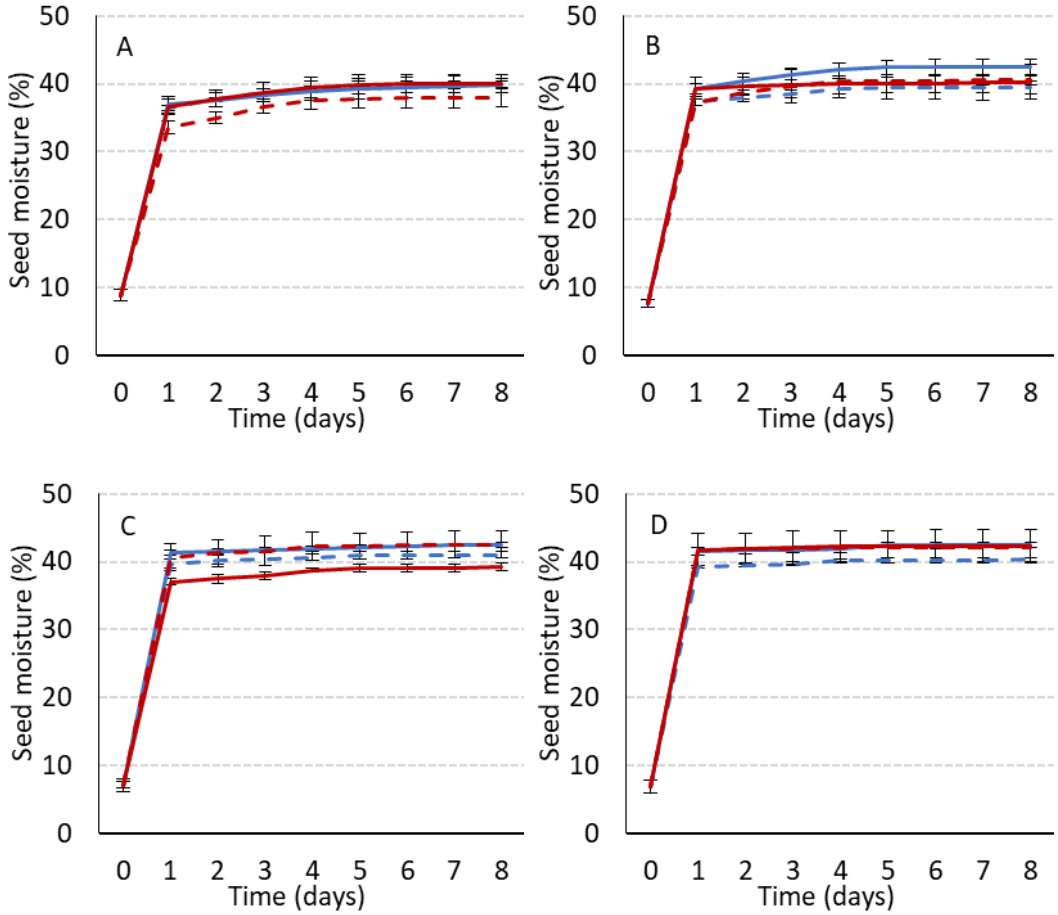

Figure 2. The seed moisture content (%) along the soaking period. Mean values for combinations of four caper seed categories (intact seeds (**A**); scrapped seeds (**B**); cracked seeds (**C**); and broken seeds (**D**)), two imbibition mediums (between paper in blue and in a 10 cm column in red), and imbibition medium (water in continuous lines and GA solution in dashed lines). Vertical bars represent the standard error.

Seeds do not hydrate uniformly during imbibition, since there is a "wetting front" that develops as the outer portions of the seed hydrates while the inner tissues are still dry; seed parts may hydrate differentially depending on their contents [37]. As in other seeds [38], caper seed coats contain the hilum (a scar formed when the funiculus detaches from the seed at maturity) and the micropyle (a scar that corresponds to the micropyle of the ovule), commonly known as the hilar region, and the rest is known as the extrahilar region. The hilar region can allow for water uptake, behaving like a water channel [12,37]. If these sites are large enough to admit dyes, the permeable areas can be visualized.

According to [38], given that the methylene blue dye molecules are larger than those of water, dye entry into the seed coat indicates that this is a penetration point for water. Figure 3b shows that seed hydration begins through the hilar region, while the extrahilar region is impermeable. Caper seeds do not have a water-impermeable coat sensu stricto and they imbibe water without the seed coat being disrupted. When this region is damaged, by scrapping (Figure 3c) or by cracking (Figure 3d,e), hydration begins through the damaged area, contrary to what was reported for *Opuntia tomentosa* seeds [22].

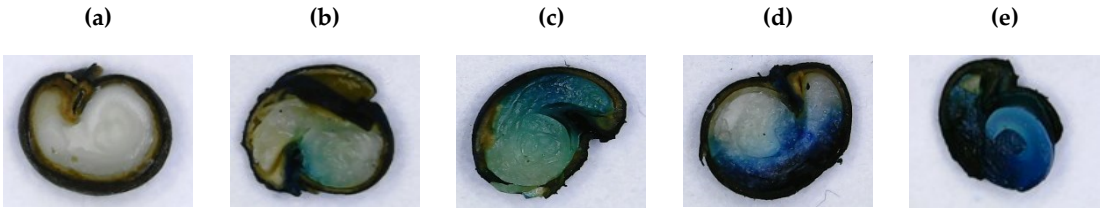

Figure 3. Longitudinal sections of *Capparis spinosa* seeds. (**a**) Intact seeds before soaking. Seeds previously immersed for 24 h in a methylene blue solution: (**b**) Intact seeds; (**c**) scrapped seeds; (**d**) cracked seeds; and (**e**) broken seeds.

Table 3 presents the analysis of variance for the seed moisture content corresponding to the first, fourth, and eighth day of imbibition. After one day of soaking, BS reached a higher seed moisture content than CS ($p \leq 0.05$), which in turn, presented higher values than SS ($p \leq 0.05$), and those were greater than IS ($p \leq 0.05$). These differences decreased with the imbibition period. At the fourth and eighth day, only IS presented a lower seed moisture content ($p \leq 0.05$; on average 2.6% and 2.2%, respectively) than the other categories, which did not differ between them.

Table 3. Effects of the caper seed category, imbibition medium, and solution on the seed water content(%) after 1, 4, and 8 days of soaking. Mean values.

	Seed moisture (%)		
	Day 1	**Day 4**	**Day 8**
Seed category (C)			
Intact seeds	35.36 d	38.44 b	39.09 b
Scraped seeds	38.32 c	40.46 a	40.71 a
Cracked seeds	39.71 b	40.98 a	41.34 a
Broken seeds	41.10 a	41.61 a	41.82 a
Imbibition medium (M)			
Between paper	39.01	40.53	41.00
10 cm column	38.23	40.22	40.47
Saturation solution (S)			
Water	38.97	40.52	40.98
GA	38.28	40.23	40.50
Analysis of variance			
Source (degrees of freedom)	% Sum of squares		
C (3)	49.86 **	24.56 **	18.86 **
M (1)	1.68 NS	0.41 NS	1.24 NS
S (1)	1.31 NS	0.37 NS	1.01 NS
C × M (3)	6.71 *	3.75 NS	3.20 NS
C × S (3)	3.16 NS	3.33 NS	3.63 NS
M × S (1)	2.02 NS	4.97 NS	5.87 *
C × M × S (3)	3.73 NS	3.57 NS	4.75 NS
Residual (48)	31.53	59.03	61.46
Standard deviation	2.0	2.1	2.1

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** (*) Indicates significant differences at $p \leq 0.01$ ($p \leq 0.05$). NS indicates not significant differences.

In seeds with a permeable seed coat, seed hydration is determined by the gradient of the water potential between the medium (in this case the solution column in which the seeds are soaked or the two filter papers) and the seed. Initially, the matric potential of dry seeds is very low, and water is absorbed on the dry coat surface, on the cell walls, and in the polymeric reserve compounds,

principally proteins [22], mainly due to the gradient between the osmotic potential of the seeds and the osmotic and pressure potential of the medium [39]. When the seeds have a "water channel", this process occurs through it. No differences at the $p \leq 0.05$ level in the seed moisture content were detected in relation to the imbibition medium (filter paper or liquid column) or the saturation solution (water or GA), which indicated that neither the saturated filter paper, nor the use of GA, restricted the solution uptake with respect to soaking in water; i.e., the osmotic potential of the seeds was much lower than the osmotic and pressure potential of the medium. In this experiment, the water column above the seeds had a height of approximately 10 cm, water Ψo = −0.13 MPa, and GA solution Ψo = −0.65 MPa.

The viability of the four seed categories was very low, ranging from 0 to 20% ($p \leq 0.01$; Table 4) when healthy seeds (H) were considered, and between 2.5% and 32.5% in seeds with viable tissues (H + WV). Considering H, two groups ($p \leq 0.05$) were clearly detected, one consisting of IS and SS, which had greater viability than those of the other group, consisting of seeds with appreciable damage in their coat (CS and BS). The viability of BS seeds was nil. When considering all the seeds with viable tissues (H + WV categories), the viability of the BS seeds was lower ($p \leq 0.05$) than that of the other seeds.

Table 4. Effects of the seed category on the viability (%) of the seeds considering only the healthy seeds (H) and considering both the healthy and viable but with weak tissues (H +WV). Mean values.

Seed category (C)	Viability (H)	Viability (H + WV)
Intact seeds	20.0 a	32.5 a
Scraped seeds	17.5 a	27.5 a
Cracked seeds	7.5 b	25.0 a
Broken seeds	0 .0 b	2.5 b
Analysis of variance		
Source (degrees of freedom)	% Sum of squares	
C (3)	74.5 **	61.5 **
Residual (12)	25.5	38.5
Standard deviation	5.4	10.5

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** Indicates significant differences at $p \leq 0.01$.

As in Experiment I, *A* was very low or nil; specifically it was null for BS, practically null (on average 0.3‰) for CS and SS, and very low for IS; therefore, it was only possible for IS to adjust the cumulative germination to the logistic model. Coefficients of determination (R^2) for the eight curves (four replicates from two combinations of variation sources) were greater than 0.94. The average parameters of the germination curves for IS were: $A = 4.3$, $Gt_{50} = 23.4$ days, and $k/2 = 0.4$ day^{-1}. The use of GA increased the A value in relation to the use of water, as in previously cited studies, practically doubling its value (3.0% for water, 5.6% for GA). These results agreed with those obtained in Experiment I for the 0.5-year old seeds, which was the original seed lot. As stated above, the results were also consistent with the low viability of these seeds, particularly considering the healthy seeds.

As far as we know, this is the first time that data on caper seed imbibition has been reported. In this experiment, seeds imbibed the corresponding solution, and the embryo likely did not have enough growth potential to germinate [22], as it was mentioned in the previous experiment. It remained to be confirmed whether the moisture content reached in these seeds was enough to allow their germination; thus, the third experiment was conducted.

2.1.4.3. Experiment III

The initial seed moisture content of the two types of seeds was rather similar (8.85% for standard seeds and 8.92% for the owned seeds), and evidently this difference was not significant ($p \leq 0.05$). As in Experiment II, (Figure 4) the two first phases of germination were described by the water uptake, imbibition, and lag phase.

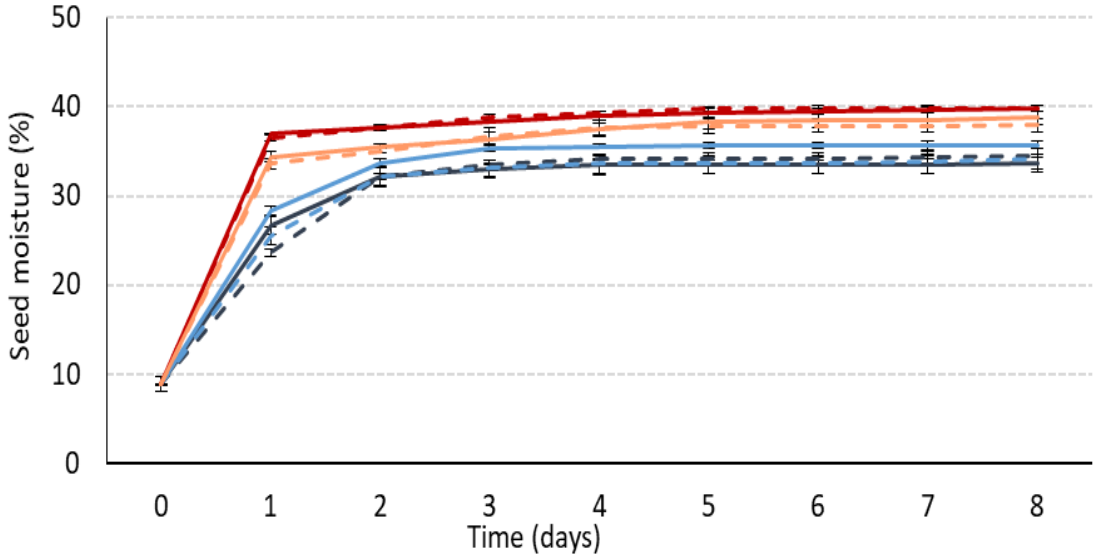

Figure 4. The seed moisture content (%) along the soaking period. Mean values for the combinations of two caper seed lots (owned seeds in blue lines and standard seeds in red lines), two imbibition mediums (between the paper in dark lines and in a 10 cm column in light lines), and two substrate saturation solutions (water in continuous lines and the GA solution in dashed lines). Vertical bars represent the standard error.

The analysis of variance for the seed moisture content corresponding to the first, fourth, and eighth day of imbibition are shown in Table 5. Although dry seeds of the two types showed no differences in moisture content, differences ($p \leq 0.01$) appeared with imbibition, particularly after one day of soaking (9.3%), decreasing with the soaking period down to 4.6% at the eighth day. As in Experiment II, neither the saturated filter paper nor the use of GA restricted the solution uptake with respect to soaking in water. However, the seed lot × saturation method interaction was significant ($p \leq 0.05$) for all the analyzed dates, as the seed moisture reached in the BP method was greater than that in the 10 cm solution column, however, only in standard seeds.

Table 5. Effects of the seed lot and imbibition medium, and solution on the seed water content (%) after 1, 4, and 8 days of soaking. Mean values.

	Seed moisture (%)		
	Day 1	**Day 4**	**Day 8**
Seed lot (L)			
Owned seeds	26.06 b	34.18 b	34.48 b
Standard seeds	35.36 a	38.34 a	39.09 a
Imbibition medium (M)			
Between paper	30.94	36.46	36.92
10 cm column	30.48	36.06	36.65
Saturation solution (S)			
Water	31.60 a	36.35	36.89
GA	29.82 b	36.17	36.15
Analysis of variance			
Source (degrees of freedom)	% Sum of squares		
L (1)	85.37 **	69.80 **	76.81 **
M (1)	0.21 NS	0.63 NS	0.27 NS
S (1)	3.18 **	0.13 NS	0.44 NS
L × M (1)	5.05 **	5.72 *	4.98 **
L × S (1)	1.43 **	0.83 NS	0.00 NS
M × S (1)	0.00 NS	1.76 NS	2.34 NS
L × M × S (1)	0.02 NS	1.68 NS	0.49 NS
Residual (24)	3.73	19.44	14.67
Standard deviation	1.1	1.3	1.2

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. **(*) Indicates significant differences at $p \leq 0.01$ ($p \leq 0.05$). NS indicates not significant differences.

The viability was greater in the owned seeds ($p \leq 0.01$; Table 6) than in the standard seeds, considering both H and H + WV. The high viability of the owned seeds was expected since, on the one hand, they were manually extracted from the fruits, and mature dark-brown seeds were selected and, on the other hand, these seeds were only 0.5-years old. As mentioned above, the authors in [9] reported that germination declined for caper seeds after 12 months of storage at room temperature, and [34] demonstrated viability over 84% after three years of storage. Thus, a high viability was expected for 0.5-year old seeds.

Table 6. Effects of the seed lot on the viability (%) of the seeds, considering only the healthy seeds (H) and considering both the healthy and viable but with weak tissues (H + WV). Mean values.

Seed lot (L)	**Viability (H)**	**Viability (H + WV)**
Owned seeds	87.5 a	90.0 a
Standard seeds	20.0 b	32.5 b
Analysis of variance		
Source (degrees of freedom)	% Sum of squares	
L (1)	95.0 **	87.5 **
Residual (6)	5.0	20.0
Standard deviation	8.9	12.1

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** Indicates significant differences at $p \leq 0.01$.

Figure 5 presents the cumulative germination curves fitted to the logistic model obtained for the average values of each seed type and saturation solution combinations. The coefficients of determination (R^2) for the 16 curves (four replicates from four combinations of variation sources) were greater than 0.92.

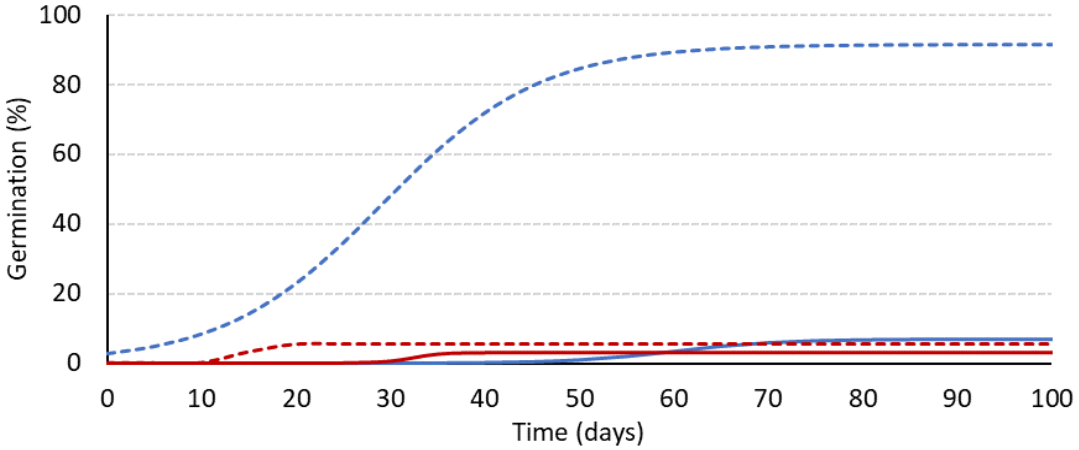

Figure 5. Logistic model fitted to cumulative germination curves of caper seeds. Mean values for the combinations of seed lot (owned seeds in blue lines, standard seeds in red lines) and the substrate saturation solution (water in continuous lines and the GA solution in dashed lines).

Large differences ($p \leq 0.01$; Table 7) were observed for A between the owned seeds and the standard ones, in favor of the former. The use of GA also had a significant effect ($p \leq 0.01$), multiplying by two the germination in the standard seeds and by 16 in the owned seeds; in the latter, the viability and final germination values coincided totally. The seed lot x saturation solution interaction was significant ($p \leq 0.01$) due to the fact that GA increased A more intensively in the owned seeds than in the standard ones, presenting greater viability. In addition to the higher germination, Gt_{50} was longer for the owned seeds ($p \leq 0.01$) than for the standard seeds and, in both cases, was reduced using GA. With the use of GA all viable seeds germinated (including category WV), which implies that GA increased the "push power" of the weak embryo, so that it was able to expand, allowing the radicle protrusion, i.e., to germinate, and/or it also could reduce the mechanical resistance to expansion of the embryo. This should be studied in depth.

Given that the owned seeds reached a germination of 90% (all the viable ones), and taking into account that seed moisture was greater in the standard seeds than in the owned ones, we determined that caper seeds do not have a water-impermeable coat *sensu stricto* and that they imbibe water without the seed coat being disrupted.

Table 7. Effects of the seed lot and the saturation solution on the germination parameters: The final germination percentage (*A*, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate (*k/2*; d^{-1}). Mean values.

	A	Gt_{50}	*k/2*
Seed lot (L)			
Owned seeds	49.05 a	44.66 a	0.08 b
Standard seeds	4.29 b	23.43 b	0.38 a
Saturation solution (S)			
Water	4.89 b	46.21 a	0.21
GA	48.46 a	21.88 b	0.25
Analysis of variance			
Source (degrees of freedom)	% Sum of squares		
L (1)	35.8 **	32.5 **	52.1 *
S (1)	34.0 **	42.7 **	1.0 NS
L × S (1)	30.0 **	3.2 NS	2.4 NS
Residual (12)	0.2	21.6	44.5
Standard deviation	2.0	10.6	0.2

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** (*) Indicates significant differences at $p \leq 0.01$ ($p \leq 0.05$). NS indicates not significant differences.

2.1.5. Conclusions

Imbibition in caper seeds is not a determining factor in their germination, given that the seed moisture content reached in the owned seeds allowed germination percentages up to 90%, and all the viable seeds germinated. Thus, caper seeds do not have a water-impermeable coat sensu stricto and they imbibe water without the seed coat being disrupted, i.e., caper seeds do not show physical dormancy. To obtain a high percentage of germination, the use of GA was required, which indicates the presence of a physiological dormancy. The presence of scrapped and cracked coat seeds in the lot of standard seeds is one of the causes of low viability and germination of standard commercialized caper seeds, although it is not the only cause. The deterioration, visibly apparent or not, produced in the extraction from the fruits, cleaning, drying, and storing processes, decreased the viability and vigor, and consequently the germinative power of these seeds.

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2.2. Influence of seed-covering layers on caper seed germination

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2.2.1. Abstract

Caper is a perennial shrub that is widespread in the Mediterranean Basin. Although the fruits contain many seeds, they germinate slowly and with very low percentages, due to their nondeep physiological dormancy. The influence of the testa and endosperm, as well as the effect of applying gibberellic acid (GA_3) solutions on seed germination to release its dormancy, are reported in this study. The mechanical resistance exerted by the testa and endosperm against radicle protrusion in mature caper seeds was measured. The best germination results were obtained with seeds devoid of testa wetted with water and with intact seeds wetted with a 500 mg L^{-1} GA_3 solution, without statistical differences between them. The GA_3 addition triggers an increase in both the content of endogenous gibberellins (GA) and the GA/abscisic acid ratio, increasing germination. Its germination consists of two temporally separated events: testa cracking and endosperm piercing. Testa cracking begins in the hilum-micropillar area; it involves a signal from the embryo, which GA can replace, possibly by increasing the growth potential of the embryo. After testa cracking, the radicle emerges through a hole in the micropylar endosperm. The puncture force necessary to pierce the micropylar endosperm decreased drastically during the first day of imbibition, remaining practically constant until testa cracking, decreasing afterwards, regardless of the addition or not of gibberellins.

Keywords: abscisic acid; gibberellins; micropylar endosperm; puncture force; seed dormancy

2.2.2. Introduction

The caper (*Capparis spinosa* L.) is a perennial shrub that is naturally widespread throughout the Mediterranean Basin, with a significant ecological and economic role in traditional and specialized systems for commercial production [1]. It is mainly cultivated in arid environments in Morocco, Turkey, the Pantelleria and Salina Islands in Italy and the Balearic Islands in Spain, as well as on the southeastern Iberian Peninsula [2]. It has a great variety of uses, having a high agricultural potential, particularly in food (principally for its flower buds and semimature fruits, pickled in brine) and in the pharmaceutical (to prevent cardiovascular and gastrointestinal diseases) and cosmetic industries [3]. Some researchers have proposed the use of nonedible caper seed oils to obtain biodiesel as an alternative and renewable energy source, which could contribute to lowering the impact on the environment, thus reducing the effects of climate change by reducing the use of fossil fuels [4,5]. Given the resistance of caper to drought and its ability to reduce soil erosion, in the Mediterranean Basin, it is cultivated in intercrops with cereals, vines and both olive and almond trees [6] and is of particular interest on marginal arid lands. Furthermore, drought resistance and its ornamental value make caper an interesting plant to be used in xerogardening and landscaping [3].

This plant has developed mechanisms to survive in Mediterranean conditions [2], including the already mentioned tolerance to drought and seed dormancy. Although the fruits contain many (more than 150 seeds $fruit^{-1}$), small (with an average maximum Feret diameter of 3.3 mm), reniform, and dark brown seeds (Figure 1), they have a very low germination percentage.

The caper seed coat is bitegmic, formed by testa and tegmen, being impermeable and hard to the touch. Its thickness is approximately 0.2 mm, and it has two anatomical structures, the hilum and micropyle (Figure 1). The testa is formed by a layer of 1-2 thick cells with very lignified and thickened walls; the external epidermis has stomata [7]. The tegmen consists of a lignified exotegmen composed of a layer of several brachysclereid cells and a lignified, fibrous endotegmen composed of a few layers of cells. The set of testa and tegmen are referred to simply as testa in this paper. The endosperm comprises several cell layers surrounded by a tightly embedded cuticle. The embryo has a long, robust, coiled hypocotyl, a short radicle, and coiled cotyledons (Figure 1).

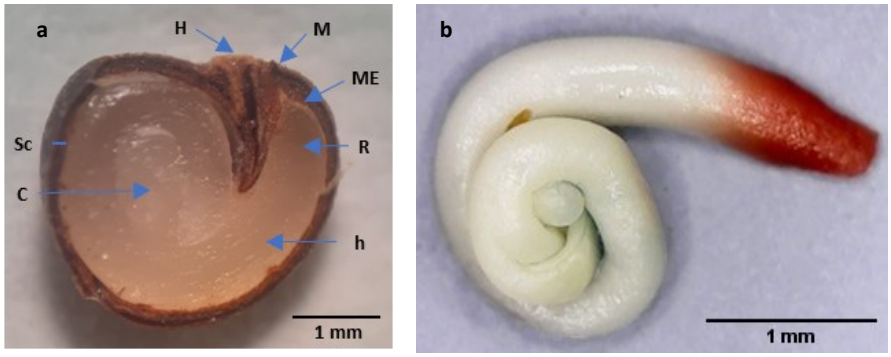

Figure 1. Caper seed. (a) Longitudinal section of a mature caper seed after soaking in water for four days. H, hilum; M, micropyle; ME, micropylar endosperm; R, radicle; h, hypocotyl; Sc, seed coat; and C, cotyledons; (b) Embryo with a stained radicle with tetrazolium.

The germination process starts with water uptake by the dry seed, which is followed by embryo expansion growth and concludes with the rupture of the covering layers and emergence of the radicle, which is considered the completion of germination [8]. Under optimal supply conditions, the typical uptake of water by seeds is triphasic [9]. There is initially a phase of rapid water absorption (Phase I), followed by a second phase (Phase II or lag phase) in which the plant stabilizes and begins the activation of metabolism and the mobilization of nutrients. Seeds that complete germination enter the third phase (Phase III), which begins with radicle protrusion.

Dormancy is the temporary failure of seeds to germinate under favorable conditions. There are different types of dormancy: (i) physical, mechanical, or chemical inhibition caused by the covering layers of the embryo; (ii) the inability to germinate because of an undifferentiated or immature embryo; and (iii) germination repression originating from metabolic restrictions [9]. Based on the Nikolaeva system to classify dormancy types, Baskin and Baskin [10] proposed a hierarchical classification system that includes five classes of seed dormancy: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY) and combinational (PY + PD). According to these authors, water- impermeable layers of palisade cells in the seed that control water movement cause PY. This could be the case for caper seeds, given that their testa structure could affect their dormancy persistence [11,12], which the authors of [2] also hypothesized, pointing to the caper seed coat as one of the causes of the low germination rate. However, our research team [3,12] stated that the lack of imbibition is not a determining factor for the germination of caper seeds, since seed hydration begins through the hilum-micropylar region until reaching the endosperm and the embryo; therefore, these seeds do not present PY due to the impermeability of the seed coat.

To obtain high germination percentages, the use of gibberellins (GA) is required [13,14], which suggests the presence of PD in the embryo by requiring a softening of the seed-covering layers (the testa and endosperm) and/or an increase in force or potential of embryo growth to achieve seed germination. PD is the most abundant form of dormancy found in seeds of the major angiosperm clades [15]. PD has been divided into three levels: deep, intermediate and nondeep [10].

According to the internal morphology of the embryo and endosperm in mature angiosperm seeds, caper seeds belong to the FA-2 seed type [16]. Some species within this type, including *Arabidopsis thaliana*, have nondeep PD [15]. Seeds that have nondeep PD may produce normal seedlings from embryos excised from seeds; depending on the species, GA can release this dormancy, which can also be released by scarification, after-ripening in dry storage and cold or warm stratification. Hormones play an essential role in dormancy status, maintaining or releasing it. Although other dormancy-releasing chemicals (ethylene, cytokinin, auxin, brassinosteroids, nitrogenous compounds and butenolides) exist, GA and abscisic acid (ABA) profoundly influence seed dormancy and germination. This influence is affected during the lag phase of germination by

hormone biosynthetic and catabolic enzymes, whose abundance is controlled primarily at the transcriptional level [9].

The embryo-covering layers in seeds that have PD can confer a mechanical restriction (coat dormancy) that the embryo growth potential must overcome [9]. The success of germination in seeds with PD-type coat dormancy demands that the embryo growth potential increases to overcome the mechanical restriction and/or that the mechanical re-striction associated with the seed-covering layer(s) is reduced. These two mechanisms are known as 'embryo dormancy' and 'coat dormancy', respectively [15], being both components of PD and determined by their sum and interaction, the degree of the 'whole-seed' PD. Embryo dormancy inhibits extension growth; thus, excised embryos do not grow. Non-dormant embryos excised from coat-dormant seeds can therefore extend and grow. 'Coat' is used loosely, referring to any embryo-covering structure, such as the testa and/or endosperm. The testa consists mainly of dead tissues, and it is the seed's interface with the external environment, protecting the embryo against adverse environmental conditions; it imposes a mechanical restriction in coat-imposed seed dormancy that controls germination timing [17]. In many species, such as caper, a living layer of endosperm is interposed between the testa and the embryo; endosperm weakening occurs before germination, and the tissue can produce enzymes for initiating this process [15].

The main aim of the present study was to determine the influence that the testa and the endosperm exert against radicle protrusion, the influence of these covering layers and the influence of an application of a gibberellic acid (GA_3) solution on seed germination. Therefore, two germination tests were carried out using seeds with and without testa (consisting of endosperm and embryo), wetting the substrate with water and a GA_3 solution. The hormone (GA and ABA) content of caper seeds was determined in different phases of the germination process. The mechanical resistance that the testa and endosperm exert against radicle protrusion in dry, mature caper seeds was also determined by measuring the puncture force necessary to perforate the testa and endosperm, which was determined from the beginning of imbibition until radicle protrusion.

2.2.3. Materials and Methods

2.2.3.1. Plant Material

The caper seed lot used in this study was obtained from adult plants grown in Llíria (39º 38′54.2′′ N, 0º 37′3.5′′ W; Valencia, Spain). The fruit collection was carried out in September 2020, and the experiments started on 1 April 2021. Once collected, the fruits were transferred to the laboratories of the Plant Production Department of the Universitat Politècnica de València (Valencia, Spain), where all the experiments were carried out. The seeds were extracted from ripe fruits collected on the day of their dehiscence and from fruits located in the anterior and posterior positions, as reported in previous studies [18]. These seeds were then disinfected with sodium hypochlorite (2 min) and rinsed in tap water. The mature seeds were selected through flotation in tap water, which is a standard method for separating viable from non-viable seeds [35]. Selected seeds were dried in the shade at room temperature (23 - 25 ºC) for two weeks and then kept in closed airtight containers at 7 $\pm$ 0.5 ºC in a domestic refrigerator (Beko, Beko Electronics España, Barcelona, Spain) until the tests started.

2.2.3.2. Viability and Germination Tests

According to the ISTA [27], the seed lot viability was determined by the tetrazolium test, as reported by the research team [3], using four replications of fifty seeds.

The germination test was carried out using intact seeds and seeds devoid of the testa (consisting of endosperm and embryo), which were obtained using tweezers and a scalpel. The germination tests were carried out following the between paper method (BP; [27]) in 9 cm Petri dishes. The substrate was wetted with ultra-pure water (Wasserlab G. R Type II analytical grade

water system, hereinafter referred to as water) or a 500 mg L^{-1} GA_3 solution (Semefil L, Nufarm Spain., Barcelona, Spain). To prevent fungal problems, in all cases, 2 g L^{-1} captan (Captan 50, Bayer) was added to the solution. Petri dishes were placed in a growth chamber (Zimbueze model, Seville, Spain) at 30 ± 1 / 20 ± 1ºC and 85 ± 1% relative humidity for a photoperiod of 12 h (cold white fluorescent tubes, Philips TL-D 36W/54, providing 81.1 µmol m^{-2} s^{-1}) for a maximum of 120 days. The seeds were considered germinated when the radicle protruded through the structures surrounding it [9,35], and germinated seeds were eliminated from the Petri dish; evaluation was carried out every three days. Four replicates of 100 seeds each were used. For each replicate, the germination data were fitted to the logistic function [38,39], defined as a particular case of Richards' function [40]: $G = A/1 + e^{(\beta - kt)}$, where G is the cumulative germination (%), A represents the final germination percentage, t is the germination time (d; days) and β and k are function parameters used to determine the time (in d) required to reach 50% of G ($Gt_{50} = \beta/k$) and the mean relative cumulative germination rate ($k/2$, d^{-1}). Considering the results obtained in the germination test, it was repeated including lower GA_3 concentrations (0, 5, 50 and 500 mg L^{-1}).

2.2.3.3. Hormone Content

Active GA (GA_1, GA_3, GA_4 and GA_7) and ABA contents were determined (in duplicate) in mature dry and imbibed seeds at 10, 20 and 75 (only for seeds wetted with water) days of the germination test, both in seeds with cracked and uncracked testa. For quantification, seeds were frozen in liquid nitrogen and stored at -80 ºC until use at the Plant Hormone Quantification Service (IBMCP, Valencia, Spain). Then, they were ground into powder and suspended in an extraction solvent containing internal standards and mixed by shaking. The extracts were centrifuged, and the supernatant was dried in a vacuum evaporator. The dry residues were dissolved and passed through an Oasis HLB column [41]. The dried eluates were dissolved, and the active GA and ABA were separated using an auto sampler and reversed-phase UHPLC chromatography (2.6 µm Accucore RP-MS column, 100 mm length × 2.1 mm i.d.; Thermo Fisher Scientific, Waltham, MA, USA). GA and ABA were analyzed with a Q-Exactive mass spectrometer (Orbitrap detector; Thermo Fisher Scientific, Waltham, MA, USA) by targeted selected ion monitoring (SIM). The concentrations in the extracts were determined using embedded calibration curves and Xcalibur 4.0 and TraceFinder 4.1 SP1 programs.

2.2.3.4. Seed Water Uptake

To describe water uptake during the germination process, the fresh seed weight was periodically determined [35]. This periodicity varied throughout the experiment; measurements were carried out daily except between Day 20 and 75 in the seeds wetted with water (time with practically constant weight) when the measurements were taken every five days. Four replicates of 10 seeds each were weighed on an analytical balance (Sartorius, Model B 120S, Barcelona, Spain), following the methodology previously reported [12], including both the intact seeds and testa and the endosperm (including the embryo), separately.

2.2.3.5. Mechanical Resistance Determination

The puncture force necessary to pierce both the testa (in the hilum-micropylar area) and the empty endosperm (without the embryo) was measured after the indicated incu- bation period, following a similar methodology to that reported by Müller et al. [20] and Zhang et al. [42], using a digital fruit firmness tester (53205, TR Turoni, Foirli, Italy) with a 0.6 mm diameter metallic blunt-tipped needle. Empty endosperms were obtained by cutting the testa in half with a sharp scalpel and carefully extracting the endosperm, from which the embryo was carefully excised as in the excised-embryo test [35], leaving the ME intact, where the puncture force was measured. Four replicates of 10 seeds and 10 endosperms were measured for each treatment. The puncture force

necessary to pierce the testa was measured daily during the first 20 days of the germination test, both in seeds wetted with GA_3 solution or water and then every five days until 75 days only in seeds wetted with water. These dates, 20 and 75 d, correspond to the Gt_{50} obtained with the use of the GA_3 solution or water to wet the substrate, respectively, since this time is when more seeds crack their testa per day, and mainly because after the cracking of the testa, it is not appropriate to determine its resistance. The puncture force necessary to pierce the ME was determined daily during the first 25 days and then every five days until 75 days in seeds wetted with water and was measured again daily afterwards. After 20 d for seeds wetted with GA_3 and 75 d for those wetted with water, the puncture force necessary to pierce the ME corresponded to the seeds in which testa had naturally cracked and before the radicle protruded.

2.2.3.6. Data Analysis

The differences between the maximum and minimum values of the four replicates obtained in all tests met the tolerance levels [27]. The results were analyzed by multi- way analyses of variance (ANOVA [43]). The percentage data were arcsin transformed before analysis. The normality distribution was analyzed by verifying the residual normal distribution [44] by the Shapiro–Wilk test [43]. A probability $p \leq 0.05$ was considered significant. Mean separations were performed when appropriate using Fisher's least significance difference (LSD test) at $p \leq 0.05$.

2.2.4. Results and Discussion

2.2.4.1. Viability and Germination Tests

The viability of the seeds was 87.5% ± 4.8%, similar to that obtained in other lots of caper seeds by the research team [3,18], who related the high viability to the fact that the fruits were collected at their physiological maturity and to the careful extraction, cleaning, handling and storage of the seeds.

Caper seed germination consists of two temporally separated events: first, testa cracking occurs, starting at the hilum-micropylar area and requiring water absorption and swelling of the embryo and endosperm, and then rupture of the endosperm occurs (Figure 2), as stated by Moghaddasi Mohammad et al. [19]. These two events also occur in many seeds of the Solanaceae (as *Nicotiana tabacum* [15]) or Brassicaceae (as *Lepidium sativum* and *Arabidopsis thaliana* [20]) families. These two events are mechanically distinct processes [15], because, as previously indicated, the testa is dead in these species, and the endosperm is living tissue.

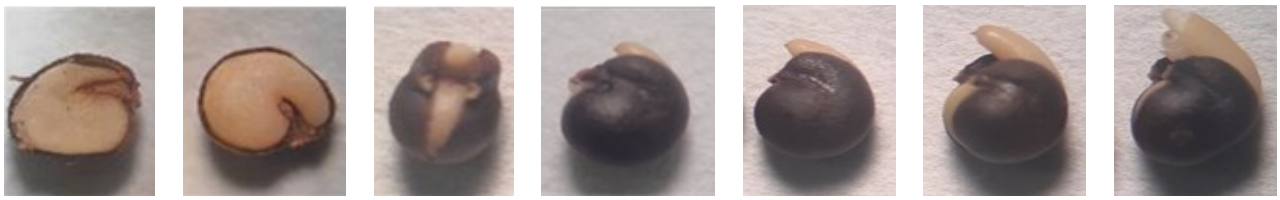

Figure 2. From left to right, photographs correspond to the number of days elapsed since the start of the germination test (Day 0; dry mature seed) through to the opening of the testa (Day 20) and elongation of the hypocotyl and radicle inside the endosperm (from Day 20 to 23), until the rupture of the micropylar endosperm (Day 24). On Days 0 and 4, half of the testa was removed to observe the state of the endosperm.

The coefficients of determination (R^2) obtained for the germination data fitted to the logistic function ($p \leq 0.01$) for each replicate from the four combinations of variation sources were greater than 0.94 (Figure 3), allowing the use of the variable A (instead of G), as well as other variables, such as Gt_{50} and $k/2$, as performed in previous studies of caper seed germination [14,21]. Significant differences were obtained ($p \leq 0.01$; Table 1) for the presence of testa and the solution used, as well as for their interaction ($p \leq 0.01$; Table 1). Given that this interaction explains 44% of the variability of the test, it is presentedin Figure 4.

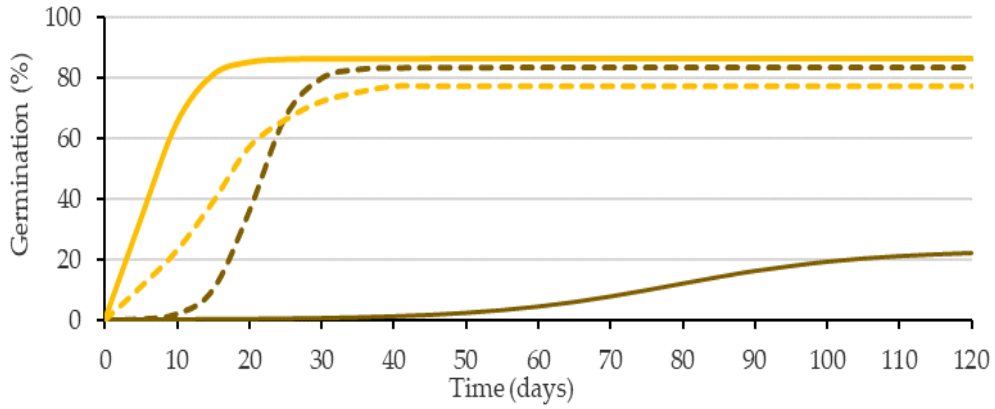

Figure 3. Logistic model adjusted to the germination curves made for seeds with intact testa (brown) and without testa (yellow) with substrate wetted with water (solid line) or GA_3 solution (500 mg L^{-1}; dashed line). Average values of four replicates.

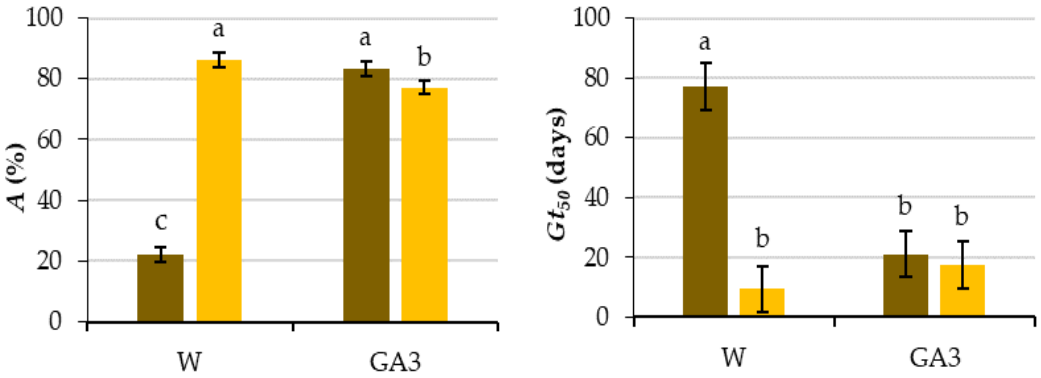

Figure 4. Analysis of the significant interactions of the analysis of variance in Table 1 between the presence of the testa (with testa (brown) and without testa (yellow)) and the solution used to wetthe substrate (water (W) or GA_3) on the final germination (A) and the number of days needed to reach 50% of the final germination (Gt_{50}). Average values of four replicates. Different letters indicate significant differences according to the LSD test. Error bars represent the LSD ($p \leq 0.05$).

Table 1. Effect of the presence of the testa and the solution used to wet the substrate on the final germination (*A*, %), the number of days needed to reach 50% of the final germination (Gt_{50}, d) and the relative average rate of accumulated germination ($k/2$, d^{-1}). Average values of four replicates.

	A	***Gt_{50}***	***k/2***
Testa (T)			
With testa	52.78 b	49.06 a	0.113
Without testa	81.61 a	13.42 b	0.126
Solution (S)			
Water	54.16 b	43.24 a	0.109
GA_3	80.22 a	19.25 b	0.130
Analysis of variance			
Factors (degrees of freedom)	% Sum of squares		
T (1)	30.05 **	40.01 **	0.64 NS
S (1)	24.55 **	18.12 **	1.52 NS
T × S (1)	44.43 **	32.43 **	29.52 NS
Residual (12)	0.97	9.44	68.33
Standard deviation ($^{+}$)	3.00	9.99	0.08

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$, NS: Not significant. ($^{+}$) The standard deviation was calculated as the square root of the residual mean square.

The difference obtained between the germination of seeds with (22%) and without testa (86%, i.e., all viable seeds germinated) when water was used to wet the germination substrate suggests coat-imposed dormancy due to mechanical characteristics, since previous studies [3] have ruled out insufficient seed coat permeability. In the case of coat- imposed dormancy, removal of the tissues surrounding the embryo (e.g., testa and/or endosperm) in some species (in this experiment, the testa) is enough for successful completion of germination [15,22]. However, the testa-removal procedure is very laborious, and it may damage the embryos [23]; therefore, in some studies, such as those carried out in caper seeds [11,24,25], only an incision in the seed coat was made with a scalpel. Our results are in line with those obtained by Sozzi and Chiesa [11], who performed surgical treatment on caper seeds that had failed to respond to dormancy-breaking pretreatments to improve the germination of caper seeds, performing an incision with a scalpel close to the radicle. With this piercing, 100% of viable embryos germinated within 3–4 days, being released from seed dormancy. The results presented herein also agree with the high germination percentages obtained by Chalak et al. [24] for dormancy-breaking treatment for in vitro propagation of caper seeds whose coat was scarified with a scalpel (up to 71%). Elazazi [25] achieved the highest germination percentage (98%) and the fastest germination using mechanical scarification in freshly collected caper seeds. When the seed coat is damaged (cracked or removed), the force it imposes is sufficiently reduced to no longer be a constraint on the low growth potential of the embryo [26].

The seeds devoid of the testa germinated earlier ($p \leq 0.05$), both in water (Gt_{50} = 9.4 d) and in the GA_3 solution (Gt_{50} = 17.5 d), than those intact seeds germinated in water (Gt_{50} = 77.1 d). The Gt_{50} for seeds with testa wetted with GA_3 (Gt_{50} = 21 d) was lower ($p \leq 0.05$) than those wetted with water, not differing ($p \leq 0.05$) from the time required by the seeds without the testa (Figure 4).

Considering the lower germination achieved in seeds devoid of the testa wetted with the GA_3 solution (77%) than that achieved with water (86%), on 1 July 2021, a new germination test was started with seeds belonging to the same lot to analyze the response of the seeds to different

GA_3 concentrations (0; 5; 50 and 500 mg L^{-1}) used for wetting the substrate. Figure 5 presents the logistic model adjusted to the different treatment combinations, and Table 2 presents the statistical analysis of the germination parameters. The difference between the germination values obtained in the two tests does not exceed the tolerance established by International Rules for Seed Testing (ISTA) [27].

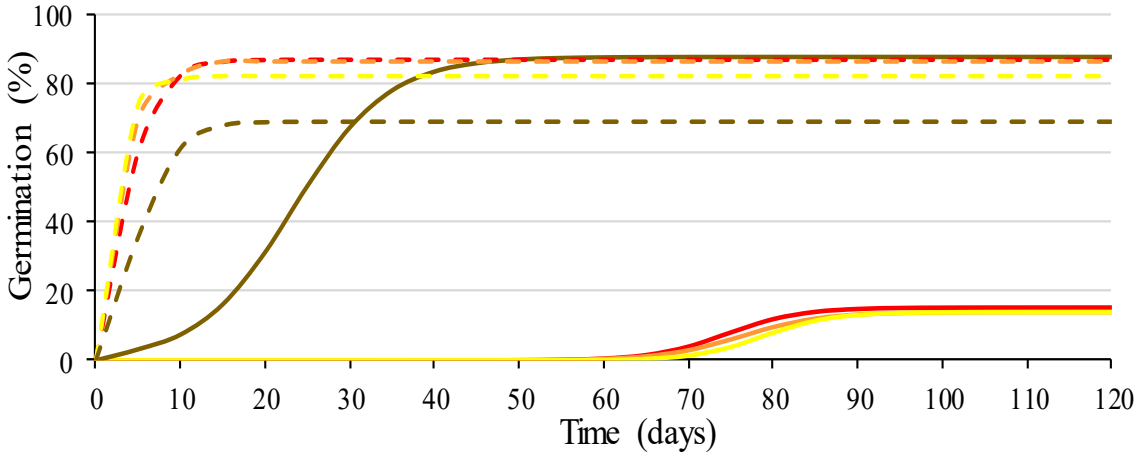

Figure 5. Logistic model adjusted to the germination curves of the seeds with (solid lines) and without testa (dashed lines) with substrate wetted with different GA_3 concentration solutions (0, 5, 50 and 500 mg L^{-1} in red, orange, yellow and brown, respectively). Average values of four replicates.

Table 2. Effect of the presence of the testa and the concentration of the GA_3 solution used to wet the substrate (0, 5, 50 and 500 mg L^{-1}) on the final germination (*A*, %), the number of days needed to reach 50% of the final germination (Gt_{50}, d) and the relative average rate of accumulated germination (*k/2*, d^{-1}). Average values of four replicates.

	A	***Gt_{50}***	***k/2***
Testa (T)			
With testa	32.69 b	63.86 a	0.247 b
Without testa	82.07 a	3.37 b	1.032 a
GA_3 Concentration (C)			
0	50.89 b	39.21 a	0.205 b
5	52.30 b	40.06 a	0.990 ab
50	47.96 b	40.36 a	1.219 a
500	78.38 a	15.00 b	0.145 b
Analysis of variance			
Factors (Degrees of freedom)	% Sum of squares		
T (1)	52.00 **	77.30 **	15.14 *
C (3)	12.74 **	9.78 **	21.91 *
T × C (3)	33.57 **	11.92 **	13.12 NS
Residuals (24)	1.68	1.00	49.83
Standard deviation ($^+$)	5.12	15.3	0.8

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. ** (*): significance level $p \leq 0.01$ (0.05), NS: Not significant. ($^+$) The standard deviation was calculated as the square root of the residual mean square.

The presence–absence of testa explains 52% ($p \leq 0.01$) of the total variability for A. Its interaction with the GA_3 concentration of the wetting solution explains 33.6% ($p \leq 0.01$), and it is presented in Figure 6.

Regarding intact seeds, the only GA_3 concentration that improved ($p \leq 0.05$) germination compared to the control was the 500 mg L^{-1} solution, increasing ($p \leq 0.05$) *A* and decreasing ($p \leq 0.05$) Gt_{50}, with the lower concentrations being inefficient, which agrees with previous studies carried out by our research team [28]. For the seeds devoid of the testa, the GA_3 addition did not improve ($p \leq 0.05$) germination compared to water, even decreasing *A* ($p \leq 0.05$) by 500 mg L^{-1}.

Müller et al. [20] extracted the embryos from the endosperms of the seeds of *Lepidium sativum* and obtained an interaction between the embryo and endosperm during a time frame between 2 and 5 h that could induce endosperm weakening by a signal exercised by the embryo; after this period, this weakening did not require the presence of the embryo. They stated that the embryo signal could be replaced by GA, causing the complete weakening of ME isolated at 2 h. Similarly, caper seeds may not need the imbibition of the GA_3 solution throughout the germination test, as demonstrated in several studies in which statistically greater germination percentages were obtained with caper seeds soaked for 12-24 h in GA_3 solutions than in control seeds and similar to those obtained by wetting the germination substrate throughout the germination test [28]. The seeds devoid of testa did not need the imbibition of GA_3; in addition, keeping these seeds devoid of testa wet throughout the test using a solution of such a high concentration of GA_3 could cause a decrease in the germination rate (Figure 6), which could be considered as an onset of GA_3 toxicity [29–31].

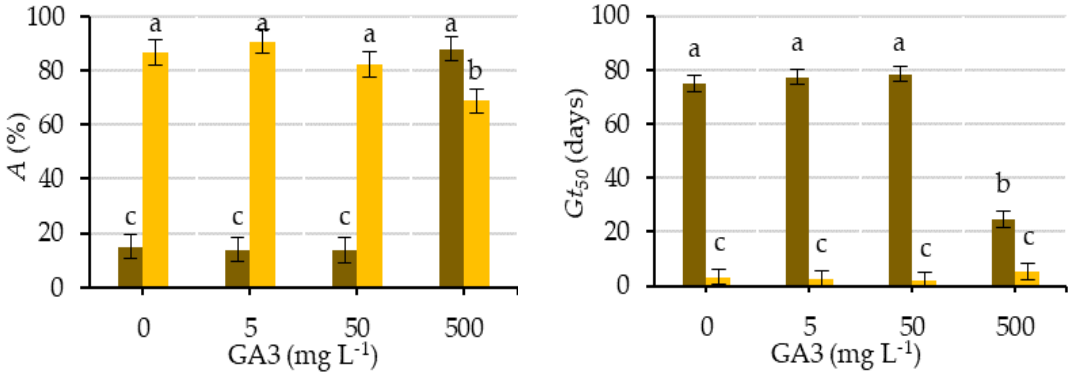

Figure 6. Analysis of the significant interactions of the analysis of variance in Table 2 between the presence of the testa [with testa (brown) and without testa (yellow)] and the concentration of the GA_3 solution used to wet the substrate (0, 5, 50 and 500 mg L^{-1}) on *A* and Gt_{50}. Average values of four replicates. Different letters indicate significant differences according to the LSD test. Error bars represent the LSD ($p \leq 0.05$).

2.2.4.2. Hormone Content

Figure 7 shows the time course of GA_1 (a), GA_3 (b), GA_4 (c), GA_7 (d) and ABA (e) contents and the GA/ABA ratio (f). The content of GA, especially that of GA_1, increased with the germination process, whereas the concentration of ABA decreased. This increase in GA content was faster when the GA_3 solution was used than when water was used to wet the substrate, whereas the decrease in ABA content was similar in both cases.

Exogenous GA_3 applications increased the contents of endogenous bioactive GA, particularly GA_1, agreeing with Yuxi et al. [32], who reported that GA_1 was the most abundant GA after applying GA_3 to peony plants. The increasing content of endogenous GA was probably due to de novo biosynthesis rather than a substantial conversion of GA_3 to GA_1 and GA_4 (Servicio de Cuantificación de Hormonas Vegetales, personal communication). These increasing contents of endogenous GA probably regulate GA biosynthesis and signal transduction through feedback [32].

Bioactive GA (such as GA_1 and GA_4) are biosynthesized from geranylgeranyl diphosphate (GGDP) via a three-step process: (i) synthesis of ent-kaurene from GGDP; (ii) conversion of ent-kaurene to GA_{12}; and (iii) synthesis of 19- and 20-carbon GA from GA_{12}. In the third step, the process can mainly follow two pathways, which vary according to the species [33,34]: GA_4 is synthesized through the non-13-hydroxylation pathway, and GA_1 is synthesized through the early 13-hydroxylation pathway. Although GA_4 exists in most species and is thought to be the main bioactive GA in *Arabidopsis thaliana* and some Cucurbitaceae members [33], GA_1 is the major bioactive form in other species, as occurs in caper. Most likely, the early 13-hydroxylation pathway is the predominant synthesis pathway in caper seed germination.

As Bewley et al. [9], stated, there is coregulation or 'cross-talk' between GA and ABA, i.e., ABA regulates GA metabolism and signal transduction, and GA affect ABA metabolism and signal transduction reciprocally. This coregulation implies that each signal is amplified rapidly (in this case, GA_3 triggers an increase in GA content and response by eliminating ABA production and signal transduction). ABA determines seed dormancy and inhibits seed germination, and GA can release nondeep PD. The GA/ABA balance is as important as the hormone levels for dormancy release [35–37]. Indeed, during the germination process, the GA/ABA balance increased, reaching values greater than 100 in the newly germinated seeds (start of Phase III), both when the GA_3 solution was used (at Day 20) and when water was used (at Day 75). In addition to the hormone content, the transition from the nondeep PD state to germination is accompanied by a decrease in sensitivity to ABA concomitant with an increase in sensitivity to GA [36].

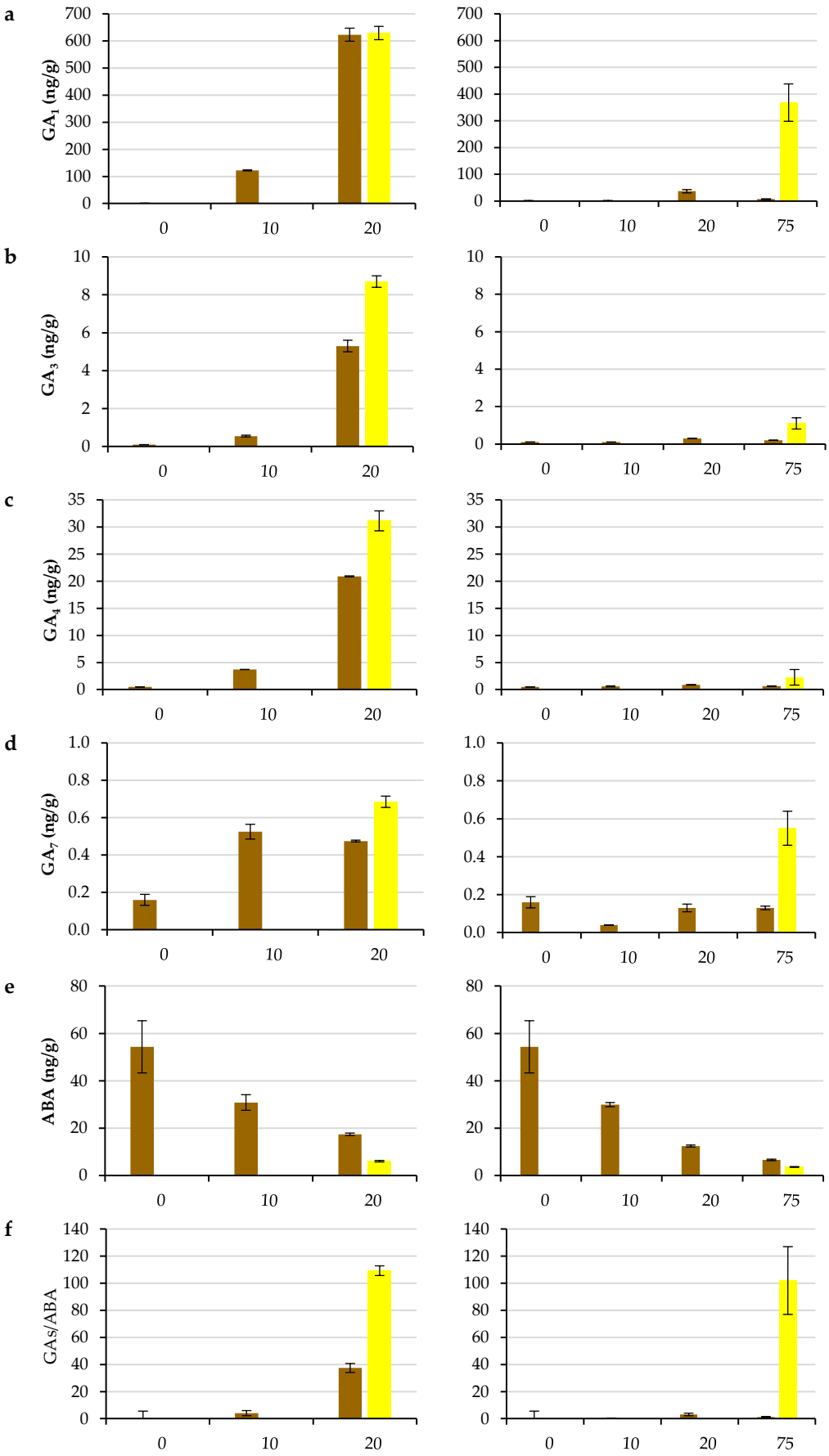

Figure 7. GA_1 (**a**), GA_3 (**b**), GA_4 (**c**), GA_7 (**d**), ABA (**e**) contents, and the GAs/ABA ratio (**f**) at 0, 10, 20 and 75 (if applicable) days after of the start of the germination test, using a GA_3 (500 mg L^{-1}) solution (left) or water (right) to wet the substrate. Day 20 (75) corresponds to the protrusion of the radicle of the seeds wetted with GA_3 (water). Non-germinated seeds in brown, and germinated seeds in yellow. Mean values ± SE of 40 seeds (two replicates of 20 seeds).

2.2.4.3. Seed Water Uptake

Figure 8 shows the typical triphasic increase in seed fresh weight during the germination process: the imbibition phase (Phase I), characterized by rapid water uptake; the lag phase (Phase II) with active metabolic activity and little water uptake; and radicle protrusion (Phase III), in which additional water uptake leads to fresh weight gain, cell enlargement and radicle protrusion [35].

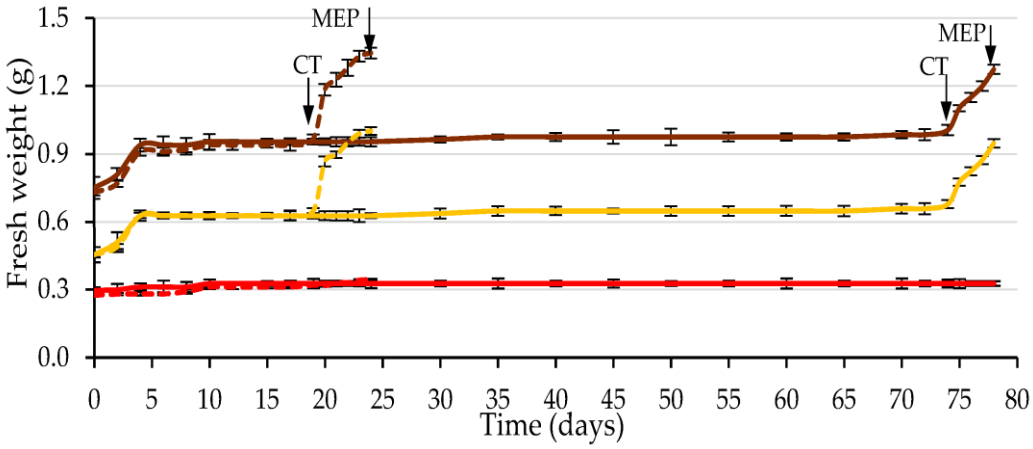

Figure 8. Time course of fresh weight (water uptake) during the germination test of caper seeds, considering seeds with testa (brown), the testa (red) and the endosperm containing the embryo (yellow). Seeds wetted with water are shown as solid lines, and those wetted with the 500 mg L^{-1} GA_3 solution are shown as dashed lines (up to Day 19, solid and dashed lines practically overlap). The arrows indicate the dates on which the cracking of the testa (CT) and the piercing of the micropylar endosperm (MEP) occurred. Average values of four replicates of 10 seeds each. Vertical bars represent the standard error.

This seed water uptake pattern coincides with previous studies on caper seeds [12]. It was observed that the testa fresh weight remained constant. Its water content remained constant, and therefore, the water increase only occurred in the endosperm and embryo, both using water and GA_3. Phase II lasted from Day 5 to Day 19 when the GA_3 solution was used, and from Day 5 to Day 74 when water was used. After testa cracking, the water absorption and swelling of the embryo and endosperm increased, and the radicle emerged through a hole in the ME. This was hypothetically due to tissue dissolution and/or increased growth potential of the emerging radicle.

2.2.4.4. Puncture Force Needed to Crack the Testa

Most biological materials (including diverse seed coats) are anisotropic, i.e., their mechanical properties differ for different load directions [17]. Although the forces to pierce and crack the testa would probably be different, they would probably be correlated; therefore, the puncture force was measured. Until the testa cracked (Day 20 and 75 for seeds wetted with GA_3 and water, respectively), the puncture force required to pierce it remained practically constant, with average values (of those determined as indicated in Section 3) of 2.85 N in seeds wetted with GA_3 and 2.86 N when wetted with water. Testa cracking occurred when the embryo's growth potential overcame the resistance presented by the testa, producing cracking in the hilum-micropylar area and leaving the endosperm visible. The effect of the wetting solution on the puncture force required to pierce the testa at 0, 4 (corresponding to the maximum water uptake) and 20 (cracking of the testa in seeds wetted with GA_3) days was statistically analyzed (Table 3).

Table 3. Effect of the imbibition period (days from the beginning of the germination process) and the solution used to wet the germination substrate in the germination test on the puncture force necessary to pierce the testa at 0, 4 and 20 days after the start of the germination test. Average values of four replicates of 10 seeds each.

	Puncture force (N)
Imbibition period (P)	
0	2.83
4	2.87
20	2.89
Solution (S)	
Water	2.88
GA_3	2.85
Analysis of variance	
Factors (Degrees of freedom)	% Sum of squares
P (2)	4.29 NS
S (1)	1.31 NS
P × S (2)	1.93 NS
Residuals (18)	92.47
Standard deviation ($^+$)	0.13

NS: Not significant at $p \leq 0.05$. ($^+$) The standard deviation was calculated as the square root of the residual mean square.

There is a general trend in which an increase in the seed moisture content causes a de- crease in fracture toughness [17], but in the present study, there was no weakening ($p \leq 0.05$) of the testa with imbibition prior to cracking or differences ($p \leq 0.05$) between the use of water and GA_3. If the germination results (Table 1) and those of the puncture force required to pierce the testa (Table 3) were analyzed together, it was observed that there were no differences ($p \leq 0.05$) between the use of water and the GA_3 solution in terms of puncture force (2.88 and 2.85 N, respectively), but there was higher and faster germination ($p \leq 0.05$) when GA_3 was applied (A = 83%, Gt_{50} = 21 d) than when water was used (A = 22%, Gt_{50} = 77 d; Figure 4). The germination capacity of the seeds is the result of a balance between the physical restrictions imposed by the tissues surrounding the embryo (testa and endosperm) and the ability of the embryo to grow and protrude, allowing the elongation of the radicle [15]. Given these results and according to Davies et al. [35], gibberellins increase the growth potential of the embryo, increasing the swelling of the endosperm so that it leads to overcoming the mechanical resistance imposed by the testa and therefore to its cracking without weakening it. The increase in embryo growth potential required to crack the testa occurs after a long period after initial water imbibition and requires a signal from the embryo, which can be replaced by the addition of GA_3 (that triggers the increase in both the content of endogenous GA and the GA/ABA ratio), which, in turn, shortens the period required to crack the testa.

2.2.4.5. Puncture Force Needed to Pierce the Endosperm

Figure 9 shows the time course of the puncture force required to pierce the ME. The required puncture force decreased drastically during the first day of imbibition (from 0.35 to 0.09 N, on average), using water and the GA_3 solution, remaining practically constant and with identical values in both cases (approximately 0.08 N) until the testa cracked.

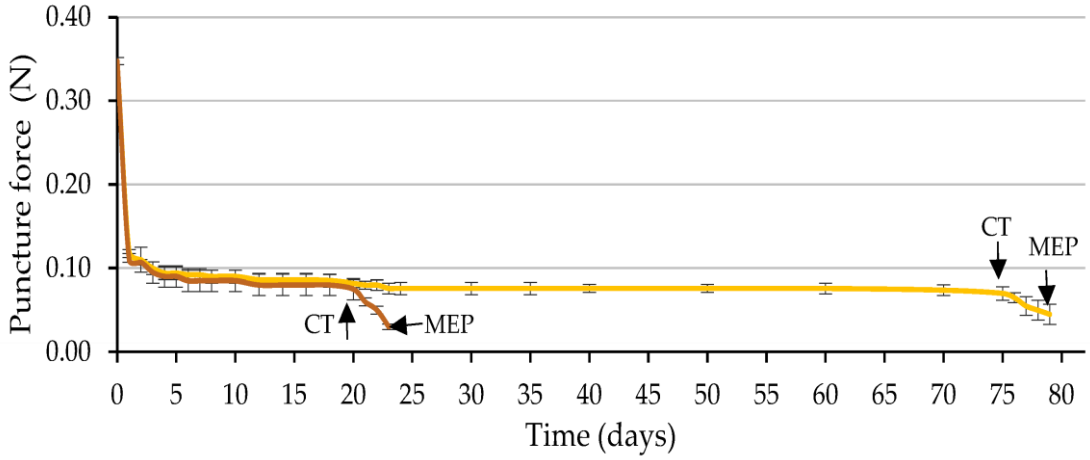

Figure 9. Time courses of endosperm weakening measured by the puncture force required to pierce the micropylar endosperm (ME) during the germination test, using a GA_3 solution (500 mg L^{-1}, in brown) or water (in yellow) to wet the substrate prior to ME piercing (MEP) by the radicle (3 days after testa cracking). The arrows indicate the dates on which the cracking of the testa (CT) and MEP occurred. Up to each CT, the values correspond to seeds with uncracked testa and from CT to seeds with cracked testa (prior to MEP by the radicle). Average values of four replicates of 10 seeds each. Vertical bars represent the standard error.

Of the two factors analyzed, testa cracking and the solution used to wet the substrate, only the first factor significantly affected the puncture force ($p \leq 0.05$; Table 4 and Figure 10). Following testa rupture, the puncture force to pierce the ME decreased to 0.030 N with the use of GA_3 and 0.048 N with the use of water, not differing significantly ($p \leq 0.05$; Table 4 and Figure 10). The radicle emerged through a hole in the ME, which was fundamentally a consequence of the weakening of the tissue and, to a lesser extent, of the increase in the growth potential of the emerging radicle, as stated in the literature [8], for tobacco seeds.

Table 4. Effect of the testa cracking and the solution used to wet the germination substrate in the germination test on the puncture force required to pierce the micropylar endosperm at three days after testa cracking. Mean values of 4 replicates of 10 seeds each.

	Puncture force (N)
Testa (T)	
Uncracked	0.080 a
Cracked	0.039 b
Solution (S)	
Water	0.065
GA_3	0.054
Analysis of variance	
Factors (Degrees of freedom)	% Sum of squares
T (1)	73.23 **
S (1)	5.45 NS
T × S (1)	1.68 NS
Residual (12)	19.64
Standard deviation (+)	0.01

Different letters within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$, NS: Not significant. (+) The standard deviation was calculated as the square root of the residual mean square.

As in *Lepidium sativum* [20] seeds, during Phase II of germination, the embryo could exercise a signal to induce endosperm weakening; therefore, after testa cracking, GA application is not necessary to induce ME weakening. According to the general trend [17], ME weakening involves cell-wall loosening, cell separation, and programmed cell death to provide a decrease in localized ME tissue resistance, autolysis and finally, the formation of the ME hole required for radicle emergence. Unravelling how various aspects of seed germination are controlled, such as water uptake, dormancy and its release, seed viability and seedling vigor, as noted in a general way [17], will remain a major topic for research. Future studies will focus on unravelling the molecular mechanisms underlying caper seed germination.

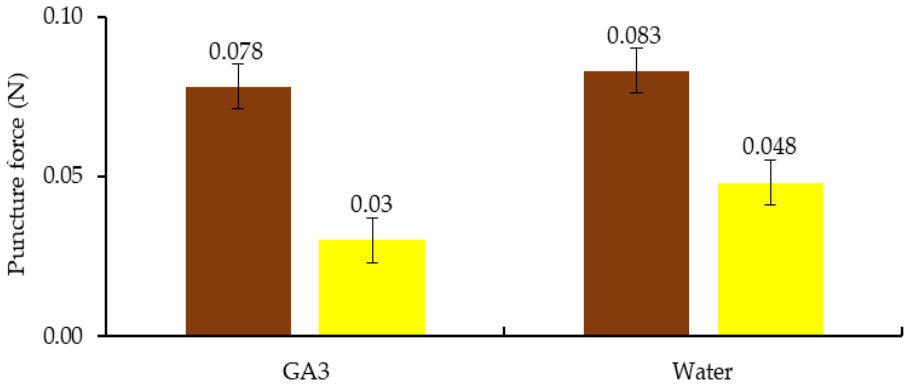

Figure 10. Analysis of the interaction of the analysis of variance in Table 4 between the cracking of the testa (uncracked testa (brown) and cracked testa (yellow)) and the solution used to wet the substrate on the puncture force required to pierce the micropylar endosperm at three days after the cracking of the testa. Average values of four replicates of 10 seeds each. Error bars represent the LSD ($p \leq 0.05$).

2.2.5. Conclusions

Caper seeds have a nondeep physiological dormancy, which can be released by adding GA_3 to the germination substrate. This addition triggers an increase in the content of endogenous GA and, to a greater extent, the GA/ABA ratio, advancing and increasing the germination percentage. Its germination consists of two temporally separated events: testa cracking and endosperm piercing. The cracking of the testa begins in the hilum-micropylar area, requires water absorption, and involves a signal from the embryo, which the addition of gibberellins can replace, increasing and advancing the percentage of seeds in which cracking occurs. This cracking is not due to a decrease in the mechanical constraint of the testa but rather to an increase in the growth potential of the embryo to overcome it. With 500 mg L^{-1} GA_3, germination percentages similar to those obtained by eliminating the testa were obtained. After testa cracking, the radicle emerges through a hole in the micropylar endosperm. The puncture force necessary to pierce the micropylar endosperm decreased drastically during the first day of imbibition, remaining practically constant until testa cracking, decreasing afterwards, regardless of the addition or not of gibberellins.

2.2.6. References

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2.3. Gibberellins improve caper seeds germination: guidelines for their application

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2.3.1. Abstract

The caper is an important plant in the Mediterranean area, tolerant to drought, used for its flower buds and fruits, and recently for its bioactive principles. The seeds present a low germination percentage (G), which has been related to physiological dormancy because dormant seeds germinate in the presence of gibberellic acid (GA_3) solutions. The objective of this study was to provide practical guidelines for gibberellin use to improve G. Three experiments were carried out. In the first experiments, two GA_3 application methods (1 – wetting the germination substrate of the between-paper method with a GA_3 solution, and 2 – soaking the seeds in the GA_3) using five GA_3 concentrations (0, 100, 500, 1000 and 2000 mg L^{-1}) were compared. The best result was obtained by wetting the substrate with the 500 mg L^{-1} GA_3 solution (76.3%). In the second experiment, different methods for wetting the substrate with a 500 mg L^{-1} GA_3 were compared. The method of continuous wetting, used in the first experiment, was compared with 1 – changing the germination substrate fortnightly, and 2 – with wetting the substrate with the GA_3 solution for 30 days and with water afterwards. The best result was obtained by changing the substrate (86%) or wetting it with GA3 only for 30 days (83%). In the last experiment, lower GA_3 and technical gibberellin (A_4 and A_3) concentrations were compared (0, 0.1, 1, 5, 10 and 50 mg L^{-1}). A4 led to higher G than control, even at the lowest concentration, and the best results were achieved from 5 mg L^{-1} without significant differences between concentrations (up to 92.3%). Overall to improve caper seed germination it is recommended to apply an A_4 solution at low concentrations (minimum 5 mg L^{-1}) or to use a 500 mg L^{-1} GA_3 replacing the germination substrate fortnightly, or to apply such a solution for 30 days, and then continue to wetthe substrate with water.

Keywords: germination percentage, *Capparis spinosa* L., gibberellic acid, seed viability

2.3.2. Introduction

Caper (*Capparis spinosa* L.) is a characteristic plant of the Mediterranean flora that belongs to the Capparaceae family. It is an evergreen, creeping shrubby plant, which can reach up to 1 m in height. Its floral buds (capers), its immature fruits (caper fruits) and to a lesser extent its tender cuts are used in Mediterranean cuisine, mainly in pickles (Sozzi et al., 2012). They have a high nutritional value given their content of vitamins, minerals and bioactive compounds that are present in different organs of the plant. In addition, nowadays, these are also in high demand by the cosmetic and pharmacological industries (Juan, 2017). Due to these characteristics, the cultivation of *C. spinosa* can have high economic value (Chedraoui et al., 2017).

The caper fruits are berries that contain numerous seeds that are used for its sexual propagation, which is the simplest and cheapest way to multiply this species (Juan, 2017; Sottile et al., 2021). However, these seeds present a low germination percentage, which may be due to non-deep physiological dormancy (Baskin and Baskin, 2004; Foschi et al., 2020). This dormancy, which is partly caused by the interaction between the plant genotype and different environmental factors (temperature, soil moisture, light and nutrient availability) is mainly consequence of the regulation of the metabolism and signaling of two phytohormones with antagonistic roles: gibberellins and abscisic acid (Müller et al., 2006; Carrera-Castaño et al., 2020). For seeds with coat-imposed dormancy, as may also be the case for caper seeds, abscisic acid is primarily produced in the endosperm tissue and exported into the embryo (reviewed in Penfield, 2017).

In previous studies carried out by the research team (Pascual et al., 2009; Juan, 2017; Foschi et al., 2022), it was possible to increase and advance the germination process with theapplication of exogenous gibberellic acid (GA_3). Germination percentages greater than 90% were obtained with the application of a GA_3 solution (500 mg L^{-1}) to the substrate. Other gibberellins such as laboratory grade gibberellin A_4 or A_3 could be more effective than the commercial GA_3. For example, in tomato seeds gibberellin A_{4+7} was more active in inducing germination in seeds than commercial GA_3 (Groot and Karssen, 1987).

The objective of this study was to provide practical guidelines for the use of gibberellins to improve germination in caper seeds, for which three experiments were carried out. In the first one, two methods for the GA_3 application were compared: 1) applying the GA_3 solution to the germination substrate using the between-paper method (BP), and 2) soaking the seeds for 72 h prior to place them BP. In both methods, five concentrations of GA_3 were used. The second experiment compared different methodologies for applying the GA_3 using the BP method. In the third experiment, the effect of lower concentrations of GA_3 and two gibberellins, A_4 and A_3, was assessed.

2.3.3. Materials and Methods

Seeds were obtained from adult caper plants (subsp. *rupestris*) grown in Llíria (Valencia, Spain), collected in 2019, throughout the months August and September. They were selected by rejecting the less dense using the flotation method using tap water (Davies et al., 2018). Seeds were then disinfected for 2 min with a 25% solution of sodium hypochlorite, followed by three washes with distilled water. The seeds were dried at room temperature and stored in airtight glass containers at 7 °C until the time of testing (September 2020).

Viability was determined by the tetrazolium test; caper seed was considered viable when the seed were fully stained or the radicle tip was the maximum area without staining (ISTA, 2003). The germination tests lasted 120 days and were carried out using the BP method, following the ISTA Rules (ISTA, 2018). For each treatment, four repetitions of 100 seeds each, evenly distributed, were performed in 9-cm Petri dishes. The dishes were kept in a germination chamber (Climatronic), at 30 °C during the day and 20 °C during the night, with a photoperiod of 12 h and 85% RH. Lighting was performed with white fluorescent tubes (Philips TL-D 36W/54), providing 81.1 μmol m^{-2} s^{-1}. Data were adjusted to the logistics function (Torres and Frutos, 1990; Pascual et al., 2004): $G = A\ [1+ \exp(\beta - kt)]^{-1}$, where G is the percentage of accumulated germination, A is the maximum percentage of germination, t is the germination period in days, β is a parameter referring to the position of the curve relative to the time axis, and k is a velocity parameter. From β and k, parameters with biological significance were obtained, such as the number of days needed to reach 50% of the percentage of final germination ($\beta/k = Gt_{50}$) and the relative average speed of accumulated germination ($k/2$, day^{-1}). Viability and germination tests were carried out in all three experiments.

In experiment 1, two methods of application of commercial GA_3 (Semefil, Nufarm) were compared: application of the GA_3 solution to the BP germination substrate and soaking the seeds in a GA_3 solution for 72 h, prior to the germination tests, in which the germination substrate was wetted with ultra-pure-water (Wasserlab GR type II analytical grade water system; herein referred to as water). Both methodologies were applied to five different GA_3 concentrations: 0, 100, 500, 1000 and 2000 mg L^{-1}.

In experiment 2, four methods for wetting the germination substrate, with a 500 mg L^{-1} GA_3 solution, were compared: T1, control (wetting the substrate with water); T2, continuous application of the GA_3 solution to the substrate; T3, continuous application of GA_3 to the substrate, which was changed fortnightly; T4, continuous application of GA_3 to the substrate only for 30 days and with water afterwards.

Experiment 3 compared six concentrations (0, 0.1, 1, 5, 10 and 50 mg L^{-1}) of commercial GA_3 and two technical products: A_4 and A_3 gibberellins (Sigma-Aldrich).

Results were analysed by the analysis of variance (ANOVA) performed with the Statgraphics Centurion 18 program (Statgraphics, 2018), and the separation of means with Fisher's LSD test, for a 95% confidence level. The percentage data were transformed before the analysis to achieve the assumption of normality with the arc√x function.

2.3.4. Results and Discussion

The initial viability of the seed collected was high, 95±2.9%, agreeing with that reported in (Foschi et al., 2022), who relates high viability of caper seeds to the fruits being harvested at their physiological maturity, and to the careful extraction, cleaning, handling and storage of the seeds.

Experiment 1

The 40 germination curves were adjusted using the logistic model, with an R^2 greater than 94.8%. This allowed the use of the variable *A* for the analysis, instead of G, as well as the constants β and *k* to calculate the biological parameters *β/k* and *k*/2, as done in previous studies on germination of caper seeds (Juan, 2017; Foschi et al., 2022). Figure 1 shows the curves fitted using the logistic model for the different treatment combinations.

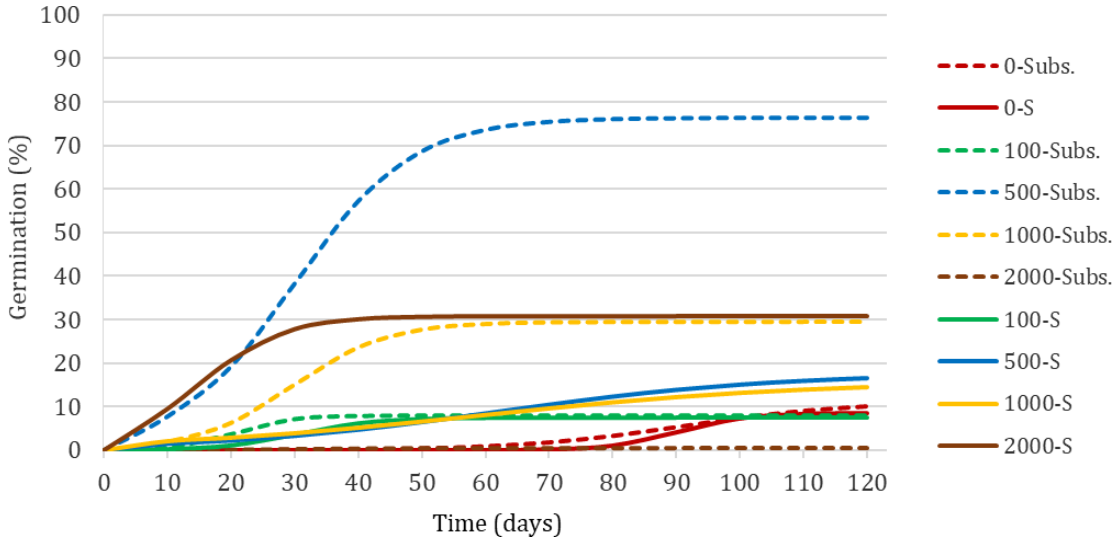

Figure 1. Curves fitted using the logistic model for germination for the GA_3 application method (applied to the substrate (Subs.) and soaking (S)) and GA_3 concentrations (0, 100, 500, 1000 and 2000 mg L^{-1}).

Table 1 presents the statistical results for the germination parameters. The maximum germination ($p \leq 0.01$) was obtained with the 500 mg L^{-1} of GA_3 solution applied to the substrate (76%), with a Gt_{50} of 30.5 days, reducing the germination time by 64 days relative to the control. Arefi et al. (2012) reported similar germination percentages (72%) when wetting the germination paper with in 250 mg L^{-1} GA_3 after treatment with 8000 mg L^{-1} KNO_3 for 24 h. The higher doses of GA_3 applied to the substrate in this study inhibited the germination, possibly due to an overexposure to such a high concentration, since at the end of the tests more than 70% of the non-germinated seeds appeared to be dead (data not shown). Although soaking with the highest GA_3 concentration led to an increase in germination compared with control, it barely exceeded 30% (67% for the non-germinated seeds were dead at the end of the germination test; data not shown). The germination percentage obtained with the 500 mg L^{-1} GA_3 concentration applied to the substrate, is similar to that obtained by Imbernón (2000) in similar conditions. However, Imbernón (2000) recorded higher germination percentages, 74 and 72%, by soaking the seeds in a 750 mg L^{-1} concentration for 24 h, and in 1500 mg L^{-1} for 2 h, respectively, while control germination was 27.7%. Žutić et al. (2020), increased the germination percentage in *Capparis orientalis* Veill., by up to 20% by soaking the seeds for 12 h in a 2000 mg L^{-1} GA_3, compared with 1.7% obtained without GA_3.

Table 1. Effect of the GA_3 application method and of the GA_3 concentrations (mg L^{-1}) on final germination (*A*; %), number of days needed to reach 50% of *A* (Gt_{50}; d) and relative average rate of accumulated germination (*k/2*, day^{-1}).

	A	**Gt_{50}**	***k/2***
Application method (M)			
Substrate	25.1 a	38.2 b	0.079
Soaking	16.1 b	52.4 a	0.057
Concentration (C)			
0	9.9 d	88.7 a	0.066
100	7.7 d	26.6 c	0.087
500	46.9 a	46.1 b	0.068
1000	22.7 b	44.2 b	0.068
2000	15.7 c	20.9 c	0.051
Interaction (M×C)			
Substrate – 0	11.3 cd	94.4 a	0.064
Soaking – 0	8.6 c	83.0 a	0.069
Substrate – 100	7.9 c	22.4 c	0.093
Substrate – 500	76.3 a	30.5 c	0.089
Substrate – 1000	29.6 b	29.5 c	0.092
Substrate – 2000	0.5 e	14.6 c	0.058
Soaking – 100	7.6 c	30.9 c	0.082
Soaking – 500	17.6 cd	61.8 b	0.048
Soaking – 1000	15.8 cd	59.0 b	0.045
Soaking – 2000	30.8 b	27.3 c	0.043
LSD ($p \leq 0.05$)	6.3	14.7	NS
Analysis of variance			
Factors (degrees of freedom)	% Sum of squares		
M (1)	4.6**	6.6**	5.2 NS
C (4)	45.3**	75.1**	5.9 NS
M×C (4)	46.9**	8.0**	4.2 NS
Residual (30)	3.3	10.3	84.7
Standard deviation [a]	4.4	10.2	0.05

Different letters in the same column, within each factor, indicate significant differences ($p \leq 0.05$) according to the LSD test. ** significance level $p \leq 0.01$, NS: not significant. [a]The standard deviation has been calculated as the square root of the residual mean square.

Experiment 2

In this experiment, the method that in the previous experiment gave the best results (GA_3 solution of 500 mg L^{-1} applied to the germination substrate), was compared with other application methods. The 16 germination curves were adjusted to the logistic model with a correlation coefficient R^2 greater than 98.1%. Figure 2 shows the curves fitted to the logistic model for each treatment.

The new methods applied in this experiment, increased the germination percentage ($p \leq 0.05$; Table 2), both changing the substrate every 15 days or wetting it with the GA_3 solution only for the first 30 days (86 and 83%, respectively), compared with continuous application of the GA_3 solution (76%), which in turn was much higher than that obtained for the control treatment (11%). There were no differences for Gt_{50} between the treatments that applied the GA_3 solution ($p \leq 0.05$; Table 2), with all approximately a third of the Gt_{50} for control seeds. The germination rate was not affected by the application method ($p \leq 0.05$; Table 2). The two new methods (T3 and T4) led to a reduction in GA_3 exposure during imbibition, which could reduce any toxic effects on caper seeds, thus increasing the germination.

Table 2. Effect of the GA_3 application method to the substrate on final germination (*A*; %), number of days needed to reach 50% of *A* (Gt_{50}; d) and the relative average rate of accumulated germination (*k/2*, day^{-1}).

	A	***Gt*$_{50}$**	***k/2***
Application method			
T1	11.3 c	96.3 a	0.04
T2	76.3 b	30.4 b	0.06
T3	85.9 a	30.4 b	0.07
T4	82.5 a	34.4 b	0.05
Analysis of variance			
Factors (degrees of freedom)		% Sum of squares	
Application method (3)	99.5**	96.9**	41.0 NS
Residual (12)	0.5	3.1	59
Standard deviation [a]	2.6	5.7	0.01

Different letters in the same column indicate significant differences ($p \leq 0.05$) according to the LSD test. ** significance level $p \leq 0.01$, NS: not significant. [a]The standard deviation has been calculated as the square root of the residual mean square. T1: control (ultra-pure-water); T2: continuous wetting; T3: fortnight germination substrate change; T4: continuous wetting for 30 days and then applying ultra-pure-water.

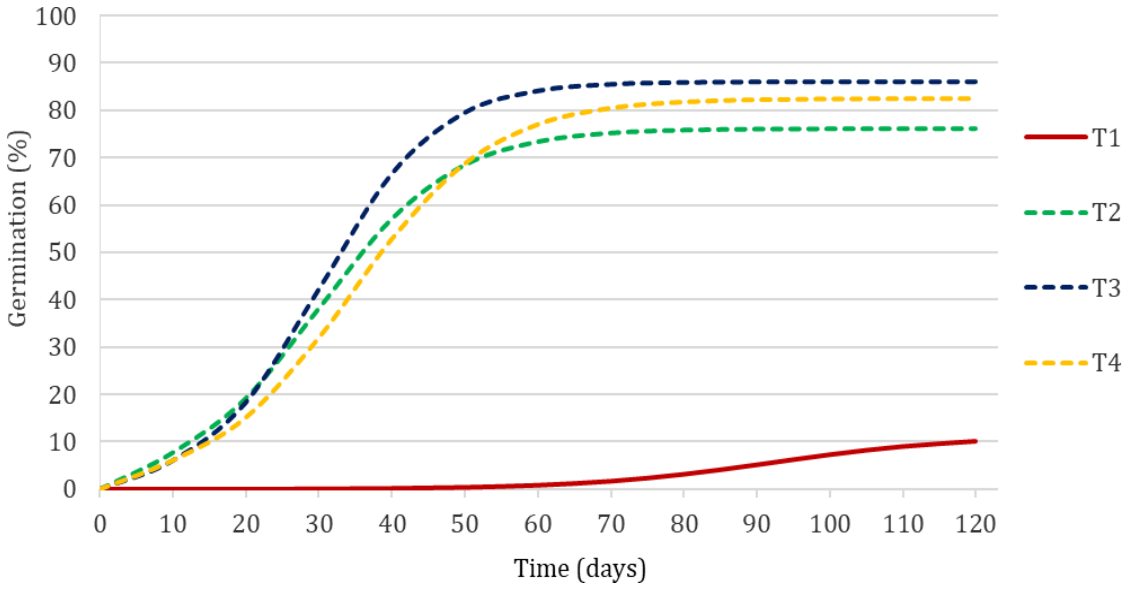

Figure 2. Curves fitted using the logistic model for germination according to the GA_3 (500 mg L^{-1}) application method: T1: control (water); T2: continuous wetting; T3: fortnight germination substrate change; T4: continuous wetting for 30 days and then applying water.

Experiment 3

The 72 germination curves obtained for the different gibberellins and concentrations were fitted to the logistic model with a correlation coefficient R^2 greater than 96.8%. Figure 3 presents the logistic model for the combination of the different gibberellins and concentrations.

The germination parameters (*A* and Gt_{50}) were affected by the gibberellin type, by the solution concentration and by the interaction of gibberellin type and concentration ($p \leq 0.05$; Table 3). The highest and quickest germination was obtained with gibberellin A_4 with no differences at concentrations greater than 5 mg L^{-1}. For gibberellin A_4 at concentrations greater than 5 mg L^{-1}

germination percentages were higher than 90% and Gt_{50} less than 35 days. The germination reached with the different concentrations of A_3 and GA_3 was significantly lower ($p \leq 0.05$; Table 3), with percentages below 19%. This study shows gibberellin A_4 was 10 to 100 times more active than A_3 and GA_3 in enabling germination. This agrees with studies carried out by Groot and Karssen (1987), in tomato seeds which indicated that gibberellin A_{4+7} was 1000 times more active than commercial GA_3. Germination obtained for A_4, from 1 mg L^{-1}, is greater than those reported by Sozzi and Chiesa (1995), who obtained germination percentages close to 70% by soaking caper seeds in a 100 mg L^{-1} A_{4+7} solution for 90 min after being scarified with concentrated sulfuric acid; the better result obtained in this experiment may be related with the method used to apply the gibberellins, as obtained in Experiment 1. Considering these results and those obtained in Experiment 1, in agreement with Imbernón (2000) the recommended concentration for GA_3 is 500 mg L^{-1}.

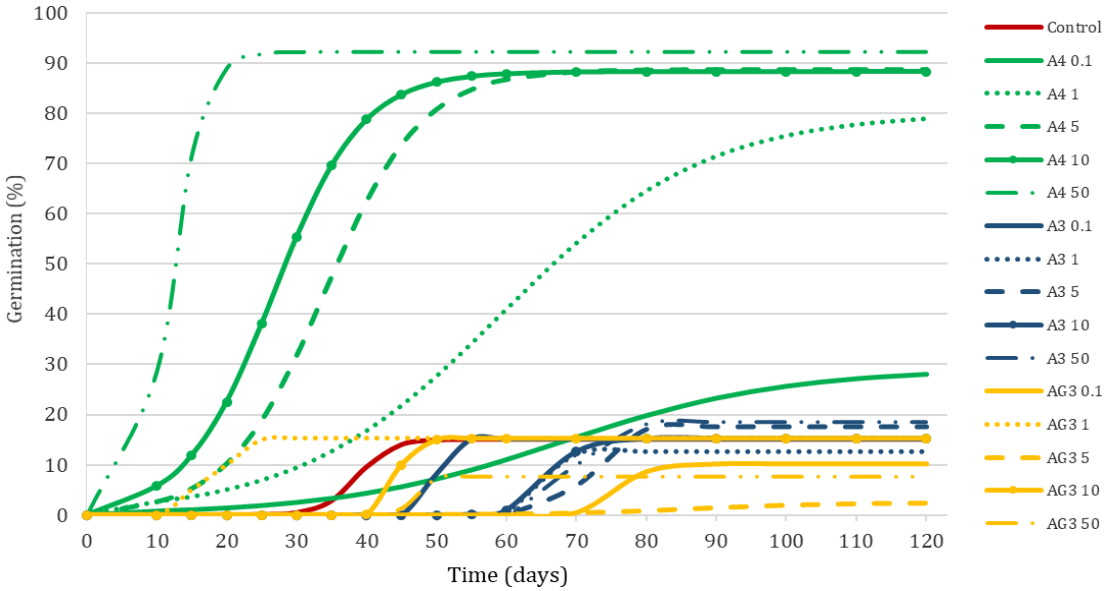

Figure 3. Curves fitted using the logistic model for germination according to gibberellin molecule (A_4, A_3 and GA_3) and concentration (0, 0.1, 1, 5, 10 and 50 mg L^{-1}) applied.

Overall, it can be stated that, among the gibberellin application methods assessed, wetting the germination substrate with an A_4 solution (5 mg L^{-1} being sufficient) led to the maximum germination percentage and in the shortest time. If this gibberellin is not readily available, or it is too expensive, the GA_3 at 500 mg L^{-1} applied to the substrate during the first month of the germination process or changing fortnightly the germination substrate is also recommended as an alternative.

Table 3. Effect of the gibberellin applied and the solution concentration (mg L^{-1}) on final germination (*A*; %), number of days needed to reach 50% of *A* (Gt_{50}; d) and the relative average rate of accumulated germination (*k/2*, day^{-1}).

	A	**Gt_{50}**	***k/2***
Gibberellin (G)			
A_4	65.6 a	42.9 b	0.104
A_3	15.7 b	62.2 a	0.300
GA_3	11.0 c	60.7 a	0.765
Concentration (C)			
0	15.1 c	53.0 bc	0.203
0.1	18.1 c	71.4 a	0.29
1	36.0 b	50.7 bc	1.063
5	36.3 ab	61.9 ab	0.163
10	39.6 a	44.5 c	0.263
50	39.5 a	50.0 bc	0.358
Interaction (G×C)			
Control – 0	15.1 d	53.0 bcd	0.203
A_4 – 0.1	29.0 c	68.5 abc	0.031
A_4 – 1	80.1 b	61.1 bc	0.035
A_4 – 5	88.7 a	35.1 de	0.073
A_4 – 10	88.3 a	27.1 e	0.080
A_4 – 50	92.3 a	12.5 e	0.204
A_3 – 0.1	15.2 d	69.7 abc	0.603
A_3 – 1	12.6 d	62.0 bc	0.316
A_3 – 5	17.5 cd	64.0 abc	0.212
A_3 – 10	15.3 d	56.6 bcd	0.212
A_3 – 50	18.6 cd	67.8 abc	0.257
GA_3 – 0.1	10.2 d	76.2 ab	0.236
GA_3 – 1	15.3 d	28.9 e	2.838
GA_3 – 5	2.6 e	86.5 a	0.205
GA_3 – 10	15.3 d	49.8 cd	0.497
GA_3 – 50	7.6 de	69.8 abc	0.613
LSD ($p \leq 0.05$)	8.5	23.9	NS
Analysis of variance			
Factors (degrees of freedom)		% Sum of squares	
G (2)	62.8**	13.9**	5.3 NS
C (5)	10.6**	14.4**	6.5 NS
G×C (10)	23.8**	33.0**	14.3 NS
Residual (54)	2.8	38.7	74
Standard deviationa	6.0	16.9	1.2

Different letters in the same column indicate significant differences ($p \leq 0.05$) according to the LSD test. ** significance level $p \leq 0.01$, NS: not significant. [a]The standard deviation has been calculated as the square root of the residual mean square. T1: control (ultra-pure-water); T2: continuous wetting; T3: fortnight germination substrate change; T4: continuous wetting for 30 days and then applying ultra-pure-water.

2.3.5. Conclusions

The best germination results for caper seeds are obtained with the application of the gibberellin A_4 to the germination substrate, at concentrations from 5 mg L^{-1}. Wetting the germination substrate with a GA_3 solution at 500 mg L^{-1} increases and advances the germination of caper seeds. Neither lower nor higher GA_3 concentrations improved germination. Soaking the seeds in GA_3 solutions for 72 h prior to the germination tests, did not improve the germination regardless of GA_3 concentration. The best germination results for the 500 mg L^{-1} GA_3 solution are obtained when the germination substrate is changed fortnightly or when GA_3 solution was applied only during the first month, applying water afterwards.

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Capítulo 3. Criterios de recolección, selección y período de almacenamiento en semillas de alcaparra

3.1. Collection guidelines to achieve a viable caper commercial propagation

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3.1.1. Abstract

The caper (*Capparis spinosa* L.) is a perennial plant characteristic of the Mediterranean region that presents difficulties in its propagation, both vegetatively and by seeds. The main aim of this study is to provide collection guidelines to achieve a viable caper commercial propagation, for which three experiments were undertaken to determine the viability and germination in different seed lots. In the first experiment, commercial and own produced seeds (collected with the same criteria as commercial seeds) were analysed; the commercial seeds presented the lowest viability and germination. The second experiment analysed the effect of the fruit (from which the seeds were extracted) at its maturation stage, obtaining the lowest seed viability and germination in the seeds extracted from the dry fruits. In the third experiment, seed viability and germination were analysed immediately after collection, following a short drying period (3 d), and after six storage months. Viability and germination decreased with seed storage. Overall, it can be stated that caper seeds are sensitive to desiccation; consequently, a general rule of thumb is to collect the fruits once a week, to extract the seeds, and to plant them immediately for germination.

Keywords: *Capparis spinosa* L.; seed viability; germination percentage; seed moisture content; gibberellic acid

3.1.2. Introduction

The caper (*Capparis spinosa* L.) is a perennial plant characteristic of the Mediterranean region, which can grow spontaneously or cultivate in arid or semi-arid climates. It is a creeping shrub that can reach a height of up to 0.5 m with branches up to 3 m long. It has a high agricultural potential and a great demand for exploitation [1] due to its various uses, highlighting food (flower buds and immature fruits, pickled in brine; [2]), pharmaceutical industry [1,3,4], and in xerogardening and landscaping for its ornamental value [5], its resistance to drought, and its ability to reduce soil erosion [1,6–8].

Traditionally, caper has been propagated both vegetatively, using stem cuttings, andby seeds, presenting important difficulties in both cases. Recently, Sottile et al. [9] reported the state-of-the-art of the main techniques of sexual and vegetative propagation, including *in vitro* propagation, used in capers.

In vegetative propagation, our working group [10,11] obtained results that can be considered acceptable: up to 41% of cuttings rooted and sprouted with cuttings obtained in October from the basal section of the branches, and up to 83% of cuttings rooted in July from apical section of the branches. This led the group to conclude that the presence of active buds in each cutting is essential, so that in addition to rooting, sprouting occurs. The group obtained positive results using *in vitro* propagation by means of nodal shoot explants (data not published).

Regarding the propagation of caper by seed, caper fruits have many seeds, which could facilitate sexual propagation; however, these seeds present low germination percentages, and the germination is a slow process [12,13], making it difficult to be used in commercial propagation. This low seed germination has been related to the presence of dormancy. Baskin and Baskin [14] pointed out the presence of physiological dormancy by reporting six references from different research groups, including three references from our research group. Pascual et al. [15] stated that physical and physiological dormancy occurs by obtaining high germination percentages (90%) with sulphuric acid scarification, which is followed by the addition of a gibberellic acid solution (GA_3) to the germination substrate. Later, it was also obtained that sulphuric acid and a GA_3 solution application under alternate temperatures was the best method for breaking the caper seed dormancy [16]. Žutic´ et al. [17] stated that the GA_3 addition could partly eliminate the dormancy of non-scarified caper seed. In the last few years, our work team has achieved

germination percentages up to 99% with the unique application of a GA_3 solution to the germination substrate [5]; furthermore, we have verified that imbibition takes place through the hilar region [18], thus, considering both studies, we stated that dormancy caused by a water-impermeable seed coat should not be considered.

This research team found great variability in the germination percentage obtained in caper seeds from different lots [13]. Foschi et al. [18] stated the low germination of some lots of commercial seeds ($\leq$5%), while using mature dark brown seeds extracted from ripe fruits collected both at dehiscence and immediately before or after it led to germination percentages up to 99% (with the application of a GA_3 solution to the germination substrate [5]). Collecting the fruits immediately before or after dehiscence requires substantial labour; although feasible in carrying out research, it may be unfeasible in commercial seed production. Generally, fruits with different degrees of maturity are collected in each collecting pass, containing apparently ripe fruits before or after its dehiscence, including both fruits with fresh and dry pulp after its dehiscence. In prior studies (unpublished data), we stated a higher and speedy germination of recently collected caper seeds than those obtained with seeds collected a few weeks before starting the germination tests. In this sense, a short period of drying (2–3 days) may increase the ability of seeds to respond to dormancy-breaking treatments [14].

The main aim of this study is to provide collection guidelines to achieve a viable caper commercial propagation by considering the results obtained in three experiments. In the first experiment, the viability and germination of caper seeds from various lots, both commercial and of their own production, are analysed. In the latter, a distinction was made between seeds (and fruits) collected, with the same criteria as commercial seeds, and seeds obtained from dry fruits; in all three cases, the seeds were produced in 2019 and 2020. The second experiment was carried out to analyse the effect of the fruit (from which the seeds were extracted) at the maturation stage on the viability and germination of seeds of their own production. In the third experiment, seed viability and germination were analysed immediately after collection, following a short drying period (3 days), and after six storage months. Given that in previous studies, the application of GA_3 to the substrate significantly increased the caper seeds germination, the substrate was moistened both with water or a GA_3 solution in the germination test.

3.1.3. Materials and Methods

3.1.3.1. Experiment 1

In the first experiment, two standard category commercial lots (CS, commercial seeds) were used; these were purchased from a private company (Cantueso Natural Seeds). According to the information provided by the company, they were produced in 2019 and 2020 (each lot corresponding to one year) in national parks in the province of Córdoba (Spain). Seeds were extracted without any chemical treatment and came mainly from apparently ripe fruits before dehiscence, although some seeds could also come from open fruits but prior to the fruits deteriorating by pulp degradation (personal communication).

The own produced seeds (OS) were produced in the same years in Llíria (Valencia, Spain). They were collected from adult plants in September, following the criterion indicated by the private company for the CS, including seeds extracted from ripe fruits collected on the day of dehiscence and from fruits located in the position before and after it. In addition, seeds extracted from deteriorated fruits with dry pulp (type IV) were considered separately. Thus, three different seed lots were used each year: CS, OS, and type IV.

All of our own produced seeds were extracted from the fruits, disinfected with sodium hypochlorite for 10 min, rinsed twice in tap water, and dried in the shade at room temperature (23–25 ºC, 20–50% relative humidity) for two weeks, after which they were stored in closed airtight plastic containers at 7 ± 0.5 ºC until the tests were conducted. Before drying, mature seeds were selected by

rejecting the light seeds by flotation in tap water. Flotation is a common method for separating viable from non-viable seeds; it involves placing seeds in water so that heavy, sound seeds sink to the bottom and the lightweight and unfilled seeds float to the top [19].

The seed moisture content for each lot was determined by the constant temperature oven method; samples of 50 seeds were dried in quadruplicate for 24 h at 103 ºC [20] in a forced-air oven (Selecta 297; Selecta, Barcelona, Spain). The calculation was determined on a fresh mass basis [20]:

$$\text{Seed moisture (\%)} = 100 \times (\text{Fresh mass} - \text{Dry mass})/\text{Fresh mass}. \quad (1)$$

Seed viability was determined by the tetrazolium test [21]. Preconditioning of the seeds was applied by soaking them in water at 20 ºC during 18 h, after which they were cut longitudinally off at the widest Feret diameter and soaked in a 1% tetrazolium solution at 30 ºC during 18 h. Seeds were observed with a photomicroscope (U500X Digital Microscope; Cooling Tech, Guangdong, China). For the evaluation, the maximum area of unstained tissue permitted to consider a seed as viable was the radicle tip. Following [22], three tissue categories were considered according to their characteristics: sound (S. Staining proceeds gradually and uniformly from the exposed surfaces inward. Changes in the colour intensity are gradual without distinct boundaries); weak but viable (WV. Stain greyish red or brighter red than normal); and weak, not viable (WNV. Tissue colour can be mottled. Cut surfaces may appear white, while the inner tissues may appear dark red. The unstained dead tissues look flaccid, liquid-logged, blurred, chalky white, and lack-lustre). Samples consisted of 200 seeds (four replications of 50 seeds each).

The germination tests were carried out by the Between Paper method (BP) as described in the International Rules for Seed Testing [20], in Petri dishes with a diameter of 9 cm. The substrate was moistened with pure water (Wasserlab G.R type II analytical grade water system, hereinafter referred to as water) or 500 mg L^{-1} GA_3 solution (Berelex L.; herein referred to as GA_3). To prevent fungal problems, in both cases, 2 g L^{-1} captan (CAPTAN 50 BAYER) was added. Petri dishes were placed in a growth chamber (model Climatronic, Barcelona, Spain) under an alternating temperature and light regime: 12 h at 20 ± 1 ºC in the dark and 12 h at 30 ± 1 ºC under a photosynthetic photon flux density of 324 µmol m^{-2} s^{-1} for a maximum of 150 days. The seeds were considered germinated when the radicle protruded from the seed coat [20] and germinated seeds were eliminated from the Petri dish; evaluation was carried out every three days. Four replicates were used, consisting of 100 seeds. For each replicate, the germination data were fitted to the logistic function [12,23], defined as a special case of Richards' function [24]:

$$G = A/1 + e^{(\beta - kt)} \quad (2)$$

where G is the cumulative germination (%); A represents the final germination percentage; t is the germination time (d; days); and β and k are function parameters. These parameters were used to determine the time (in d) required to reach 50% of G ($Gt_{50} = \beta/k$) and the mean relative cumulative germination rate ($k/2$, d^{-1}).

Moisture content, viability, and germination tests started on March 2020 and 2021, lasting 24 h, 48 h, and 150 d, respectively.

3.1.3.2. Experiment 2

In the second experiment, seeds were classified depending on the fruits from which they were extracted according to their maturation stage: Types I, II, III, and IV (Figure 1). Type II included the seeds extracted from fruits collected the day of their dehiscence, while the seeds from fruits in the anterior and posterior position (with dehiscence up to 3 days after and before, respectively) were classified as Type I and III, respectively. Type IV, as in Experiment 1, were seeds obtained from deteriorated fruits with dry pulp. This experiment was carried out in 2019 and 2020, using in each year four seed lots.

Moisture content, viability, and germination tests were carried out as indicated in Experiment 1, starting after seed collection in September 2019 and 2020.

Figure 1. Fruit types from which the seeds were extracted according to their maturation stage. From left to right: ripe fruits collected before dehiscence (type I; 42 days after anthesis (daa)), ripe fruits immediately after dehiscence (type II; 45 daa), overripe dehiscent fruits before they deteriorate due to pulp degradation (type III; 48 daa), and deteriorated fruits with dry pulp (type IV; 55 daa).

3.1.3.3. Experiment 3

In the third experiment, seed moisture content, viability, and germination tests were carried out immediately after collection (fresh seeds; FS), after a short (3 d) drying period (dried seeds, DS), and after 6 storage months (stored seeds; SS). All these seeds belonged to a seed lot (type II) collected in September 2020.

Seed moisture content, viability, and germination tests were carried out as indicated in Experiment 1 after the storage period (0, 3, and 180 d).

3.1.3.4. Statistical Analysis

The differences between the maximum and minimum values of the four replicates obtained in all the tests met the tolerance levels of the International Seed Testing Association [20]. In all three experiments, results were analysed by multi-way analyses of variance (ANOVA [25]). The percentage data were arcsin transformed before analysis. The viability and seed moisture analysis were analysed by two-way ANOVA, considering the factors type of seed and year of production (except in Experiment 3), while for the germination data analysis, the AG_3 addition to the germination substrate was also considered. A probability of $\leq 0.05\%$ was considered significant. Mean separations were performed when appropriate, using the Fisher's least significance difference (LSD test) at $p \leq 0.05$.

3.1.4. Results and Discussion

3.1.4.1. Experiment 1

Neither the type of seed nor the year of production significantly affected ($p \leq 0.05$) the moisture content of the seed at the beginning of the viability and germination tests, ranging, on average, from 9.35% and 9.51% (Table 1).

Table 1. Effect of the type of seed (own produced (OS), type IV, and commercial (CS)) and the year of production on the seed moisture content (%) at starting the viability and germination tests and on the viability of the seeds (%), considering both the sound seeds (S) and the sound and weak but viable tissues (S + WV) criteria; mean values.

	Seed Moisture Content	**Viability (S)**	**Viability (S + WV)**
Type of seed (TS)			
OS	9.51	52.50 a	73.75 a
IV	9.36	31.25 b	43.75 b
CS	9.40	6.25 c	17.50 c
Year of production (Y)			
2019	9.48	27.50	44.17
2020	9.35	32.50	45.83
Analysis of variance			
Source (degrees of freedom)	% Sum of squares		
TS (2)	6.06 NS	72.67 **	74.56 **
Y (1)	10.82 NS	1.27 NS	0.10 NS
TS × Y (2)	3.75 NS	1.48 NS	1.52 NS
Residual (18)	79.37	24.58	23.82
Standard deviation	0.20	12.69	15.00

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

The type of seed was the only factor that significantly ($p \leq 0.05$) influenced the seed viability, considering both S and S + WV (Table 2), corresponding to the highest value to OS, which is followed by type IV seeds, and the lowest viability corresponded to CS. The low viability of type IV seeds (31.3% for S) and particularly that of CS (6.3% for S) is notable, since they should not present viability nor germination restrictions given their age (6 months) [13].

Although the viability obtained for CS was extremely low, it doubles that obtained by [18] (on average 3.15% for S) in a commercial seed lot produced in 2018 and that was provided by the same company. Using a stereoscopic microscope, based on the integrity of the seed coat, these authors classified the seeds of that lot into four groups: intact, scraped, cracked, and broken seeds. In the 2018 seed lot, the deterioration of the seed coat considerably contributed to the low viability of the seeds, since cracked and broken seeds supposed 31% and 16%, respectively, of the total seeds, their respective viability being low (7.5% and 0%, respectively, for S criterion). This suggested that in the 2019 and 2020 lots, the deterioration of the seed coat could have contributed considerably to the low viability of the seeds; nevertheless, cracked and broken seeds were considerably reduced in these lots, the cracked seeds accounting for 1.3% and the 1.8% and the broken seeds accounting for 0.5% and 1.0% of the total seeds in 2019 and 2020, respectively. It seems that, although slowly, this company has been improving the quality of their caper seeds.

The low viability of both type IV and CS seeds agrees with [26], who stated that some studies confirmed that the seed quality (i.e., viability, germination rate) in a number of species, such as Arabidopsis, beans, melon, rape, pepper, and tomato, continues to increase after physiological maturity, but once the seeds have dried below about 20% moisture content, their metabolism has ceased, and deterioration may begin. Probably for this reason, according to [26], the seeds of some crops are harvested with relatively high moisture contents and then carefully dried to avoid deterioration in the field and to preserve the highest quality. Seeds of type IV and probably also part of CS were quickly dried in the field, which could lead to a reduced viability.

Table 2. Effects of the type of seeds (own produced (OS), type IV, and commercial (CS) seeds), the year of production, and the substrate saturation solution on the germination parameters: cumulative germination (*G*, %), final germination percentage (*A*, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate (*k/2*; d^{-1}); mean values.

	G	***A***	***Gt*$_{50}$**	***k*/2**
Type of seed (ST)				
OS	39.31 a	39.42 a	50.74	0.058 b
IV	10.69 b	10.90 b	58.98	0.096 b
CS	1.56 c	1.59 c	50.37	0.148 a
Year of production (Y)				
2019	16.79	16.77	55.26	0.086
2020	17.58	17.84	51.46	0.116
Saturation solution (S)				
Water	5.33 b	5.65 b	74.37 a	0.087
GA_3	29.04 a	28.96 a	32.35 b	0.115
Analysis of variance				
Source (degrees of freedom)			% Sum of squares	
ST (2)	44.91 **	45.63 **	2.02 NS	22.71 **
Y (1)	0.03 NS	0.05 NS	0.46 NS	3.74 NS
S (1)	24.40 **	23.94 **	56.34 **	3.25 NS
ST × Y (2)	0.03 NS	0.08 NS	2.21 NS	2.39 NS
ST × S (2)	29.11 **	28.77 **	0.94 NS	4.48 NS
Y × S (1)	0.07 NS	0.06 NS	5.11 **	1.85 NS
ST × Y × S (2)	0.43 **	0.34 **	11.85 **	12.83 *
Residual (36)	1.02	1.13	21.07	48.75
Standard deviation	2.80	2.93	14.84	0.06

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** (*) Indicates significant differences at $p \leq 0.01$ ($p \leq 0.05$).

As expected, given the low viability, the accumulated germination (*G*) values were low for OS and very low for IV and CS. The coefficient of determination (R^2) for 48 curves (four replicates from three combinations of variation sources: three types of seeds, GA_3 or water addition to the germination substrate and both years of production) ranged from 0.891 to 0.995, with F ratio values of the model statistically significant ($p \leq 0.01$; data not shown). This indicates that the use of the logistic function is suitable for analysing caper seed germination as it was in similar studies carried out both in caper [12,18] as in other crops [23,27], weeds [28], and fungi [29].

Figure 2 presents the cumulative germination curves fitted to the logistic model obtained for the average values of each type of seed and saturation solution combination.

The cumulative germination (*G*) and the final germination percentage (*A*) values are similar (both in this experiment, Table 2, and in experiments 2 and 3), being both significantly affected by the same factors; thus, only *A* values will be discussed.

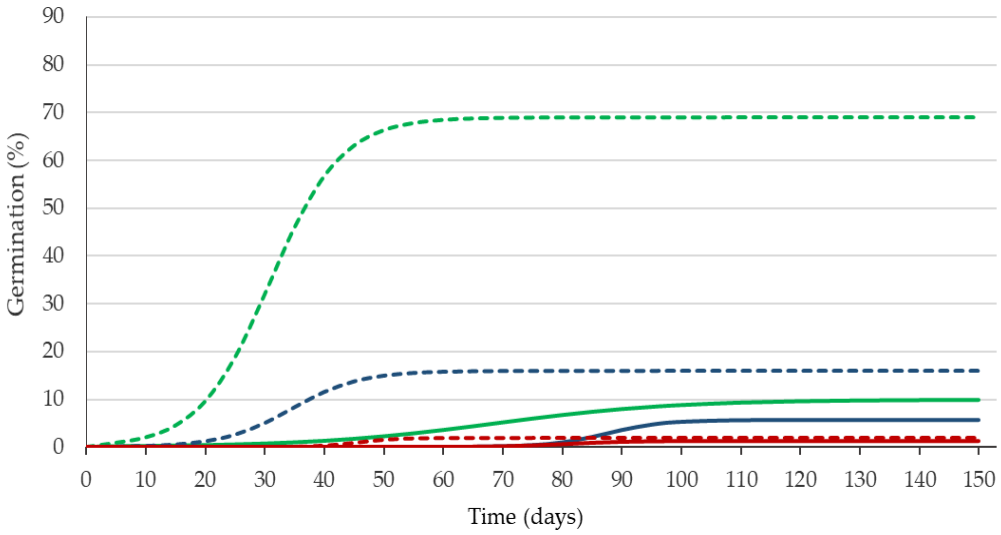

Figure 2. Logistic model fitted to the cumulative germination curves of caper seeds. Mean values for the combination of the type of seed (own produced (OS), type IV, and commercial (CS) seeds in green, blue and red, respectively) and substrate saturation solution (water in continuous lines and GA_3 solution in dashed lines).

Both the type of seed and the substrate saturation solution as well as their interaction influenced ($p \leq 0.05$) in A, the highest value corresponding to OS and the lowest value corresponding to CS. The GA_3 addition to the substrate increased the value of A. When analysing their interaction (Figure 2), it is observed that GA_3 considerably increased germination in OS and, to a lesser extent, in the type IV seeds, but it did not increase germination in the CS. The germination obtained with the GA_3 addition can be considered acceptable (69.3%) in OS, very low in the type IV seeds (16%), and practically negligible in CS (1.9%). These values agree with the viability obtained (S + WV, 73.8% for OS). It seems that as previously stated and according to [26], deterioration begins with the seed drying after reaching its physiological maturity.

As can be seen in Figure2 and Table 2, the caper seeds germination is very slow, so that on average, the time required to reach 50% of G has exceeded 50 d, as has been obtained in previous studies [15,18]. The GA_3 application reduced the Gt_{50} (on average from 74.4 to 32.4 d; $p \leq 0.01$), and the significant interaction of this factor with the year of production ($p \leq 0.01$) indicates that this decrease was greater in seeds produced in 2019 than in those produced in 2020.

The $k/2$ was affected ($p \leq 0.01$) by the type of seeds, the highest value corresponding to CS, but it is necessary to emphasise that only less than 2% of these seeds germinated. As in viability, it can be stated that overall, the values of the germination parameters obtained with the CS agree with those obtained in previous studies [18].

3.1.4.2. Experiment 2

There were significant differences ($p \leq 0.01$) in the moisture content depending on the type of seeds, decreasing with increasing fruit maturity (Table 3). The moisture content of type IV seeds (14.7%) was clearly lower than that of other types of seeds (on average 27.8%). Logically, this seed moisture content was much higher than that presented in Experiment 1 (on average 9.4%), since results were obtained after drying and storing for six months.

Table 3. Effects of the type of seeds and the year of their production on the seed moisture content (%) and viability (%), considering only the sound seeds (S) and both the sound and viable but with weak tissues (S + WV); mean values.

	Seed Moisture Content	Viability (S)	Viability (S + WV)
Type of seed (ST)			
I	28.45 a	80.00 a	93.75 a
II	27.55 b	90.00 a	95.00 a
III	26.82 c	58.75 b	68.75 b
IV	14.65 d	33.75 c	37.50 c
Year of production (Y)			
2019	24.27	61.89	69.38
2020	24.46	69.38	78.13
Analysis of variance			
Source (degrees of freedom)	% Sum of squares		
ST (3)	98.95 **	62.17 **	75.70 **
Y (1)	0.03 NS	1.88 NS	2.65 NS
ST × Y (3)	0.28 NS	6.98 NS	4.81 NS
Residual (24)	0.75	25.97	16.85
Standard deviation	0.57	17.02	12.75

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

In the case of fruits that are kept in the field after dehiscence, the seeds dry out before being collected, reducing their weight and, consequently, their density. These seeds can be both mature and immature. When the seeds have a density below a threshold, they float in the water; thus, it is not feasible to separate the mature seeds from the immature seeds by flotation method [19]. On average, the percentage (on weight basis) of mature seeds in seed types I, II, and III (not floating in tap water) represented 61.5% and 67.0% of the total seeds of 2019 and 2020, respectively. In the type IV seeds, the desiccation led to the flotation of all the seeds. This fact agrees with the lower seed moisture content of the type IV seeds (14.7%) in relation to the other seed types (on average 27.6%). According to the results obtained for the flotation method, the mature seeds (those that did not float) of types I, II, and III, and all seeds of the type IV, were used in the viability and germination tests.

The viability of type IV seeds, considering both the seeds of category S and those of category S + WV, was lower ($p \leq 0.05$) than that of type III, which in turn was lower ($p \leq 0.05$) than of types I and II, with no differences ($p \leq 0.05$) between them (Table 3). Although the difference between the viability of the seeds of types I and II was not significant ($p \leq 0.05$), the proportion of WV was lower in seeds of type II (5%) than in those of type I (13.8%). As previously cited, ref. [26] reported that the quality of the seed is maximum during and shortly after its physiological maturity.

The viability of the type IV seeds was very low (<38%, considering S + WV), although it must be considered that these lots include both mature and immature seeds (because all of them floated in tap water); thus, these figures could underestimate (although in a small percentage) the viability of the mature seeds, as mentioned afterwards. In addition, it is common for ants, wasps, and birds to take seeds, especially the best quality seeds, when the fruits dry in the field.

The coefficients of determination (R^2) for 64 curves (four replicates from three combinations of variation sources: four types of seeds, GA_3 or water addition to the germination substrate, and two years of production) ranged from 0.931 to 0.996, with F ratio values of the model statistically significant ($p \leq 0.01$; data not shown), meaning that the use of the logistic function is suitable to analyse the germination of caper seeds in this experiment, as shown in Experiment 1 (Figure 3).

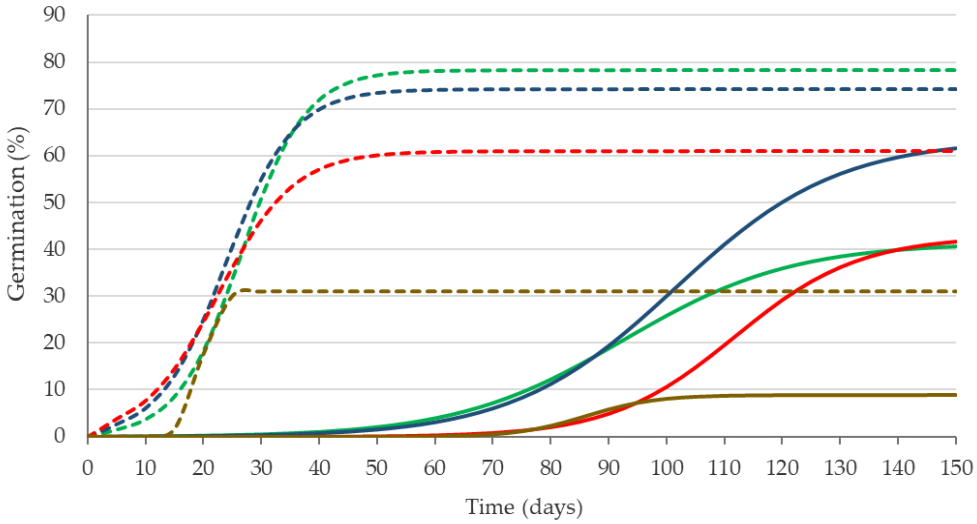

Figure 3. Logistic model fitted to the cumulative germination curves of caper seeds. Mean values for the combination of the type of seed (I, II, III, and IV in green, blue, red, and brown, respectively) and substrate saturation solution (water in continuous lines and GA_3 solution in dashed lines).

The type of seed influenced ($p \leq 0.05$) its germination, so that the type II seeds presented the highest A values ($p \leq 0.05$), while the lowest value was obtained for the type IV seeds. The year of seed production did not affect A ($p \leq 0.05$). As mentioned, the GA_3 application increased ($p \leq 0.05$) the A values, as well as the $k/2$, decreasing Gt_{50} ($p \leq 0.05$).

The interaction between the type of seed and the GA_3 application significantly affected A ($p \leq 0.01$; Table 4). It can be observed in Figure 3 that while with the GA_3 application, there were no differences ($p \leq 0.05$) between the germination obtained in seeds of type I (78.3%) and type II (74.3%), being high in both cases, when only water was applied to the substrate, the germination obtained by the type II seeds (63.5%) was higher ($p \leq 0.05$) than that obtained by those of type I (41.4%). On the other hand, the A value was higher ($p \leq 0.05$) with the GA_3 application in type I seeds (78.3%) than in type III seeds (61.0%), while there were no differences when only water was applied (41.4% and 42.8% for I and III, respectively; $p \leq 0.05$). It is worth noting the high germination obtained with type II seeds, both with water and GA_3 application, which agrees with that stated by [26], in the sense that the ideal state for the seed collection is just in the fruit dehiscence, since the seed quality is maximum during and shortly after the physiological maturity of the seeds.

Germination of the type IV seeds may be considered as very low (30.9% with GA_3 and 9.0% with water; on average $A \approx 20\%$), although it must be considered that these lots included both mature and immature seeds (because all of them floated in tap water, as mentioned above). Thus, these figures could underestimate, by a small percentage, the germination of the mature seeds. Specifically, considering for the seed types I, II, and III that on average, 35.75% (on weight basis) of the seeds floated, and that 13.15% of these seeds germinated (parallel studies; data not shown), the cited underestimation could be around 5% (specifically 4.7%). Therefore, the germination of the mature seeds in type IV could reach percentages up to 36% with the AG_3 application.

The interaction between the type of seed and the GA_3 application also significantly affected ($p \leq 0.01$) the Gt_{50} (Table 4), in the sense that Gt_{50} values obtained in the four types of seeds with the GA_3 application did not differ between them, requiring, on average, 23.3 d to reach the 50% of the corresponding final germination. However, when only water was applied to the germination substrate, the highest time ($p \leq 0.05$) was required by type III seeds (113.2 d) and the lowest time ($p \leq 0.01$) was required by type IV (80.6 d), but this shorter time of the latter is related with its low germination percentage (Figure 3).

Table 4. Effects of the type of seeds, the year of production, and the substrate saturation solution on the germination parameters: cumulative germination (*G*, %), final germination percentage (*A*, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate ($k/2$; d^{-1}); mean value.

	G	***A***	Gt_{50}	***k/2***
Type of seeds (ST)				
I	59.06 b	59.85 b	61.73 a	0.06
II	67.19 a	68.87 a	63.09 a	0.06
III	50.69 c	51.86 c	68.19 a	0.06
IV	20.06 d	19.95 d	52.55 b	0.07
Year of production (Y)				
2020	49.06	49.96	60.99	0.06
2019	49.44	50.30	61.78	0.06
Saturation solution (S)				
Water	37.09 b	39.13 b	97.96 a	0.04 b
GA_3	61.41 a	61.13 a	24.82 b	0.09 a
Analysis of variance				
Source (degrees of freedom)	% Sum of squares			
ST (3)	57.58 **	60.92 **	2.14 **	2.83 NS
Y (1)	0.01 NS	0.01 NS	0.01 NS	0.03 NS
S (1)	26.76 **	21.69 **	89.88 **	65.46 **
ST × Y (3)	1.35 NS	1.29 S	0.17 NS	2.72 NS
ST × S (3)	3.32 **	4.08 **	2.46 **	2.56 NS
Y × S (1)	0.06 NS	0.11 NS	0.00 NS	0.07 NS
ST × H × S (3)	0.59 NS	0.64 NS	0.22 NS	10.01 **
Residual (48)	10.33	11.27	5.12	16.33
Standard deviation	8.72	9.16	10.08	0.01

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

3.1.4.3. Experiment 3

As expected, the state of the seed at the start of the viability and germination test (consequence of the period between the extraction of the seeds from the fruits, which was carried out immediately after the collection, and the start of the tests) significantly affected ($p \leq 0.01$) the moisture content of the seed (Table 5). The moisture content of the FS was higher ($p \leq 0.05$) than that of DS, which in turn was higher ($p \leq 0.05$) than that of SS, although the difference between the last two are small in absolute value (about 1%), which indicates that the desiccation of the seed occurs mainly in the first days of drying.

The state of the seed significantly affected ($p \leq 0.01$) its viability, considering both the S and S + WV criteria (Table 5). The lowest values corresponded to the SS without differences between the FS and DS ($p \leq 0.05$), which means that seed viability decreases with storage, decreasing the proportion of S and increasing those of WV (0%, 10%, and 30% in FS, DS, and SS, respectively).

Both the state of the seeds and substrate saturation solution, as well as their interaction, influenced the final germination (Figure 4 and Table 6), the highest *A* value corresponding to FS and the lowest corresponding to SS ($p \leq 0.05$; Table 6). As occurred in experiment 2, the GA_3 application to the substrate increased the value of *A*. When analysing the interaction of both factors (Figure 4), the final germination percentage obtained with the GA_3 addition can be considered as very high in FS (89.2%) and high in DS and SS (72.1% and 72.6%, respectively). These *A* values were expected considering the viability obtained with the S criterion for FS and DS (97.5% and 82.5%, respectively) and with that obtained with the S + WV criterion for SS (77.5%). It can be

observed in Figure 4 that GA_3 considerably increased germination percentages compared to those germinated with water, in SS (64.2%), to a lesser extent in DS (35.6%), and even less in FS (25.8%). This seems to be related to a weakening of a part of the seed coat or to the low embryo growth potential, so that the increase in germination obtained with the GA_3 addition (compared to water) increases with storage, that is, the GA_3 effect was more important when the seeds had been stored and their viability had decreased. The high germination percentage obtained with seeds stored for one month (with the GA_3 application), which allows the distribution and sow of the seeds during this period, not being necessary to apply techniques such as priming. Priming is a practice used by the seed industry to increase the performance of commercial seed lots, improving the germination rate and uniformity [19,30]. Nevertheless, priming tends to shorten seed life in storage, and the benefits of priming can be lost during storage [19]. Recently harvested seeds (FS and DS) presented higher viability and germination values than those harvested six months before (SS), thereby agreeing with [13], who recommended a storage period for caper seeds no longer than two years because during this period, its viability does not significantly decrease, and high germination percentages can be obtained, although Gt_{50} increased for the seeds stored for one year compared to those stored for just one month. This highest viability and germination obtained in FS differs from seeds of other common families such as Asteraceae and Poaceae, among others [31], which present nondeep physiological dormancy and undergo after-ripening, that is, dormancy break during dry storage. The herein presented results, as well as those reported in [13,18], indicate that caper seed viability and germination not only do not increase with dry storage, but they decrease.

The GA_3 addition to the substrate reduced Gt_{50} ($p \leq 0.01$), obtaining similar values for the three periods (23.6 d on average, with no differences ($p \leq 0.05$) between them), while without the GA_3 application, the highest value ($p \leq 0.05$) corresponded to FS (103.7 d; with the highest final germination) and the lowest value ($p \leq 0.05$) corresponded to SS (56 d; related to its low germination value). The GA_3 addition to the germination substrate increased $k/2$ ($p \leq 0.01$).

Table 5. Effect of the state of the seed (fresh seeds (FS), dried seeds (DS), and stored seeds (SS)) on the seed moisture content (%) at starting the viability and germination tests and on the viability of the seeds (%), considering both the sound seeds (S) and the sound and weak but viable tissues (S + WV) criteria; mean values.

	Seed Moisture	**Viability (S)**	**Viability (S + WV)**
State of the seed			
FS	27.80 a	97.5 a	97.5 a
DS	9.95 b	82.5 a	92.5 a
SS	8.86 c	47.5 b	77.5 b
Analysis of variance			
Source (degrees of freedom)		% Sum of squares	
State (2)	99.55 **	72.2 **	79.4 **
Residual (9)	0.45	27.8	20.6
Standard deviation	0.68	15.0	5.0

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using Fisher's least significance difference (LSD) test. ** Indicates significant differences at $p \leq 0.01$.

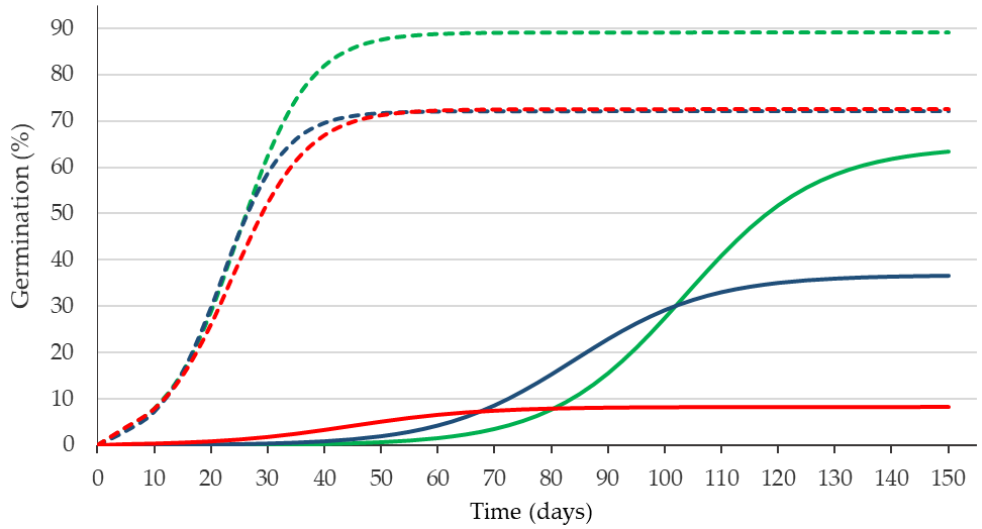

Figure 4. Logistic model fitted to the cumulative germination curves of caper seeds. Mean values for the combination of the state of the seeds (fresh seeds (FS), dried seeds (DS), and stored seeds (SS) in green, blue, and red, respectively) and substrate saturation solution (water in continuous lines and GA_3 solution in dashed lines).

Table 6. Effect of the state of the seed (fresh seeds (FS), dried seeds (DS), and stored seeds (SS)) and the substrate saturation solution on the germination parameters: cumulative germination (*G*, %), final germination percentage (*A*, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate ($k/2$; d^{-1}); mean values.

	G	***A***	***Gt*$_{50}$**	***k/2***
State of the seed				
FS	75.37 a	76.89 a	64.39 a	0.06
DS	54.62 b	54.38 b	53.04 a	0.07
SS	40.50 c	40.48 c	39.92 b	0.06
Saturation solution				
Water	34.92 b	36.54 b	81.28 a	0.04 b
GA_3	78.75 a	77.96 a	23.63 b	0.08 a
Analysis of variance				
Source (degrees of freedom)	% Sum of squares			
State (2)	26.33 **	29.48 **	8.94 **	2.11 NS
Solution (1)	61.66 **	56.19 **	74.32 **	61.87 **
State × Solution (2)	7.45 **	9.18 **	8.25 **	1.64 NS
Residual (18)	4.56	5.15	8.49	34.39
Standard deviation	6.88	7.24	11.25	0.02

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

3.1.5. Conclusions

In view of the obtained results, it is advisable to collect the caper fruits on the day of dehiscence; however, if the required labour is considered excessive, the fruits located immediately before and after the dehiscent one could also be collected. In practice, this is the equivalent of carrying out one pass per week. Caper seeds are sensitive to desiccation; accordingly, a general rule of thumb is to collect the fruits once a week, to extract the seeds, and to plant them immediately for germination by using a GA_3 solution to moisten the substrate.

3.1.6. References

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3.2. Criteria for the caper seed collection and selection for commercial use

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3.2.1. Abstract

The fruits of the caper (*Capparis spinosa* L.) have many seeds, but these shown a low germination percentage, especially in commercial batches. Although this research team has obtained germination percentages up to 95% in own-produced seeds, it has not exceeded 6% in commercial batches (both with GA_3 addition), probably due to the different collection and selection criteria. In our prior studies, the mature own- produced seeds were selected by flotation, presenting all of them a dark brown color (DB). However, in commercial batches it is common to find also brown (B) and light brown (LB) seeds. This study analyzes the germination response of seeds extracted from different stages of apparently mature fruits (before dehiscence (type I), immediately after dehiscence (type II), 2-3 days after dehiscence (type III) and dried fruits collected up to 10 days after dehiscence (type IV)) and different seed coat color (DB, B and LB). The percentage of seeds floating in the flotation test increased with the delay in collection (from 7% of type II to 100% of type IV), since the seeds are dehydrated after the fruit dehiscence, decreasing its density. It is remarkable that 95% of type IV seeds were DB, although all of them floated. The viability of the seeds that floated was not affected by the type of fruit, but it was statically related to its coat color (from 3% for LB to 24% for DB). Germination was affected by type of fruit and seed coat color and by their interaction, so that the maximum germination (22%) was obtained with DB seeds extracted from type IV fruits. The low germination obtained in the floating seeds leads to recommend to collect the ripe fruits before or immediately after dehiscence, since they presented the highest proportion (91-93%) of seeds precipitating in flotation test, and they can be considered ripe and of good quality.

Keywords: *Capparis spinosa* L., flotation method, seed coat color, seed viability, germination percentage

3.2.2. Introduction

The caper plant (*Capparis spinosa* L.) is a perennial deciduous sub-shrub, up to 1 m high, with branches up to 3 m long, and with deep roots. It is present in almost all the circum-Mediterranean countries (Juan, 2017). It is mainly known for their floral buds (capers), however, their fruits (caper berries) and to a lesser extent the vegetative shoots, are also consumed usually in pickles. The caper fruits have many seeds, but these show a low germination percentage (G) related to a non-deep physiological dormancy (Foschi et al., 2022a). Although the research team has obtained G up to 95% in own-produced seeds (Juan, 2017), it has not exceeded 6% in commercial batches (both with GA_3 addition). This low germination in commercial batches is usual (Foschi et al., 2020) and considering that seeds are used within their longevity period (3.85 years; Pascual et al., 2006), this low germination is probably due to the different collection and selection criteria. In previous research carried out by our research group, the seeds were extracted from ripe fruits collected just before or after their dehiscence and the mature seeds were selected by rejecting the light seeds through flotation method. The proportion of the seeds that floated, and therefore that were rejected was of little importance (on average less than 15%; both in number and weight; unpublished data) in relation to those that did not float and included mainly immature seeds and seeds with an undeveloped embryo (Pascual et al., 2003).

All of the selected mature seeds presented a dark brown color (DB). However, in commercial batches it is common to find also brown (B) and light brown (LB) seeds. In order to understand the low G obtained in the commercial seeds, fruits were harvested at four different maturation stages as reported in Foschi et al. (2022b), following the possible methodology applied by the seed companies. With this change in the criteria for collecting the fruits, it has been found that there is a greater percentage of seeds that float, including seeds similar in shape and color to the mature seeds that precipitate, which led us to think that if by rejecting this fraction we could be wasting seeds with a good viability and germinative power.

The objective of this study was to analyze the germination response of seeds that floated in the flotation test, that had been extracted from different stages of apparently mature fruits (before dehiscence, immediately after dehiscence, 2-3 days after dehiscence and up to 10 days after dehiscence) and with different seed coat color (LB, B and DB).

3.2.3. Materials and Methods

Seeds were extracted from ripe fruits that were collected from adult caper plants (subsp. *rupestris*), located in Llíria (València, Spain; 39°38'07.8" N, 0°35'53.4" W), in September 2020. Seeds were classified depending on the fruits from which they were extracted, according to their maturation stage: types I, II, III and IV. Type II included the seeds extracted from fruits collected the day of their dehiscence, while the seeds from fruits in the anterior (with dehiscence up to 3 days before the collection) and posterior position (with potential dehiscence up to 3 days after the collection) were classified as type III and I, respectively. Type IV were seeds obtained from dried fruits collected up to 10 days after dehiscence.

Seeds were extracted, and then classified by the flotation method (Davies et al., 2018), which involves placing seeds in water so that heavy, sound seeds sink to the bottom (sunk seeds) and the lightweight and empty seeds float to the top (floating seeds). Floating seeds were, in turn, classified according to their seed coat color: DB, B and LB (Figure 1). The proportion of each seed type (sunk seeds/DB/B/LB), was determined on weight basis. For each seed type, the thousand-seed weight (TSW) was determined according to International Rules for Seed Analysis (ISTA, 2018), counting and weighting 8 replications of 100 seeds. The bulk density of the seeds (Dursun and Dursun, 2005) was determined by filling, at a constant rate, a 50-mL cylindrical container with seed from a height of 150 mm and then weighing the contents; not applying any manual compaction of seeds. The bulk density was calculated from the mass of the seeds and the volume of the container. Seeds were stored in glass airtight containers at 7 ± 0.5 °C until the tests started.

Figure 1. Caper seed coat color. From left to right dark brown (DB), brown (B) and lightbrown (LB).

Color coordinates (L*, a* and b*) for each seed coat color, were measured by a Minolta CR-300 chroma meter (Konica Minolta Sensing Inc., Tokyo, Japan), placing the seeds on a flat and delimited surface to avoid the presence of gaps. For each seed coat color, measurements were done in triplicate in each of the four replications. L* represents the lightness, with values ranging from 0 to 100. With a* and b* values, the hue angle (H°) and chroma (C) were calculated as $H° = \arctan(b^*/a^*)$ (McGuire, 1992) and $C = \sqrt{(a^{*2}+b^{*2})}$ (Pathare et al., 2013), respectively. Seed coat color was compared with color chips contained in the Munsell color chart (Munsell, 1994). This system uses three indicators that refer to three attributes of color: hue, lightness and saturation.

The seed viability was determined by the tetrazolium topographic staining according to the International Rules for Seed Analysis (ISTA, 2003) using four replications of 50 seeds. Seeds were soaked in water at 20 °C for 18 h for preconditioning, after which they were cut longitudinally off at the widest Feret diameter and soaked in a 1% Tetrazolium solution (Tetrazolium Red. 2,3,5-Triphenyltetrazolium chloride; Sigma), at 30 °C for 18 h. Seeds were evaluated with a photomicroscope (U500X Digital Microscope; Cooling Tech, Guangdong, China), being the radicle

tip the maximum area of unstained tissue permitted to consider a seed as viable. The viability is the percentage of normal germinated seeds to be expected when the seed batch is germinated under favorable conditions and includes sound (staining proceeds gradually and uniformly from the exposed surfaces inward where changes in the color intensity are gradual without distinct boundaries) and weak but viable (stain greyish red or brighter red than normal) tissues (Patil and Dadlani, 1993). The proportion of empty seeds was also determined, so that the sum of the proportions of empty, viable and non-viable seeds corresponded to 100%.

Germination tests were carried out with the between-paper method, following the International Rules for Seed Testing (ISTA, 2018), which consists of using filter paper as a substrate and placing the seeds between two layers of filter paper in Petri dishes of 9 cm in diameter. The samples consisted of 400 seeds (4 replications of 100 seeds). The paper (Whatman Nº 1) was wetted with a GA_3 solution (500 mg L^{-1}; Semefil L; Nufarm), and 2 g L^{-1} of Captan (Captan 50; Bayer) was added to the treatments to avoid contamination with fungi. Petri dishes were placed in a growth chamber (model Zimbueze, Seville, Spain) at 30/20 °C, 85% relative humidity for a photoperiod of 12 h (cold white fluorescent tubes Philips TL-D 36W/54, providing 81.1 µmol m^{-2} s^{-1}), for a maximum of 120 days. The seeds were considered germinated when the emerged radicle reached a length of 2 mm. The trials were judged as satisfactory when the difference between the maximum and minimum germination percentages of the four replications did not exceed the tolerance established by the International Rules for Seed Testing (ISTA, 2018). For the analysis of the germination curves of each repetition the logistic function proposed by Torres and Frutos (1990) was applied: $G = A/1+e^{(\beta-kt)}$, where G is the percentage of accumulated germination, A is the maximum germination percentage, t is the germination period in days, β is a parameter of the function concerning the position of the curve relative to the time axis, and k is a velocity parameter. Last two parameters are used to calculate those with biological significance, such as the number of days needed to reach 50% of the final germination percentage ($\beta/k = Gt_{50}$) and the average relative rate of cumulative germination ($k/2$, day^{-1}).

The results were analyzed using analysis of variance (ANOVA) with Statgraphics Centurion 18 software (Statgraphics, 2018). The differences were considered significant for a probability of $p \leq 0.05$%. The percentage data were arcsin $\sqrt{x}$ transformed before analysis to accomplish the normality assumption. Mean separations were performed when appropriate, using the Fisher's least significance difference (LSD test) at $p \leq 0.05$.

3.2.4. Results and Discussion

Table 1 presents the proportion of seeds that sink to the bottom in the flotation test, showing that the highest percentage was obtained in type I and II fruits ($p \leq 0.05$), while no seeds sank to the bottom in type IV. Usually, seeds that sink to the bottom are those considered mature, and which are selected for their further germination, rejecting those that floated. Thus, in order to get the maximum proportion of mature seeds, fruits should be collected the day of their dehiscence or up to 3 days before it. Within the seeds that floated, there were a part that apparently were similar to those that sink, as they presented the same dark brown color; the proportion of this type of seeds was less than 2% in type I and II fruits, but it accounted for 41 and nearly the 95% in types III and IV, respectively. There were no differences in the proportion of B and LB seeds for the different fruit types.

Seeds that sank to the bottom were, as expected, those with the highest TSW and the highest bulk density (Table 2; $p \leq 0.05$), while within floating seeds, the lighter was the color of their coat, the lower ($p \leq 0.05$) was their TSW and their bulk density (without significant difference between B and LB), suggesting that the light color of the seed coat may be related with their lack of maturation. The TSW values obtained for the sunken seeds was slightly higher than those reported by Dursun and Dursun (2005) for seeds of *Capparis ovata* Desf. (ranging from 6.5 to 7.6 g). The lower TSW obtained in the other seed groups was expectable considering their flotation behavior, consequence, in turn, of their lower density. The bulk density obtained for the sunken seeds (0.47 g cm^{-3}; Table 2), was in accordance to that reported by Dursun and Dursun (2005).

Table 1. Effect of the fruit from which the seeds were extracted (up to 3 days before dehiscence (type I), immediately after dehiscence (type II), 2-3 days after dehiscence (type III) and dried fruits collected up to 10 days after dehiscence (type IV)) on the proportion (%) of seeds that sank to the bottom in the flotation test and those that floated classified by their seed coat color. Average values.

	Sank	**Floated dark brown**	**Floated brown**	**Floated light brown**
Fruit type				
I	91.0 a	1.8 c	2.2	5.0
II	93.0 a	1.4 c	0.5	5.1
III	52.9 b	41.0 b	2.2	3.9
IV	0.0 c	94.6 a	2.1	3.3
Analysis of variance				
Factors (degrees of freedom)	% Sum of squares			
Fruit type (3)	99.7**	99.4**	39.1 NS	29.8 NS
Residual (12)	0.3	0.6	60.9	70.2
Standard deviation [a]	2.6	3.5	1.0	1.3

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** indicates significant differences at $p \leq 0.01$. NS indicates no significant differences at $p \leq 0.05$. [a] The standard deviation has been calculated as the square root of the residual mean square.

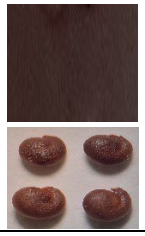
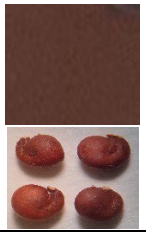
Table 2. Effect of the seed response on the flotation test, and for those that floated of their coat color on the thousand-seed weight (TSW; g) and seed bulk density (g cm^{-3}). Average values.

	TSW	**Bulk density**
Seed flotation		
Sank	8.245 a	0.474 a
Floated dark brown	5.908 b	0.249 b
Floated brown	3.325 c	0.164 c
Floated light brown	2.100 d	0.137 c
Analysis of variance		
Factors (degrees of freedom)	% Sum of squares	
Seed flotation (3)	96.6**	98.7**
Residual (12)	3.4	1.3
Standard deviation[a]	0.05	0.4

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** indicates significant differences at $p \leq 0.01$. [a]The standard deviation has been calculated as the square root of the residual mean square.

Table 3 presents the colorimetric parameters for each seed type. DB seeds, both sink and floating seeds, present the same values for H° and C, as well as the same color classification by the Munsell chart, regardless their flotation behavior. As expected, the lowest L* corresponded to the darkest seeds, and the highest L* was obtained for LB seeds. H° is rather similar for the different seeds, ranging between 51 and 58°. LB seeds presented the highest C value, indicating that its intensity or color saturation is higher, while the darkest seeds present the lowest C. The results obtained by the Munsell's chart indicate that all the different types of seeds were in the same tone of the chromatic spectrum (Hue 7.5 yellow red), and differ according to lightness and saturation, increasing both values in the lighter seeds.

Table 3. Colorimetric parameters according to the seed behavior in the flotation test and for those that floated to their seed coat color.

	Sank	Floated dark brown	Floated brown	Floated light brown
Lightness (L*)	25.1	25.0	28.3	35.5
Hue angle (H°)	57.3	57.9	50.9	58.3
Chroma (C*)	15.6	15.6	25.0	30.1
Munsell color	Dark brown 7.5 YR 3/3	Dark brown 7.5 YR 3/3	Brown 7.5 YR 4/4	Light brown 7.5 YR 5/8
Seed tonality				

The type of fruit from which the floating seeds were extracted did not affect their viability or the proportion of empty seeds (Table 4; $p \leq 0.05$). However, in the type IV fruits, 72.5% of the floating seeds were empty. This is related with the fact that these fruits were dried in the field, and many sound seeds could drop to the ground, or be eaten or carried off by birds or other animals (Davies et al., 2018), particularly, ants and bees.

The viability and the proportion of empty seeds were statistically affected by the seed coat color ($p \leq 0.01$), representing the 58 and 72% of their variability, respectively. Although, the highest viability, and the lowest percentage of empty seeds, corresponded to DB seeds, it is remarkable that the percentage of empty seeds was greater than 30%. In the LB seeds, the percentage of empty seeds was high (87.5%) and while the viability was low (3.1%), leading to negligible germination (0.9%; Table 4).

The coefficients of determination (R^2) for the 48 curves of the adjusted germination model (four repetitions of three combinations of sources of variation), presented values greater than 0.97. This allowed the use for the analysis of the variable A (instead of G), as well as the constants β and k to perform the calculations of the biological parameters Gt_{50} and $k/2$, as was done in previous studies of germination of caper seeds (Pascual et al., 2006; Juan, 2017). Figure 2 shows the cumulative germination curves adjusted to the logistic model for the seeds differentiated by color and the type of fruits they were extracted from.

The type of fruit, the seed coat color and their interaction affected A ($p \leq 0.01$; Table 4); analyzing the interaction, the highest A (Figure 2) corresponded to the DB seeds extracted from type IV fruits, which may be due to the fact that these fruits were collected when they were already dry, with both the pulp and the pericarp more degraded and dehydrated than in the other fruits, and therefore the seeds have also dried, and all of them floated, both mature and immature. Although, this A value was higher than in the other combinations ($p \leq 0.05$), germination was very low (22.2%). The 57% of the Gt_{50} variability was explained by the interaction of both studied factors (Table 4), highlighting the Gt_{50} for B seeds extracted from fruits of type IV (86 d; $p \leq 0.05$), even though it is not of great importance given the low germination obtained. As for the $k/2$, there were no differences in any of the factors evaluated.

Table 4. Effect of the fruit type and the seed coat color on the seed viability (%), percentage of empty seeds and germination parameters: final germination percentage (A, %), number of days needed to reach 50% of the final germination percentage ($\beta/k = Gt_{50}$) and the average relative rate of cumulative germination ($k/2$, day^{-1}) in the seeds that floated. Average values.

	Viability	**Empty seeds**	***A***	***Gt*$_{50}$**	***k/2***
Fruit type (T)					
I	10.8	60.9	2.1 c	16.3 b	0.07
II	12.5	60.0	4.3 b	29.5 a	0.19
III	13.3	63.4	3.3 bc	36.1 a	0.17
IV	13.3	72.5	9.7 a	35.3 a	0.18
Color (C)					
Dark brown	24.4 a	30.6 c	9.8 a	28.9 ab	0.25
Brown	10.0 b	74.4 b	3.8 b	37.7 a	0.11
Light brown	3.1 c	87.5 a	0.9 c	21.3 b	0.10
Analysis of variance					
Factors (degrees of freedom)	% Sum of squares				
T (3)	0.8 NS	3.0 NS	22.2 **	9.7 *	4.3 NS
C (2)	57.9 **	72.1 **	36.1 **	7.0 *	7.7 NS
F×C (6)	8.3 NS	0.9 NS	27.9 **	57.4 **	14.5 NS
Residual (36)	33.1	24.0	13.7	26	73.5
Standard deviationa	7.7	16.2	2.6	15.0	0.24

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. ** (*) indicates significant differences at $p \leq 0.01$ ($p \leq 0.05$). NS indicates no significant differences at $p \leq 0.05$. [a]The standard deviation has been calculated as the square root of the residual mean square.

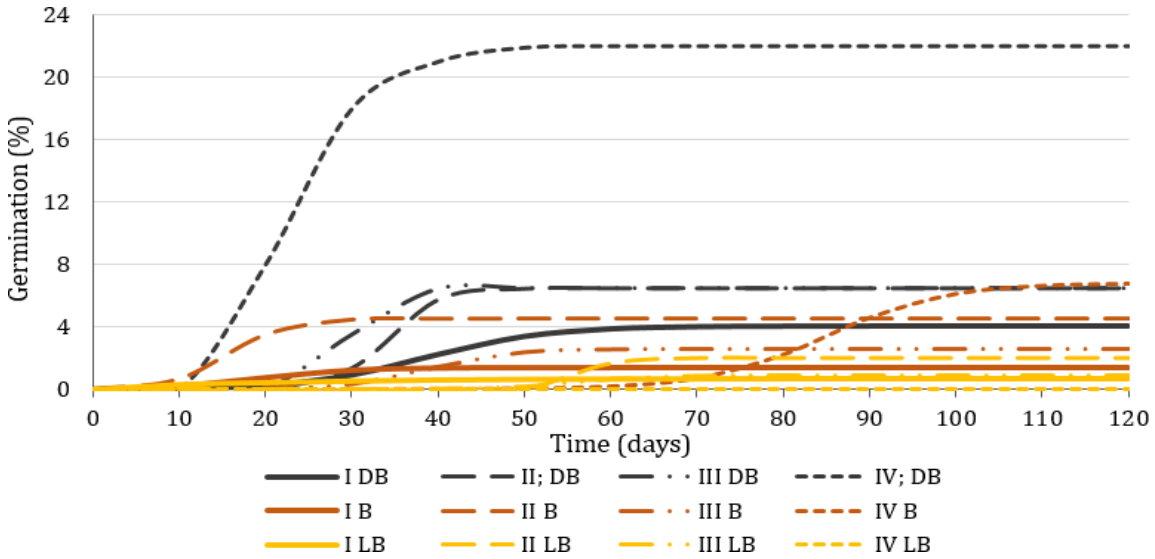


Figure 2. Logistic model adjusted to the accumulated germination of the seeds according to type of fruit (I, II, III, IV) and color of the seed coat: dark brown (DB), brown (B) and light brown (LB).

Overall, the highest germination obtained in this experiment was 22.2%, for DB seeds extracted from fruits of type IV ($p \leq 0.01$), while the rest of the seeds did not exceed 6.8%. The low germination values could be explained by their floatation features, which is considered of poor quality (Juan, 2017). The germination percentage of floating seeds extracted from type I and II fruits represents only 0.2 and 0.3%, respectively (considering that the floating fractions are 9 and 7% of the extracted seeds and that the corresponding germination percentages are 2 and 4%). These germination percentages are particularly low when compared with the maximum germination percentages

obtained with seeds that sank in the flotation test for the same fruit types, 78 and 74% for type I and II, respectively (Foschi et al., 2022b). Considering the high percentage of floating seeds obtained in fruits collected after their dehiscence (both type III and IV), it is recommended to collect the caper fruits just before or after their dehiscence and discard the floating seeds, regardless of their appearance.

3.2.5. Conclusions

According to these results, the flotation method is an effective method to get the mature and sound seeds from a seed batch after the extraction from the fruits. In order to obtain high germination percentages, the seeds that float should be discarded, regardless of their appearance, since their viability is very low. We recommended to collect the ripe fruits before or immediately after dehiscence, since they presented the highest proportion of seeds sinking in the flotation test, and considered to be of good quality.

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3.3. The imbibition, viability, and germination of caper seeds (*Capparis spinosa* L.) in the first year of storage

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3.3.1. Abstract

The caper is a shrub that adapts to harsh environments when it is established, but it presents serious difficulties in its propagation, both by cuttings and by seeds. Its seeds have low germination percentages, and germination is a very slow process. Significant increases in germination have been obtained with scarification and with the addition of gibberellic acid (GA_3) to the substrate, leading to the hypothesis that they have possible physical and physiological dormancy. However, the only way to examine the water-impermeability of the cover is through imbibition analysis. This study analyzes the imbibition, viability, and germination of two seed lots, obtained in different years and evaluated immediately after their collection (FS) and after being stored (7 ºC) for one month (DS) and one year (SS). The seed moisture content stabilizes from the fourth day, exceeding in all cases 31% in all three seed states tested (FS, DS and SS). This allows the germination of all viable seeds, only with the addition of GA_3 to the germination substrate, without the need for scarification, so that caper seeds exclusively appear to present a physiological latency. Germination decreased in storage, even with just one month. With the GA_3 addition, high germination values were obtained (up to 95% in FS).

Keywords: germination curve; germination percentage; seed moisture; tetrazolium

3.3.2. Introduction

The caper (*Capparis spinosa* L.) is a perennial shrub cultivated in the Mediterranean region, which can grow spontaneously in arid or semiarid areas. It has a creeping bearing and can reach a height of up to 0.5 m, having flexuous branches up to 3 m in length and deep roots making it resistant to drought [1,2]. It has solitary axillary flowers and flower buds; the fruit is an ovoid berry containing more than 150 seeds. The seeds are reniform, dark brown, and small with an average maximum Feret diameter of 3.3 mm.

It has a high agricultural potential since it presents a great variety of uses [3,4]. These include highlighting food (for its flower buds and immature fruits, which are usually pickled in brine [1,5]), in the pharmaceutical industry to prevent, among other things, cardiovascular and gastrointestinal diseases [6–9], and in xero-gardening and landscaping for its ornamental value [2,10], its resistance to drought, and its ability to reduce soil erosion [3,11].

One problem this species presents is the efficiency of propagation, both vegetative and sexual, through seeds. Although the fruits contain many seeds, they have a very low germination percentage. Several authors have reported various studies to improve its germination and try to break the possible physical dormancy with different types of scarification, whether mechanical, chemical, thermal, or biological, [12–15], as well as being able to break the physiological dormancy with the use of gibberellic acid (GA_3) and potassium nitrate, as reported by [16–19]. It has been proven that scarification with sulfuric acid and the addition of GA_3 have improved the germination percentage [12,20], which has led to the hypothesis that caper seeds can present physical dormancy (due to the imper- meability of their cover) and physiological dormancy imposed by the embryo. However, recently, [18] verified that the imbibition takes place through the hilum, and [14] obtained germination percentages up to 99% with only contribution of GA_3 without scarification, so they hypothesized that the dormancy caused by a waterproof seed coat should not be considered.

The longevity, also referred to as the half-life of the seeds, determined as the time taken for 50% of the seeds to die [21–24], of a caper seed lot stored at 7 ºC and obtained by our research team was around 4 years (3.85 years [20]; 4.15–4.43, for two seed lots [14]). However, both studies recommended a storage period of no longer than two years, because during this period, the viability did not decrease and high germination percentages were obtained. In previous studies [25], our group obtained higher and faster germination in freshly harvested caper seeds compared to that obtained in seeds collected several weeks before performing the germination test.

As Orozco-Segovia [26] stated, the only way to determine if seed coats are water-permeable is to conduct imbibition studies. In this sense, an imbibition test was performed according to [18] using the Between Paper method (BP) [27], reproducing the conditions of the germination test, since in previous studies [14], no significant differences were detected between the moisture contents of the seeds soaked in a 10 cm water column and moistened with the BP method, neither with the use of water nor GA_3 to moisten the seeds.

The low germination percentages obtained with seeds from commercial lots are known [18]. Thus, the research team is conducting different studies to make the use of these seeds viable and profitable. The objective of this work is to evaluate the effect of the state of the seeds in the imbibition, viability, and germination during the first year of storage, specifically the state of the seeds immediately after the collection and extraction of the fruits (fresh seeds, FS), seeds stored for 30 days (dried seeds, DS), and seeds stored for one year (stored seeds, SS). Given that in previous studies [12,18], the application of GA_3 to the substrate significantly increased the caper seeds germination, the substrate was moistened both with water or a GA_3 solution in the germination test.

3.3.3. Materials and Methods

3.3.3.1. Plant Material

The seeds used in this experiment correspond to two lots of caper seeds produced by adults grown in an experimental plot at the Universitat Politècnica de València (39º29′02.1″ N 0º 20′23.9″ W; Valencia, Spain). The harvesting of the seeds in each lot was carried out during the second half of September in 2019 and 2020, one lot for each year. The seeds were classified into three groups according to the drying (or not) and the storage period: fresh seeds (FS), extracted from the fruits, cleaned, and set to germinate immediately without letting them dry; dried seeds stored for 30 days (DS); and dried seeds stored for one year (SS).

The seeds were extracted from ripe fruits collected on the day of their dehiscence and from fruits located in the position before and after it. Then, the seeds were disinfected with sodium hypochlorite for 10 min and rinsed twice in tap water. The mature seeds were selected by rejecting the light seeds through flotation in tap water. Flotation is a common method for separating viable from nonviable seeds, which involves placing the seeds in water so that heavy, sound seeds sink to the bottom and the lightweight and unfilled seeds float to the top [28]. Once the mature seeds were separated, the tests for the FS started. The rest of the seeds were dried in the shade at room temperature (23–25 ºC, 20–50% relative humidity) for two weeks, after which they were kept in closed airtight containers at 7 ± 0.5 ºC in a domestic refrigerator (Beko, Beko Electronic España, Barcelona, Spain) until the tests were conducted.

3.3.3.2. Imbibition

The imbibition test was performed according to [18] using the BP method [27], and the seeds were soaked through the paper, which was moistened with pure water (Wasserlab G.R. Type II analytical grade water system; referred to as water). The determinations were made over 8 days to ensure maximum absorption of solution, as indicated by [26]. The BP method was performed with 9 cm diameter Petri dishes and two layers of filter paper, Whatman No 1 [27], at laboratory room conditions (23–25 ºC, 20–50% relative humidity). Four replications of ten seeds each per seed status and year of production were considered. The moisture content of the seeds was determined according to the ISTA standards [27] and the water absorption (imbibition) according to the methodology described in [26], for which, the seeds were removed from the Petri dishes hourly during the first day and once every day afterwards. They were blotted with a paper towel, immediately weighed on a precision balance (Sartorius, model B 120S, Barcelona, Spain), and returned to the Petri dish [18]. The calculation of the imbibition is presented as the accumulated percentage of absorbed water, expressed as the increase in fresh weight (%) in each day (i) with respect to the initial weight of the seeds:

$$\text{Imbibition (\%)} = 100 * (\text{Fresh weight}_i - \text{Initial fresh weight})/\text{Initial fresh weight} \quad (1)$$

After the imbibition period, the four samples of each treatment were dried for 48 h at 103 ºC in a forced-air oven (Selecta 297; Selecta, Barcelona, Spain) to determine the dry weight. The daily seed moisture content (including the initial seed moisture) was calculated on a fresh mass basis [27]:

$$\text{Seed moisture (\%)} = 100 * (\text{Fresh weight}_i - \text{Dry weight})/\text{Fresh weight}_i \quad (2)$$

3.3.3.3. Viability

The viability and vigor of the seeds were determined by the tetrazolium topographic test according to the International Rules for Seed Analysis [27]. Four replications of fifty seeds each were performed. Seeds were soaked in water at 20 ºC for 18 h for preconditioning, after which they were cut longitudinally off at the widest Feret diameter and soaked in a 1% Tetrazolium solution (Tetrazolium Red. 2,3,5-Triphenyltetrazolium chloride; Sigma) at 30 ºC for 18 h [29]. Seeds were evaluated with a photomicroscope (U500X Digital Microscope; Cooling Tech, Guangdong, China) with the radicle tip the maximum area of unstained tissue permitted to consider a seed as viable. The viability is the percentage of normal germinable seeds to be expected when the seed lot is germinated under favorable conditions and includes sound (staining proceeds gradually and uniformly from the exposed surfaces inward where changes in the color intensity are gradual without distinct boundaries) and weak but viable (stain greyish red or brighter red than normal) tissues, as reported by [30].

3.3.3.4. Germination

Germination tests were carried out with the BP method following the International Rules for Seed Testing [27], which consists of using filter paper as a substrate and placing the seeds between two layers of filter paper in Petri dishes of 9 cm in diameter. The samples consisted of 400 seeds (4 replications of 100 seeds). The paper (Whatman Nº 1) was moistened with two solutions, one of 500 mg L^{-1} of GA_3 (Semefil L; Nufarm), and another of water. In both cases, 2 g L^{-1} of Captan (Captan 50; Bayer) was added to the treatments to avoid contamination with fungi. Petri dishes were placed in a growth chamber (model Zimbueze, Seville, Spain) at 30 ± 1 / 20 ± 1 ºC, 85 ± 1% relative humidity for a photoperiod of 12 h (cold white fluorescent tubes (Philips TL-D 36W/54), providing 81.1 µmol m^{-2} s^{-1}) for a maximum of 120 days.

The seeds were considered germinated when the emerged radicle reached a length of 2 mm. The trials were judged as satisfactory when the difference between the maximum and minimum germination percentages of the four replications did not exceed the tolerance established by [27]. For the analysis of the germination curves of each repetition, according to studies carried out previously by [14,30], the model that best fits is that of the logistic function proposed by [31] and has the following expression:

$$G = A/1 + e^{(\beta - kt)} \quad (3)$$

where G is the percentage of accumulated germination, A is the maximum germination percentage, t is the germination period in days, β is a parameter of the function concerning the position of the curve relative to the time axis, and k is a velocity parameter. Both are used to calculate parameters with biological significance, such as the number of days needed to reach 50% of the final germination percentage ($\beta/k = Gt_{50}$) and the average relative rate of cumulative germination ($k/2$, day^{-1}).

3.3.3.5. Statistical Analysis

The results were analyzed using analysis of variance (ANOVA) with Statgraphics Centurion 18 software [32]. For the imbibition and viability tests, the analysis was performed as a two-way

ANOVA, and a three-way ANOVA was used for the germination test. The differences were considered significant for a probability of $p \leq 0.05\%$. The percentage data were arcsin $\sqrt{x}$ transformed before analysis to accomplish the normality assumption. In this study, the normality distribution was analyzed by verifying the residuals normal distribution [33] by the Shapiro-Wilk test [32]. The separation of means was performed using Fisher's minimum significant differences test (LSD test) in $p \leq 0.05$.

3.3.4. Results and Discussion

3.3.4.1. Imbibition

In none of the analyzed parameters (Table 1) were there significant differences between the two years of evaluation, 2019 and 2020. The initial moisture of FS was higher ($p \leq 0.05$) than that of DS and SS, with no differences ($p \leq 0.05$) between the latter two.

Table 1. Effect of the state of seeds (fresh (FS), stored for 30 days (DS), and stored for one year (SS)) on its moisture (M; %) and its accumulated imbibition (I; %) after 0, 12, 24, and 96 h in two years of production.

	0 h	**12 h**		**24 h**		**96 h**	
	M	**I**	**M**	**I**	**M**	**I**	**M**
State of the seed (S)							
FS	25.1 a	5.8 b	30.5 a	9.3 b	32.7 a	9.9 c	33.1
DS	9.8 b	22.9 a	26.1 b	28.9 a	29.6 b	33.0 a	31.8
SS	9.5 b	21.5 b	26.2 b	28.7 a	30.3 b	31.5 b	32.2
Year (Y)							
2019	14.7	16.6	26.9	22.9	30.7	25.3	32.2
2020	14.8	16.9	28.3	21.6	31.0	24.3	32.5
Analysis of Variance							
Source (degrees of freedom)	**% Sum of squares**						
S (2)	99.8 **	92.0 **	54.8 **	94.07 **	47.5 **	98.9 **	13.4 NS
Y (1)	0.0 NS	0.0 NS	6.1 NS	0.5 NS	0.8 NS	0.2 NS	1.1 NS
S × Y (2)	0.0 NS	1.2 NS	3.4 NS	0.2 NS	6.5 NS	0.0 NS	4.1 NS
Residuals (18)	0.1	6.8	35.7	4.5	45.2	0.9	81.3
Standard deviation	0.3	2.4	1.9	2.3	1.5	1.2	1.6

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

These differences in the initial seed moisture ($p \leq 0.05$) were maintained during the first 24 h, decreasing over time (Figure 1a). There were no differences ($p \leq 0.05$; Table 1) in the statistical analysis performed at 96 h, in which the moisture content of the three states seeds was about 32%. According to Juan [14], the initial moisture of DS and SS was adequate for its conservation, while the moisture reached in the three seed states was sufficient for efficient germination, which generally ranges between 25 and 50% depending on the species [34–36], and particularly for caper seeds as [14,18] stated. The evolution of the imbibition is shown in Figure 1b. The FS presented the lowest imbibition values ($p \leq 0.05$, Table 1) since at the end of the test, the seeds of the three states reached similar moisture contents (Figure 1a), while the initial moisture of FS was higher ($p \leq 0.05$) than those of DS and SS (Table 1).

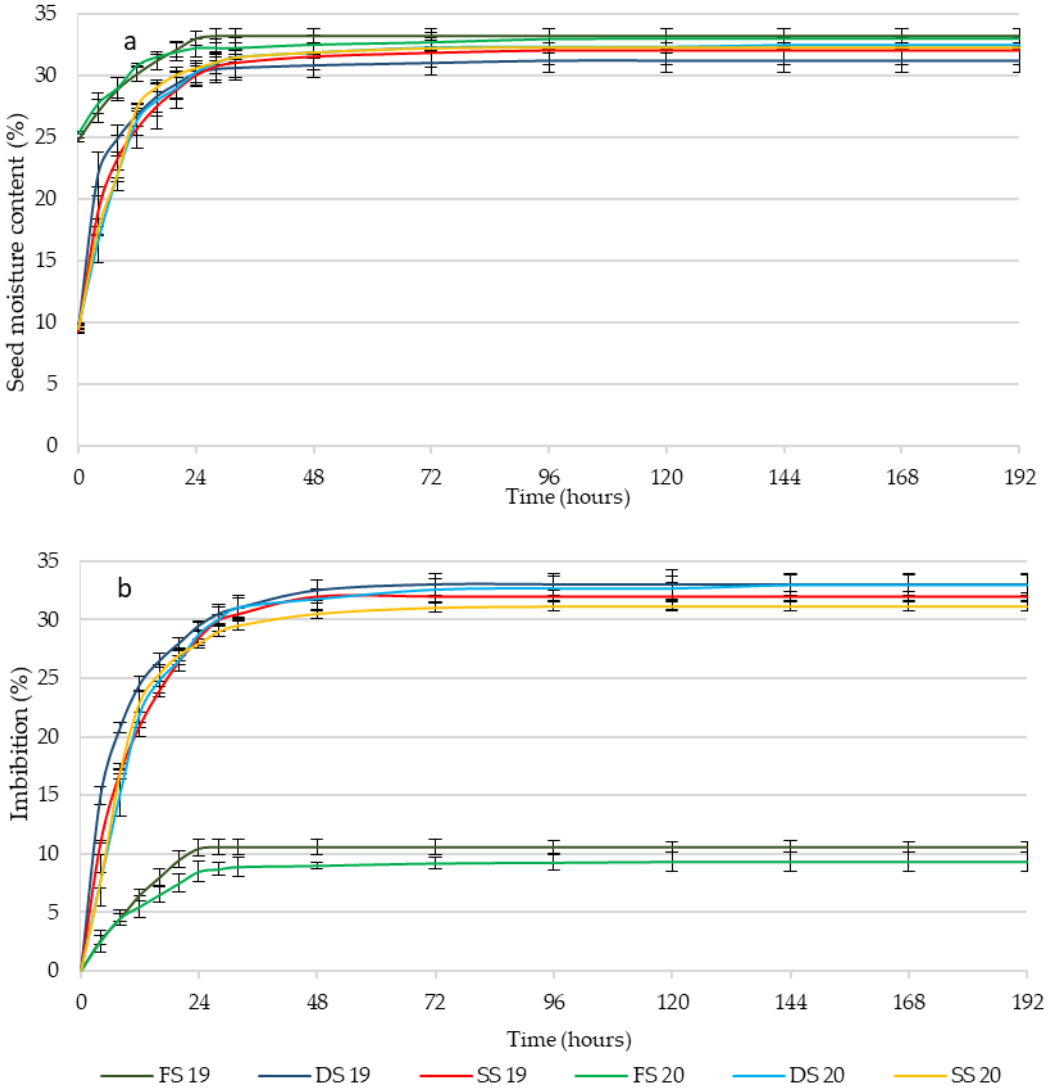

Figure 1. Evolution of the moisture content (**a**) and the accumulated imbibition (**b**) of the seeds (fresh (FS), dried and stored for 30 days (DS), and stored for one year (SS)) during the imbibition process during two years of production: 2019 and 2020. Vertical bars represent the standard error.

In the seed moisture evolution curves (Figure 1a), the first two phases of the absorption of the three-phase model of water absorption in the germination of the seeds are clearly observed. There is initially a phase of rapid water absorption, followed by a second phase in which it stabilizes and begins the activation of metabolism and the mobilization of nutrients [28,37].

3.3.4.2. Viability

As Bewley et al. [37] reported, many factors can affect the initial quality of the seeds before storage, particularly the maturity of the seeds at harvest, the conditions during drying, and the handling of the seeds before starting to monitor their viability. The initial viability of the seeds was very high (on average 95.9% for FS) as expected, due to the fruits being harvested at their physiological maturity and the careful extraction, cleaning, and handling of the seeds. There were also no differences ($p \leq 0.05$) between seed lots (seeds collected in 2019 and 2020).

Viability showed a slight tendency to decrease with drying and storage, but these differences were not significant (Table 2), so it can be indicated that viability was maintained during the first year of storage. The conditions in which seeds are dried and stored greatly affect their deterioration rate and, hence, their ability to survive in storage. Among the factors that can

influence the evolution of seed viability, the two most important are its moisture content and the storage temperature [37]. The low moisture content of the seeds can cause their deterioration due to desiccation damage [38]; deterioration is caused by lesions from drying out or a longer time of storage and by the inability to repair these lesions. Unrepaired lesions can delay or avoid the cellular changes needed for the completion of germination, leading to cell dysfunction and death. The spatial relationships between the molecules determine the level of viability of the seeds and their longevity [38,39]. In this case, the seeds were dried in the shade under laboratory conditions, and after drying, the seeds were stored at a low temperature (7 ºC), that is, under ideal conditions for drying and storage.

Table 2. Effects of the state of the seeds (fresh (FS), dried and stored for 30 days (DS), and stored for one year (SS)), of the year of production and of the saturation solution used, on viability (V; %), accumulated germination (*G*; %), final germination (*A*; %), number of days needed to reach 50% of *A* (Gt_{50}), and the average relative rate of accumulated germination ($k/2$, day^{-1}).

	V	*G*	*A*	Gt_{50}	*k/2*
State of the seed (S)					
FS	95.9	60.9 a	60.9 a	40.4	0.081
DS	90.1	48.4 b	48.1 b	38.8	0.112
SS	89.2	47.1 b	46.7 b	44.5	0.099
Year (Y)					
2019	92.5	52.5	52.4	39.4	0.108
2020	90.4	51.7	51.4	44.4	0.088
Saturation solution (Sol)					
Water	----	13.6 b	13.6 b	58.0 a	0.104
GA_3	----	90.7 a	90.2 a	25.8 b	0.091
Analysis of Variance					
Sources (degrees of freedom)		% Sum of squares			
S (2/2)	36.6 NS	2.5 **	2.7 **	1.7 NS	4.8 NS
Y (1/1)	4.0 NS	0.0 NS	0.0 NS	1.4 NS	2.8 NS
Sol (0/1)	----	96.4 **	96.1 **	58.2 **	1.2 NS
S × Y (2/2)	0.3 NS	0.0 NS	0.1 NS	4.7 NS	4.0 NS
S × Sol (0/2)	----	0.4 **	0.5 **	1.0 NS	5.6 NS
Y × Sol (0/1)	----	0.0 NS	0.1 NS	3.0 NS	2.8 NS
S × Y × Sol (0/2)	----	0.0 NS	0.1 NS	2.7 NS	11.8 NS
Residuals (18/36)	59.0	0.5	0.5	27.3	67.1
Standard deviation	4.6	3.2	3.2	12.7	0.1

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

3.3.4.3. Germination

In all cases, the germination data were adjusted to the logistic function ($p \leq 0.01$), and the coefficients of determination (R^2) for the 48 curves of the adjusted germination model presented values greater than 98.4% (data not shown). This indicates that the use of the logistic function is suitable for analyzing the germination of caper seeds (Figure 2), as in previous studies [18,20]. This allowed the use of variable *A* (instead of *G*) to analyze the germination percentage of the seeds, as well as the constants β and *k*, to be able to calculate the biological parameters of germination Gt_{50} and *k/2*, as was used in previous studies on the germination of caper seeds [18,20].

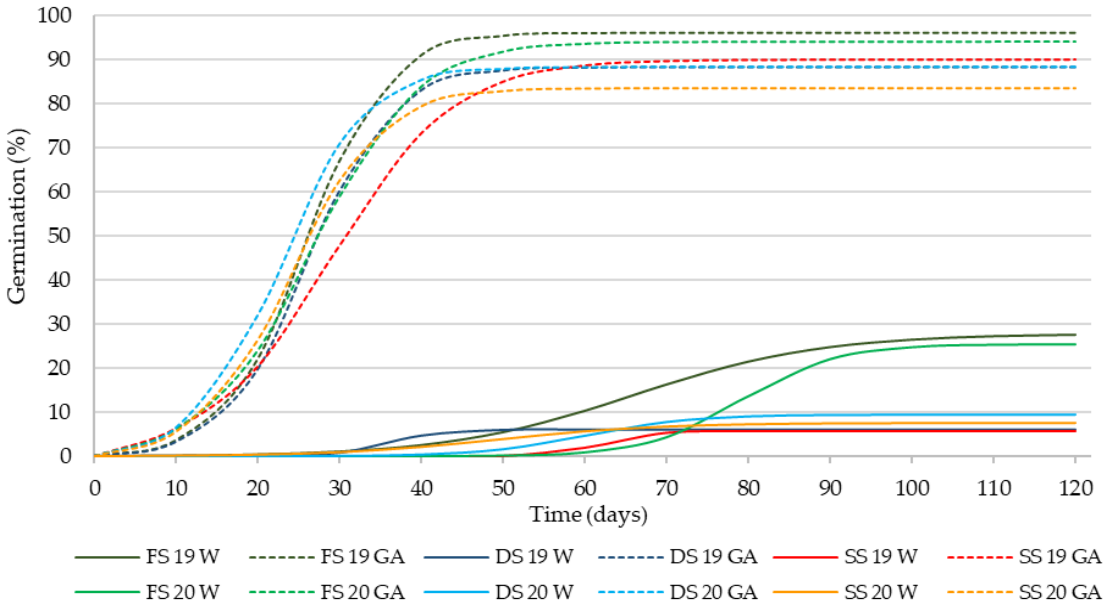

Figure 2. Logistic model adjusted to the germination curves of the seeds (fresh (FS), dried and stored for 30 days (DS), and stored for one year (SS)) for two years of harvesting (2019 and 2020), and with the substrate moistened with distilled water (W) and with a solution of GA_3.

The high percentages of germination obtained with GA_3 are consistent with those of viability (item 3.2), since the results obtained in the viability and germination tests when properly conducted are generally very close [29]. These germination percentages are also in accordance with those obtained by our team in studies previously conducted with our seeds, in which longevity of around 4 years was estimated (3.85 years, [20]; 4.15–4.43, for two seed lots [14]), in which germination remained nearly constant during an initial period, declining thereafter. The results obtained in this study, considered together with those obtained in the aforementioned works, coincide with those reported by [37], in the sense that the shape of the viability curves are often symmetrically sigmoid once viability begins to decline.

Germination was significantly higher ($p \leq 0.01$) after moistening the substrate with the GA_3 solution than with water (on average 90.2% and 13.6%, respectively). In relation to the production year, no significant differences were observed for any of the germination parameters analyzed. The factor that had the greater effect on germination was the solution used to moisten the substrate, which explained 96% of the variability of the data (Table 2). This shows the importance of using gibberellins to obtain high germination percentages in caper seeds.

From the analysis of the significant interaction ($p \leq 0.01$; Table 2 and Figure 3), "State of the seeds * Solution to moisten the substrate", it was found that the FS germinated in greater proportion than those stored, both with water (26.6%) and with the addition of GA_3 to the substrate (95.2%). The values of final germination with the addition of GA_3 practically coincided with those of viability, that is, all seeds considered viable germinated. It is worth highlighting the role played by GA_3 as mentioned above. The high germination percentages obtained with the addition of GA_3 to the substrate (≥87%) coincided with those obtained in other seed lots of own production obtained in different seasons [14,18]. The high germination percentages maintained during the first year of storage permit the seed distribution and sowing during an entire year, allowing sowing when the weather conditions are right, without requiring the use of such techniques as priming, that present greater or lesser complexity.

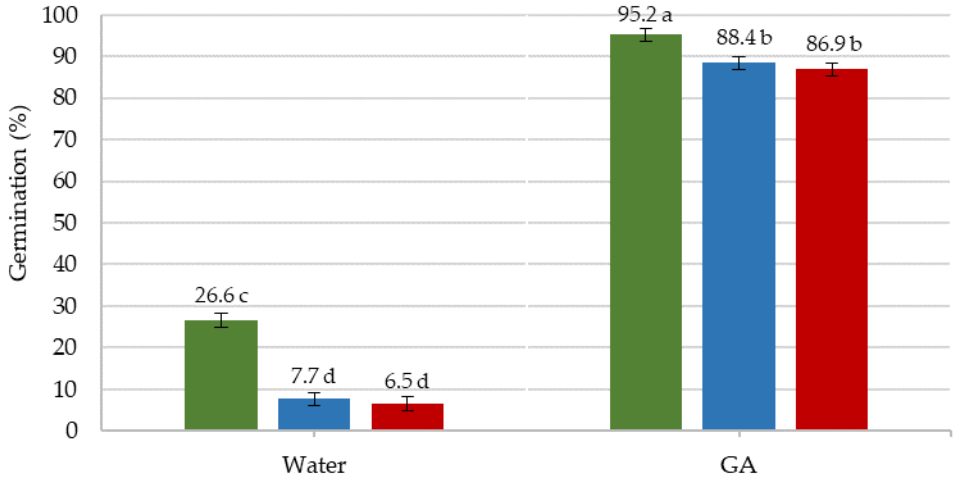

Figure 3. Analysis of the significant interaction between the state of the seeds (fresh (FS) in green, dried and stored for 30 days (DS) in blue, and for one year (SS) in red) and the saturation solution used, water and GA_3, in the final germination. Different letters in each bar indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD shown as error bar) test.

Regarding the Gt_{50}, it was only affected ($p \leq 0.01$) by the solution used, and according to [18], the GA_3 reduced the value of Gt_{50} (26 d) with respect to water (58 d). Storage for a year slightly (but not significantly) increased the Gt_{50} in relation to FS and DS, which agrees with [20]. $k/2$ was not affected ($p \leq 0.05$) by the storage period of the seeds or the solution used.

3.3.5. Conclusions

In the germination process, water absorption by seeds increased considerably in the first 24 h, reaching approximately 80% of the total water absorbed. The seed moisture content stabilized after 96 h, reaching a similar value (32%) in the three seed states tested (fresh and after being stored for one month and one year). This allows the germination of all viable seeds, only with the addition of GA_3 to the germination substrate, without the need for scarification. The use of gibberellins was essential to obtain germination percentages close to those of the viability of the seed lot. The viability of the seeds decreased slightly with drying and storage, although without statistical significance. The germination percentage decreased significantly with drying and initial storage, remaining similar in value after storage for one month and one year. From the abovementioned, it can be concluded that caper seeds do not present physical dormancy caused by the impermeability of the seed coat and that the low germination could be due to a non-deep physiological dormancy. The information obtained in this study may be of great interest for seed producer companies and nurseries to obtain viable and profitable caper propagation. Further studies will be carried out with seeds in their first year of storage applying GA_3, using seedling trays and pots, both in the greenhouse and in the field to transfer this knowledge to the process of obtaining plants in the nursery.

3.3.6. References

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Capítulo 4. Tratamientos infrautilizados en los ensayos de germinación de las semillas de alcaparra

4.1. Influence of lighting and laser irradiation on the germination of caper seeds

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4.1.1. Abstract

Caper seeds present difficulties in their germination, which has been studied by several research teams. It is known that light can release dormancy in some seeds, but its effect on caper seed germination has not yet been deeply studied. The main aim of this study was to analyze the response of caper seeds germination to light exposure. The study analyzed the germination response of seeds to lighting with different wavelengths (white, red, blue, red + blue and darkness) and to the He-Ne laser light, using both dry seeds and seeds that had been previously soaked in water. Overall, it could be stated that caper seeds are insensitive to light during the germination process. Thus, germination could be carried out in lightness or darkness, so germination in nurseries could be carried out in the darkness, leading to substantial energy savings. Caper seed irradiation with a He-Ne laser during short exposure times improved the germination percentage for the seeds previously soaked in water, germinating all viable seeds. However, applying a solution of gibberellic acid was always required in all the cases studied.

Keywords: *Capparis spinosa* L., darkness; gibberellic acid; light-emitting diodes (LEDs); light wavelength; soaking

4.1.2. Introduction

The caper (*Capparis spinosa* L.) is a deciduous creeping shrub native to Asia, which spreads throughout the Mediterranean basin, where it grows in dry lands. It is currently cultivated in Spain, Italy, France, Greece, and North Africa, as well as in South America [1,2]. It is mainly cultivated for the floral buds, called capers; however, their fruits and, to a lesser extent, their vegetative shoots are also consumed pickled or salted. As Shahrajabian et al. [3] have stated, different parts of the plant are rich sources of antioxidants and bioactive compounds beneficial to health. Furthermore, the flowers have a high ornamental value; thus, caper plants are included in gardening, particularly in xeriscape [4,5].

In a recent review article, Sottile et al. [1] stated that, as a crop, caper should not be considered a difficult crop to propagate. Pascual et al. [6] reported acceptable percentages of success in the rooting of cuttings, and Foschi et al. [7–9] obtained high germination percentages of dry seeds using gibberellic acid and without the need for any treatment in fresh seeds. These germination tests were carried out in a growth chamber under a photoperiod of 12 h.

Light is one of the main environmental signals for plants [10], being an important factor in breaking seed dormancy [11]. To respond to environmental signals, plants have developed several families of photoreceptors, which are photosensitive pigments capable of being activated by photons of specific wavelengths and, in turn, activating signal translation pathways, providing the ability to respond to light stimuli [12]. These photoreceptors include the following: (i) Phytochromes, which are red (600–700 nm) and far red (700–800 nm) light photoreceptors; (ii) Cryptochromes, which are photoreceptors for blue (400–500 nm) and ultraviolet A (320–400 nm) light; (iii) Phototropins, another group of blue and UV-A light photoreceptors [10,13].

Butler et al. [14] obtained a photo-reversible pigment from etiolated shoots of maize and named it phytochrome. Later, in studies carried out on *Arabidopsis thaliana* (L.) Heynh., Sharrock and Quail [15] identified sequences that showed that small families of genes encode phytochromes. Specifically, these authors hypothesized that the minimum number of phytochrome genes present in higher plants could be determined in studies in *A. thaliana*. They stated that phytochromes in this plant were encoded by a small gene family consisting of at least three genes and probably four or five. Subsequent studies have shown that *A. thaliana* contains five different phytochromes (*phyA, phyB, phyC, phyD* and *phyE*) [10] that are encoded by five genes (*PHYA, PHYB, PHYC, PHYD* and *PHYE*). Cryptochromes were first identified in *A. thaliana* [16]. Different organisms have different numbers of cryptochromes; plants have at least two types of cryptochromes, and this number can range up to six, as in soybean (*Glycine max* L.) [16].

According to sensitivity to white light, seeds have been classified into three categories [17]: (i) positive photoblastic (the seeds that germinate only under white light); (ii) negative photoblastic (the seeds that germinate only in the dark); (iii) light insensitive (the seeds that germinate both under white light and in darkness). A Photoblastic Index (PI; Equation (1)) has been utilized to evaluate the photoblastic responses [18]:

$$PI = (GD - GL)/(GD + GL) \quad (1)$$

GD is the germination (%) in darkness, and GL is the germination (%) under light. This index ranges from 1 (negative photoblastism) to - 1 (positive photoblastism); PI = 0 indicates that germination is not dependent on light.

Another index expressing a light requirement is the Relative Light Germination (RLG; Equation (2) [19]):

$$RLG = (GL)/(GD + GL) \quad (2)$$

GD is the germination (%) in darkness, and GL is the germination (%) under light. The RLG values vary from 0 (only seeds exposed to darkness germinate) to 1 (only seeds exposed to light germinate), with values close to 0.5 in seeds germinating both in light and darkness.

Takaki [20] proposed to replace the term photoblastism with forms of phytochrome that control germination, proposing a classification based on three mechanisms, depending on the level of fluence (total energy received by a seed in a period of time, J m^{-2} [21]) to saturate the responses [20,22]:

Low Fluency Responses (LFRs), which represent the classic reversible red/far red responses, in which Pfr (the active form of phytochrome, which absorbs light of 735 nm) production promotes plant responses and removal of Pfr reverses the response. These seeds have *phyB* controlling the germination process through LFR. The saturation of the response frequently occurs at low levels of Pfr/Ptotal (10^{-2} 0.87 Pfr/Ptotal) and intermediate fluences (1–1000 μmol m^{-2} s^{-1}).

High Irradiance Responses (HIRs), which represent responses produced by prolonged high irradiation, which do not show reciprocity or reversibility. These seeds have *phyA* controlling germination through HIR, and the maximum reaction generally occurs at wavelengths that maintain low Pfr levels for long periods of time, such as occurs in far-red- rich environments.

Very low fluence responses (VLFRs), which represent the saturation of responses by very low fluences, with reciprocity but no reversibility because the photo-equilibrium maintained by far-red light (or even safe dim green light used in experiments) produces enough Pfr to saturate these responses, which occur at low levels of Pfr (10^{-6}–10^{-3} Pfr/Ptotal). These seeds have *phyA* controlling the germination process through VLFR.

LED (Light Emitting Diode) lights are an alternative to incandescent lamps and cold white fluorescent tubes for growing plants. They have several advantages due to their small size, long lifespan, low emission temperature, high efficiency in energy conversion, and the possibility of selecting specific wavelengths [23,24]. In the last two decades, several studies have shown that LEDs of different wavelengths can modify the germination, growth, and development of seedlings in many species [11,25–30]. In this type of light, which is mainly monochromatic, it is essential to know the optimal light spectrum and the intensity required by the different species at each phenological stage to optimize yield and quality [28].

The irradiation of seeds with laser light (Light Amplification by Stimulated Emission of Radiation) can also be an alternative to improve the germination or growth and development of seedlings of various species based on the bio-stimulant effect of laser light [31–33]. Laser irradiation effects depend on many laser parameters, such as wavelength, irradiation duration, power, dose, and method (constant/pulse) [31]. However, seed properties are also important, particularly their genetic traits and physiological properties (health status, seed quality), and even their orientation during irradiation [31,32].

Among the different lasers used in agriculture, the helium-neon laser (He-Ne) is the most used [31], considering that its wavelength of 632.4 nm corresponds to the red light which is responsible for phytochrome activation [32]. The improvement and acceleration of germination have been related to an induction of the enzymatic activities, a change of thermodynamic parameters, and an acceleration of physiological and biochemical metabolism of seeds, increasing, in some cases, the levels of gemination-promoting growth hormones. such as gibberellic acid (GA_3). and decreasing inhibitors, such as abscisic acid [34–39].

Light and gibberellins can release dormancy in some seeds [40], specifically in those that present coat dormancy, promoting their germination [41]. Caper seeds have a nondeep physiological dormancy, specifically a coat-imposed dormancy due to a mechanical characteristic, which can be released by adding GA_3 to the germination substrate [7]. As far as is known, the effect of light on caper seed germination has not yet been deeply studied. The main aim of this study was to analyze the response of caper seeds germination to light exposure. Particularly, the study analyzes the germination response of seeds to lighting with different wavelengths (white, red, blue, red + blue and darkness) and to the He-Ne laser light.

4.1.3. Materials and Methods

4.1.3.1. Plant Material

Caper (*Capparis spinosa* L.) seeds were extracted from ripe fruits produced by adult plants grown in Llíria, Valencia, Spain (39º 38′ 54.2″ N, 0º 37′ 3.5″ W). The fruit collection was carried out over the first fortnight of September 2019, 2020, and 2021, each constituting a different lot.

After the extraction, mature seeds were selected using the flotation method [42] with tap water. The seeds were disinfected by soaking them in a 25% sodium hypochlorite solution and then rinsing with tap water. They were dried for 15 days at room temperature in the shade and kept in hermetically sealed glass containers at 7 ± 0.5 ºC in a domestic refrigerator (Beko, Beko Electronics España, Barcelona, Spain). At the beginning of each germination test, the three seed lots were within the recommended storage period to not affect their viability [7,43].

4.1.3.2. Viability and Germination Tests

The seed lots' viability was determined by the tetrazolium test, as Foschi et al. [7] reported (four replicates of fifty seeds each), according to the International Rules for Seed Testing [44].

Germination tests were carried out with the Between Paper method, placing 100 seeds per Petri dish of 9 cm diameter [45]. In all cases, four replicates were performed per treatment. Ultrapure water (Wasserlab G.R type II analytical grade water system; from now on referred to as water) or a solution of 500 mg L^{-1} of GA_3 (Semefil L, Nufarm L.) were used to wet the substrate. The Petri dishes were then placed under controlled conditions in a growth chamber (model Zimbueze, Seville, Spain) at 30 ± 1/20 ± 1 ºC, 85 ± 1% relative humidity for a photoperiod of 12 h (cold white fluorescent tubes Philips TL-D 36W/54), providing a photosynthetic photon flux density (PPFD) of 81.1 ± 1.7 µmol m^{-2} s^{-1}, unless stated otherwise. The germination test lasted 120 days, and germinated seeds were counted and removed periodically. Seeds were considered germinated when the radicle protruded from the testa and the micropylar endosperm, reaching a length of approximately 2 mm. Results of germination tests were fitted to the logistic function [46,47], defined as a particular case of Richards' function ([48] Equation (3)):

$$G = A/1 + e^{(\beta - kt)} \quad (3)$$

G is the cumulative germination (%), A represents the final germination percentage, t is the germination time (d), and β and k are function parameters used to determine the time required to reach 50% of G ($Gt_{50} = \beta/k$; d) and the mean relative cumulative germination rate ($k/2$; d^{-1}).

4.1.3.3. Experiment 1

This experiment evaluated the effect of light and darkness on the germination of the two caper seed lots (corresponding to seeds produced in 2019 and 2020). The experiment started in March 2021. The seeds were placed in the germination chamber with a photoperiod of 12 h of white light or continuous darkness. Light exposure was provided by cool white fluorescent tubes, as previously stated, and seeds that germinated in the dark were placed in closed opaque boxes. The Photoblastic Index (PI) and the Relative Light Germination (RLG) were determined using Equations (1) and (2).

Eight combinations of the three sources of variation were tested: 2 seed lots, light - darkness and 2 wetting solutions.

4.1.3.4. Experiment 2

The second experiment evaluated the effect of lighting with different wavelengths on caper seed germination. It started in July 2021. In light of the results obtained in the first experiment, tests were performed under white, red, blue, and red + blue lights. Color lighting was achieved with LED lights (AMZLAB GmbH), consisting of 80 LEDs (52 red, wavelength range 600–700 nm, and 28 blue, wavelength range 400–500 nm) placed 25 cm above the Petri dishes. The maximum power, when using the full spectrum of lights, was 30 W. Four types of light were tested: white was provided by fluorescent tubes (Philips TL-D 36W/54) with a power of 36 W, a distribution of the color spectrum ranging from wavelengths of 300 to 800 nm and with photosynthetically active radiation of 81.1 µmol m^{-2} s^{-1}; red (52 red LEDs; 102.5 µmol m^{-2} s^{-1}), blue (28 blue LEDs; 80.1 µmol m^{-2} s^{-1}) and red + blue (52 red LEDs + 28 blue LEDs; 125.4 µmol m^{-2} s^{-1}). Darkness was applied as in Experiment 1, placing the seeds in closed opaque boxes.

Table 1 shows the percentages and wavelengths for the color spectra of the lamps, measured with a Thorlabs spectrometer, model CCS200/M. The rates of each type of wavelength in each light were calculated using the ImageJ program [49], measuring the areas between the desired ranges of the wavelengths of the color spectrum.

Table 1. Relative percentage distribution of the different wavelengths for white light (cold white fluorescent lamp) and blue, red, and red + blue LED lights.

Wavelength	White Light	Blue LED	Red LED	Red + Blue LED
Violet 300–400 nm	1.51%	0.00%	0.00%	0.00%
Blue 400–500 nm	30.33%	97.48%	2.98%	30.25%
Green 500–600 nm	43.32%	1.05%	7.88%	5.27%
Red 600–700 nm	22.27%	1.47%	80.55%	58.41%
Far Red 700–800 nm	2.56%	0.00%	8.58%	6.06%
R/FR	8.69	0.00	9.39	9.63

This experiment analyzed 20 combinations of the three sources of variation: 2 seed lots, 5 types of lighting and 2 wetting solutions.

4.1.3.5. Experiment 3

The third experiment evaluated the influence of He-Ne laser irradiation, with different exposure times, on germination of caper seeds. Seeds from the lot harvested in 2021 were used in this experiment, which started in December 2021. The seeds were irradiated with a He-Ne laser (JDS Uniphase model 1145), with an output emission power of 22.5 mW and wavelength of 632.8 nm, belonging to the red band of the spectrum. It had a circular beam of 0.7 mm in diameter.

To define the exposure times (and therefore energies) to be analyzed in this experiment, a preliminary study was carried out to analyze the effect on seed viability of the following exposure times: 0, 1, 5, 15, 30, 60, 120 and 180 s, for which 40 seeds were irradiated during each of the times. These assayed exposure times expanded the range Juan [50] tested with the same laser. Neither of the analyzed exposure times decreased ($p \leq 0.05$) the seed viability (data not shown), thus, maximum and minimum thresholds were included in the experiment, as well as three intermediate levels: 0, 1, 15, 60 and 180 s, corresponding to 0, 22.5, 337.5, 1350, 4050 mJ applied energy levels, respectively. Intact seeds were irradiated one by one. After irradiation, the germination test was performed as previously indicated.

A total of 10 combinations of two sources of variation were tested: 5 exposure times and 2 wetting solutions.

4.1.3.6. Experiment 4

The fourth experiment evaluated the effect on germination of soaking the seeds in water before irradiation with the He-Ne laser with two timings of exposure, 1 and 15 s, which led to the best results in Experiment 3. As in the previous experiment, seeds of the 2021 lot were used and the experiment started in February 2022.

Caper seeds were soaked in water for four days before laser irradiation by a He-Ne laser for 0, 1 and 15 s. The germination test was performed as previously indicated. The germination substrate was only wetted with the GA_3 solution, as this experiment was not analyzing the effect of the wetting solution, since it haf already been stated that the GA_3 solution was required to get an acceptable germination percentage that allowed adjusting the logistic model.

This experiment tested six combinations of two sources of variation: 3 exposure times and soaked/dry seeds.

4.1.3.7. Data Analysis

All the tests verified that the tolerance required by the ISTA standards [45] was met, either between the replicates or between the germination tests. The statistical analysis program Statgraphics [51] was used to perform multi-way analyses of variance (ANOVA; $p \leq 0.05$) and verify the normality of the data. Mean separations were performed where appropriate, using Fisher's smallest significance difference (LSD test) at $p \leq 0.05$.

4.1.4. Results and Discussion

4.1.4.1. Experiment 1

The viability of the two seed lots was very high (82.5 ± 2.5% and 90.0 ± 3.1% in the seeds produced in 2019 and 2020, respectively), and, as a consequence, among other factors of careful harvesting, cleaning, and drying of the seeds, and according to that reported by Foschi et al. [9], there were no differences ($p \leq 0.01$) between both values.

Germination percentages obtained when water was used to wet the germination substrate were very low (specifically 9.5% in light and 6.8% in darkness, on average, for the two lots). These values were significantly lower ($p \leq 0.05$) than those obtained with the addition of GA_3 (81.5% in light and 81.2% in darkness, on average for the two lots). The low germination percentages obtained with water did not allow adjusting the logistic model; thus, the statistical analysis (Table 2) was only carried out for the seeds wetted with the GA_3 solution. The high germination values obtained with the GA_3 addition in the two lots are worth noting, accounting for 92% and 97% of the viable seeds. The 16 curves were adjusted to the germination logistic model with a determination coefficient higher than 99.6%, which allowed using the variable A (instead of G), as well as the

other variables derived from the logistic function, as occurred in previous studies of caper seed germination carried out by Pascual et al. [52] and Foschi et al. [8]. It applied to all the experiments reported in this manuscript. Figure 1 shows the logistic model adjusted to the average curves of accumulated germination of caper seeds from this experiment.

Table 2. Effect of the seed lot and the exposure to light–darkness on the germination parameters: final germination percentage (*A*, %), time required to reach 50% of final germination (Gt_{50}, d), and average germination rate ($k/2$; d^{-1}); average values from Experiment 1. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

	A	***Gt*$_{50}$**	***k*/2**
Seed lot (L)			
2019	79.87	24.33	0.098
2020	82.88	25.24	0.088
Exposure to (E)			
Light	81.50	23.91	0.094
Darkness	81.25	25.68	0.092
Analysis of Variance			
Factors (degrees of freedom)	% Sum of squares		
L (1)	16.30 NS	5.41 NS	10.18 NS
E (1)	0.12 NS	20.32 NS	0.61 NS
L × E (1)	2.49 NS	3.37 NS	5.14 NS
Residual (12)	81.09	70.90	84.08
Standard deviation ($^{+}$)	3.88	1.92	0.02

NS: Not significant differences ($p \leq 0.05$) according to the LSD test. ($^{+}$) The standard deviation has been calculated as the square root of the residual mean square.

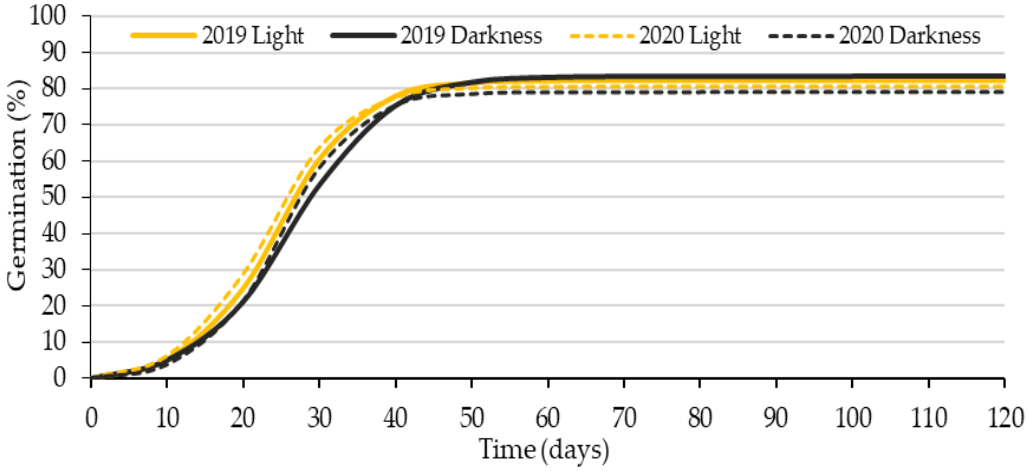

Figure 1. Logistic model adjusted to the curves of accumulated germination of caper seeds from Experiment 1. Average values of the combination of the seed lot (2019 and 2020) and the exposure to light-darkness of the seeds. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

Neither the seed lot and the seeds′ exposure to light–darkness, nor their interaction, influenced ($p \leq 0.05$) any of the determined germination parameters. These results coincided with those obtained by Germanà and Chiancone [53], in the sense that no significant difference was obtained when incubating mechanically scarified caper seeds in lightness and darkness. The non-statistical significance between the mean values of *A* obtained in light (81.5%) and darkness (81.2%) indicated that the caper seeds germinated equally in light and darkness, which was corroborated by the values of the photoblastism (PI = −0.002) and Relative Light Germination

(RLG = 0.5) indices. PI and RLG were calculated only for seeds germinated in substrate wetted with GA_3 solution and not with water to exclude calculations of both indices based on a small number of seeds, as Milberg et al. [19] did. As Takaki [20] reported, light insensitive seeds present *phyA*, corresponding to the so-called very low fluence responses (VLFR [21]). They saturate the responses by very low fluences because the photoequilibrium maintained by far-red light or extremely low light fluences in most regions of the visible spectrum produces enough Pfr to saturate this response [20,22].

The second experiment was set up with the aim of assessing the response of seeds exposed to light with different wavelengths, particularly blue light, of which the cryptochromes are photoreceptors [10,13].

4.1.4.2. Experiment 2

With the low germination percentages obtained with the seeds in water (on average, 3%, data not shown), it was not possible to fit the logistic model, so these data were not included in the subsequent statistical analysis. The germination percentage obtained with the GA_3 addition did significantly ($p \leq 0.01$) fit the logistic function, obtaining determination coefficients greater than 98.6%.

The seed lot did not affect any germination parameters ($p \leq 0.05$; Table 3); thus, in Figure 2, to facilitate its interpretation, average values for both lots are presented. As seen in Table 3 and Figure 2, the light wavelength did not influence ($p \leq 0.05$) the germination of the seeds, not differing from permanent darkness. In all cases, these germination percentages were high, comparable to those obtained in the first experiment; the difference between the germination values obtained in this and the previous experiment (both for white light and darkness) did not exceed the tolerance level established by ISTA Rules [45]. Neither the Gt_{50} nor the $k/2$ was affected by the different wavelengths. The interaction between the seed lot and the exposure to different wavelengths did not affect ($p \leq 0.05$) any of the germination parameters.

Table 3. Effect of the seed lot and the exposure to white, red, blue or red + blue lights and to darkness on the germination parameters: final germination percentage (*A*, %), time required to reach 50% of final germination (Gt_{50}; d), and average germination rate ($k/2$; d^{-1}); average values from Experiment 2. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

	A	***Gt*$_{50}$**	***k*/2**
Seed Lot (L)			
2019	78.0	28.1	0.077
2020	77.7	26.1	0.080
Exposure to Lighting (EL)			
White	80.3	29.6	0.073
Red	76.3	27.7	0.083
Blue	75.7	28.5	0.085
Red + Blue	76.8	26.2	0.081
Darkness	80.1	23.4	0.073
Analysis of variance			
Factors (degrees of freedom)	% Sum of squares		
L (1)	0.1 NS	5.2 NS	0.7 NS
EL (4)	12.3 NS	23.2 NS	13.0 NS
L × EL (4)	2.5 NS	4.5 NS	9.5 NS
Residual (30)	85.2	67.1	76.7
Standard deviation ($^+$)	5.9	4.2	0.01

NS: Not significant differences ($p \leq 0.05$) according to the LSD test. ($^+$) The standard deviation has been calculated as the square root of the residual mean square.

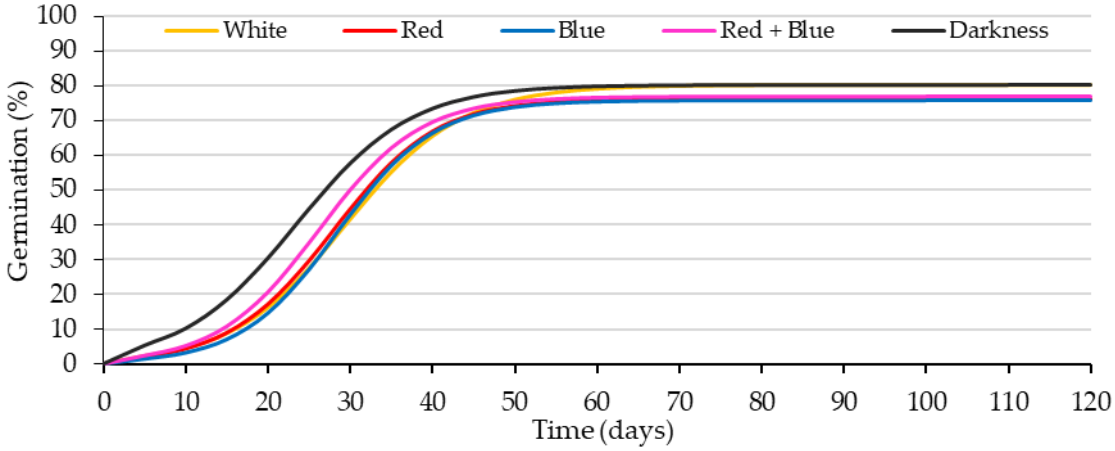

Figure 2. Logistic model adjusted to the curves of accumulated germination of caper seeds from Experiment 2. Average values for the seed exposure to white, red, blue or red + blue lights and to darkness. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

No significant differences were obtained between the *A* values obtained in white light (80.3%) and in darkness (80.1%), indicating that the caper seeds germinated equally in white light and darkness, which was corroborated by the values of the Photoblastic Index (PI = −0.001) and Relative Light Germination (RLG = 0.5). These results confirmed those obtained in the previous experiment, that caper seeds are insensitive to white light; thus, nurseries could save the energy needed to illuminate the seeds during germination.

Caper seeds were also insensitive to blue light, even though all plants had cryptochromes [16], photoreceptors of blue light (400–500 nm; [13]). They were also insensitive to red light, even though phytochromes are photoreceptors of red light (600–700 nm).

Neither Marín [54], nor Moreno et al. [55], obtained significant differences ($p \leq 0.05$) when applying white, red, and blue light in the Serrano variety of pepper (*Capssicumm annuum* L.) nor orchids *Encyclia*, respectively. Paniagua et al. [27] and Cho et al. [26] analyzed the effect of LED lights of different wavelengths on broccoli (*Brassica oleracea* Plenck var. *italica*), with none of them obtaining any statistical difference ($p \leq 0.05$) in the final germination percentage. Aguado and Álvarez [25] obtained no differences in the germination and emergence of lettuce (*Lactuca sativa* L.), basil (*Ocimum basilicum* L.), and tomato (*Solanum lycopersicum* L.) seedlings exposed to LED lights with spectra differing on the proportions of red and blue lights.

However, Enache and Livadariu [29], with the use of red LED lights in *Artemisia dracunculus* L. obtained a 10% greater germination percentage than with white light, which was, in turn, higher than those obtained under blue and green LEDs. However, the authors did not statistically compare these results.

4.1.4.3. Experiment 3

The viability of the 2021 lot was 85 ± 4%, according to what was obtained for the 2019 and 2020 lots. The germination obtained in the seeds wetted with water was very low, on average 6.5% (6.2% in irradiated and 8% in unirradiated seeds). The low germination data did not adjust to the logistic model; thus, they were not included in the analysis of variance, as in Experiments 1 and 2.

Germination data for seeds wetted with the GA_3 solution was fitted ($p \leq 0.01$) to the logistic function, presenting coefficients of determination for the 20 curves greater than 98.7%. Figure 3 shows the cumulative germination curves fitted to the logistic model obtained for the average values of each irradiation duration, the final germination percentages ranging between 69% and 82%. The tested laser irradiation durations (0, 1, 15, 60 and 180 s) did not significantly affect

($p \leq 0.05$) A or $k/2$, but an increase in Gt_{50} was observed in seeds irradiated for 180 s in relation to the control seeds (7 days delay; Table 4) and to those irradiated for 1 and 15 s (up to 10 days delay).

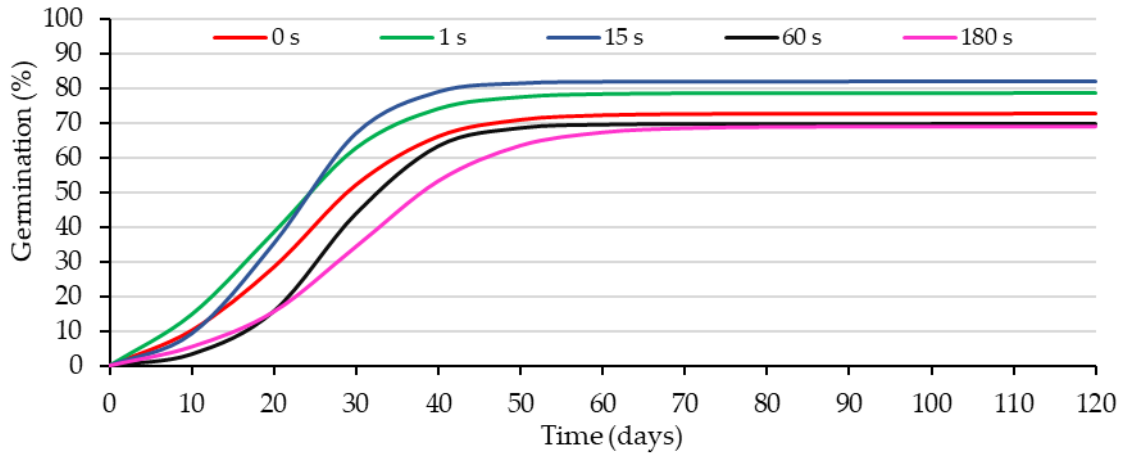

Figure 3. Logistic model adjusted to the curves of accumulated germination of caper seeds from Experiment 3. Average values of the He-Ne laser irradiation during 0, 1, 15, 60 and 180 s. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

Table 4. Effect of the He-Ne laser irradiation during 0, 1, 15, 60 and 180 s on the germination parameters: final germination percentage (A, %), time required to reach 50% of final germination (Gt_{50}; d), and average germination rate ($k/2$; d^{-1}); average values from Experiment 3. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

	A	***Gt₅₀***	***k*/2**
Time (T)			
0 s	72.81	23.05 bc	0.069
1 s	78.69	20.43 c	0.071
15 s	81.96	22.37 c	0.089
60 s	69.86	27.41 ab	0.088
180 s	69.10	30.68 a	0.061
Analysis of variance			
Factors (degrees of freedom)	% Sum of squares		
T (4)	35.58 NS	66.05 **	26.31 NS
Residual (15)	64.42	33.95	73.69
Standard deviation (+)	7.82	3.08	0.021

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$; NS: Not significant. (+) The standard deviation has been calculated as the square root of the residual mean square.

The results coincided with those that Juan, [50], obtained with the same type of laser used, in the sense that laser irradiation did not improve the germination of caper seeds. Similar results were obtained by Álvarez et al. [56] in tomato seeds irradiated with a He-Ne laser, which did not improve the germination percentages.

On the other hand, other studies on many species found that irradiation with laser light improved germination parameters, including wheat (*Triticum aestivum* L. [36]), lupine (*Lupinus albus* L.) and bean (*Vicia faba* L.) [38], radish (*Raphanus sativus* L. [57]), soybean (*Glycine max* L. [58]), safflower (*Carthamus tinctorius* L. [59]), sunflower (*Helianthus annuus* L. [37]) and Chinese woad (*Isatis indigotica* L. [34]). The laser light can affect the thermodynamic

parameters of the seeds by increasing their internal energy, affecting the enzymatic activity (mainly of amylases, proteases and glucosidases), which may positively influence the germination [36]. Another He-Ne laser effect is the acceleration of seed metabolism through increased levels of germination-promoting hormones, such as GA_3, and decreased inhibitors, such as abscisic acid, as stated by Soliman and Harith [39] in Acacia farnesiana L. and by Swathy et al. [33] in eggplant (*Solanum melongena* L.). The success of bio-stimulation caused by monochromatic laser light depends on the wavelength, irradiation duration, power, dose and method (constant or pulse), but also on the seed physiological properties and even the seed position during the laser irradiation [31]. Krawiec et al. [32] related the greatest response of seeds irradiated with a laser beam to the fact that these seeds had been previously soaked in water; thus, it was decided to analyze the effect of soaking the seeds in water before their irradiation, as presented in Experiment 4.

4.1.4.4. Experiment 4

Germination data were fitted ($p \leq 0.01$) to the logistic function, presenting coefficients of determination for the 24 curves greater than 98.1%. Figure 4 shows the germination curves adjusted to the logistic model in which germination was higher in seeds irradiated when they had been previously soaked in water compared to dry seeds and control. These differences ($p \leq 0.05$) are shown in Table 5, where it can also be seen that germination was affected ($p \leq 0.01$) by the irradiation duration. Soaking the seeds in water before irradiation represented 48% of the variation in the data, while the irradiation duration represented 26% of the variability. Figure 5 presents the significant interaction ($p \leq 0.01$) between the irradiation duration and the seed soaking before the irradiation. Germination percentages were comparable to those obtained in Experiment 3 with the same seed lot; the difference between the germination values obtained in this and the previous experiment for non-irradiated seeds, did not exceed the tolerance level established by ISTA Rules [45]. It can be seen that soaking the seeds before laser irradiation significantly increased ($p \leq 0.05$) the germination percentage, germinating all viable seeds. Neither Gt_{50} nor $k/2$ was affected by the analyzed ($p \leq 0.05$) factors.

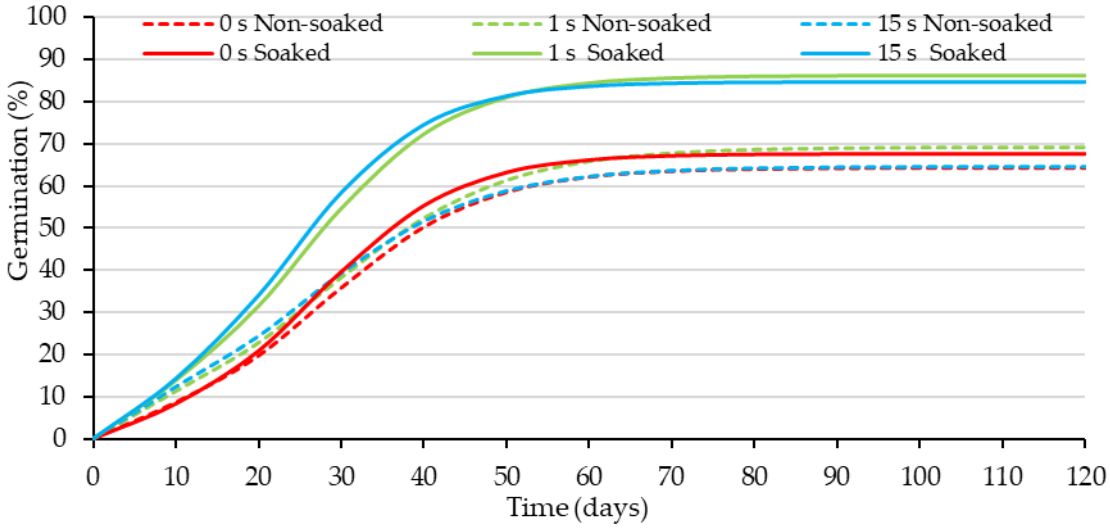

Figure 4. Logistic model adjusted to the curves of accumulated germination of caper seeds from Experiment 4. Average values corresponding to the combination of the exposure time of the seeds to the He-Ne laser irradiation (0, 1 or 15 s) after being soaked in water or not. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

Table 5. Effect of He-Ne laser irradiation during 0, 1 and 15 s applied to dry and soaking the seeds in water, for 4 days, on the germination parameters: final germination percentage (*A*, %), time required to reach 50% of final germination (Gt_{50}; d), and average germination rate ($k/2$; d^{-1}); average values from Experiment 4. A 500 mg L^{-1} GA_3 solution was used to wet the substrate.

	A	***Gt*$_{50}$**	***k*/2**
Time (T)			
0 s	66.0 b	23.8	0.055
1 s	77.6 a	22.9	0.051
15 s	74.6 a	21.8	0.054
Soaking (S)			
Soaked seeds	79.4 a	21.6	0.058
Dry seeds	65.9 b	24.1	0.049
Analysis of variance			
Factors (degrees of freedom)	% Sum of squares		
T (2)	25.6 **	3.4 NS	1.9 NS
S (1)	48.2 **	8.2 NS	11.3 NS
T × S (2)	13.8 **	3.8 NS	1.3 NS
Residual (18)	12.4	84.6	85.5
Standard deviation ($^{+}$)	4.0	4.6	0.014

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$, NS: Not significant. ($^{+}$) The standard deviation has been calculated as the square root of the residual mean square.

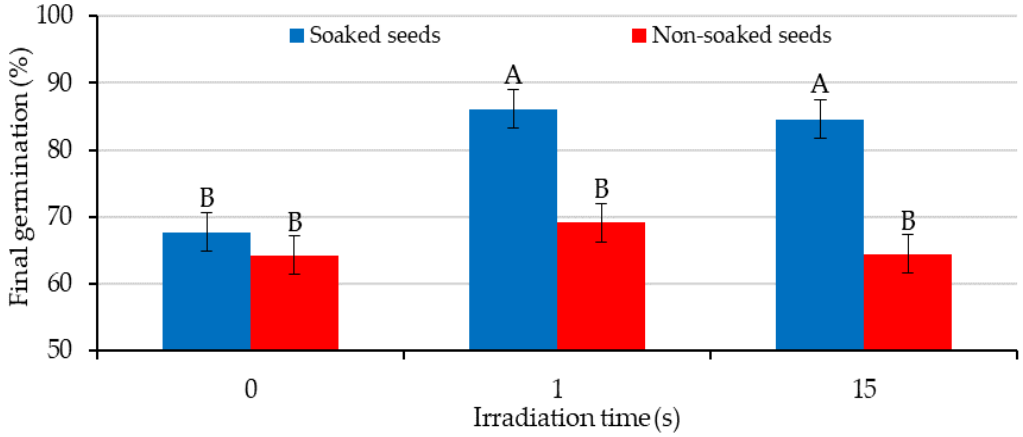

Figure 5. Analysis of the significant interactions of the analysis of variance in Table 5 between irradiation time and seed soaking prior to irradiation on the final germination. Average values of four replicates. Different letters indicate significant differences according to the LSD test. Error bars represent the LSD ($p \leq 0.05$).

As already mentioned in Experiment 3, the laser light can affect the thermodynamic parameters of the seeds [36]. It can also increase seed metabolism through increased levels of germination-promoting hormones (GA_3) and decreased inhibitors (abscisic acid), positively affecting germination [33]. In this experiment, irradiating the seeds once the germination metabolism had started, due to the prior seed soaking, improved the effectiveness of the laser irradiation in relation to that obtained in Experiment 3, resulting in significant differences ($p \leq 0.05$). This was in accordance with that reported by Perveen et al. [37,59] for sunflower and safflower, respectively, who obtained good germination results irradiating seeds that had been previously soaked in water with a He-Ne laser.

Future research will focus on studying the photoreceptors (phytochromes, cryptochromes and phototropins) present in caper seeds, as well as on analyzing the enzymatic activities and the levels of germination promoting and inhibiting hormones in seed irradiated with laser light.

4.1.5. Conclusions

Caper seeds are insensitive to exposure to white, red, blue, and red + blue lights during the germination process, not showing differences between the germination response in lightness in relation to darkness. Thus, germination can be carried out in lightness or darkness, and, therefore, germination in nurseries could be carried out in darkness, leading to important energy savings. Caper seed irradiation with a He-Ne laser during short exposure times improved the germination percentage when the seeds had been previously soaked in water, germinating all viable seeds. However, applying a solution of gibberellic acid was always required in all the cases studied.

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4.2. Effects of high intensity ultrasound stimulation on the germination performance of caper seeds

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4.2.1. Abstract

The caper bush has developed different mechanisms to survive in Mediterranean conditions, such as drought tolerance and seed dormancy. Many studies have been carried out to improve the germination of caper seeds, but ultrasound is one of the least studied methodologies in this species. This study aimed to analyze the effects of treatments with an ultrasonic probe processor on the imbibition and germination of caper seeds. After applying the ultrasound treatment using three output powers and three holding times, the seed coat's disruption level was determined, and the imbibition, viability and germination tests were carried out. Ultrasonication fastens the initial imbibition, but after 48 h of soaking, seed moisture does not present differences compared to non-sonicated seeds. It produces the scarification of the testa but does not affect the tegmen, so moistening occurs through the hilar region, as in control seeds. There is a significant linear and negative correlation between the germination of the seeds and the temperature reached during the sonication treatment, so that temperatures above 40 °C practically annulled the germination. The combination of 20 W and 60 s provided the greatest germination percentage, being the only treatment that statistically improves germination in relation to the control seeds. When the output power and/or holding time were higher, the temperature increased, and the germination percentage statistically decreased.

Keywords: *Capparis spinosa;* dormancy; holding time; output power; seed coat disruption; ultrasonic probe; viability; water uptake.

4.2.2. Introduction

The caper (*Capparis spinosa* L.) bush is a perennial shrub naturally widespread throughout the Mediterranean Basin [1]. It has developed different survival mechanisms in Mediterranean conditions [2], such as drought tolerance and seed dormancy. Even though the fruits contain many seeds, they have a very low germination percentage, which is a problem for the cultivation of capers.

The caper seed coat is impermeable and hard to the touch; the testa and tegmen form it, presenting two anatomical structures, the hilum and the micropyle. The testa consists of a layer of 1–2 thick cells with lignified and thickened walls. The tegmen presents a lignified exotegmen composed of several brachysclereid cells and a lignified, fibrous endotegmen composed of a few layers of cells [3,4].

Many studies have tried to improve caper seed germination and to break the possible physical dormancy with different types of scarifications (mechanical, chemical, thermal or biological) [5–8] and to break the physiological dormancy with the use of gibberellic acid (GA_3) and potassium nitrate [9–12]. The ultrasound application has been used in seed germination studies of both cultivated plants and weeds [13–18], but it is one of the least-studied methodologies in caper seeds.

The term “ultrasound” describes the sound waves whose frequencies are over the upper audible limit of human hearing in healthy young adults (20 kHz). Sonication is the act of applying sound energy to agitate particles in a sample. When ultrasonic frequencies are used, the process is known as ultrasonication [19].

The effects from the ultrasonication of liquids are caused by cavitation. By introducing high power ultrasound into a liquid medium, the sound waves are transmitted in the fluid and create alternating high-pressure (compression) and low-pressure (rarefaction) cycles, with rates depending on the frequency. High-intensity ultrasonic waves create small vacuum bubbles in the liquid during the low-pressure cycle. When the bubbles attain a volume at which they can no longer absorb energy, they collapse violently during a high-pressure cycle. This phenomenon is termed cavitation [20].

For ultrasonic applications, both ultrasonic probes and ultrasonic baths can be used. The probe sonication is more effective and powerful than the ultrasonic bath in the nanoparticle’s

dispersion; the ultrasonic bath device provides a weak ultrasonication and a non-uniform distribution, while the ultrasonic probe device provides a strong ultrasonication and a uniform distribution [21]. The intense sonication zone is directly beneath the probe (sonotrode) when samples are sonicated using an ultrasonic probe device; thus, the ultrasonic irradiation distance is limited to a specific area of the sonotrode's tip. Ultrasonic processes in open beakers are mainly used for sample preparation of small volumes.

The bubble collapse leads to an increase on the liquid temperature, and the pressure differences may mechanically damage the cellular and tissue structures of the seeds. If the bubbles collapse close to the seed coat, its surface may be damaged, generating pores. If the seed coat represents a physical barrier reducing the water and oxygen uptake into the inner parts of the seed [22], an increase in its porosity could increase the water and oxygen intake, and, in consequence, in germination [14]. However, if the liquid temperature or the pressure differences are excessive, the damage exerted on the tissues of the seed can negatively affect the embryo, decreasing germination. Mechanical actions, such as cutting or removing the seed coat, accelerate the germination process in caper seeds [4], so a similar result is foreseeable due to the hypothetical opening of pores that could occur in the seed coat with the application of the ultrasonication treatment, as occurred in other species. Ultrasound waves application led to greater and faster germination in barley seeds [13], increased germination percentages of deteriorated *Arabidopsis thaliana* seeds [14], accelerated the germination progress, increasing the germination rates of rice seeds [23], and induced dormancy break in *Chenopodium album* [15].

Given these positive results obtained with ultrasonication in other species, this study aimed to analyze the effects of the application of treatments with an ultrasonic probe processor on the imbibition and germination of a caper seed lot. This seed lot was own-produced by the research team, and it was known that its germination percentage was moderate, similar to that of the other own-produced lots used in previous studies.

4.2.3. Materials and Methods

4.2.3.1. Plant Material

The caper seeds used in the study were collected in September 2020 from a plot in Llíria (Valencia, Spain; 39º38′07.8″ N 0º35′53.4″ W). The viability, ultrasonication, imbibition and germination tests were carried out in April 2021, when the seeds were 6 months old.

The seeds were extracted from ripe fruits collected on the day of their dehiscence and from fruits located immediately before and after it, and they were cleaned with tap water. The mature seeds were separated by the decantation method [39]. To prevent future infections, they were disinfected with a 25% sodium hypochlorite solution (from a concentrated solution of 37 g L^{-1} of active chlorine) for 2 min, followed by three washes with ultrapure water. The seeds were dried and stored in a closed, airtight glass container at 7 ºC until the trials were carried out.

4.2.3.2. Ultrasonication

A Bioblock ultrasonic processor (sonicator), vibra cell model 75115 (Bioblock Scientific, IIIkrich, France), with a CV334 probe, was used. This sonicator works with a frequency of 20 kHz and a maximum output power of 500 W, making it possible to vary the amplitude from 0 to 100%.

First, before the treatments, the sonicator was calibrated, for which three different amplitudes (25, 50 and 75%) and four holding times (30, 60, 120 and 300 s) were tested. For calibration, 100 mL glass beakers with 80 mL of ultrapure water (hereinafter water) and 50 seeds were used. The calibration was carried out to determine the output power at which each amplitude works and, at the same time, to serve as a support to select the combinations of wave amplitude and holding time to be applied, depending on both temperature and seed viability reached with the different combinations. The energy recorded by the sonicator was used to determine the corresponding power for each holding time. After applying each amplitude–time combination, the water's temperature

and EC were measured using a handheld EC meter (Eutech Cond 6+; Eutech Instruments Pte Ltd.; Waltham, MA, USA),and the seed viability was determined as explained afterwards.

Based on the results of the calibration, it was decided to perform the treatments corresponding to the following combinations: output powers of 20, 50 and 100 W (corresponding to the wave amplitudes of 20%, 50% and 75%) for 60, 120 and 180 s, as well as a control treatment (not subjected to the ultrasounds). After ultrasound treatments were applied, the seed coat's disruption level was determined, and the imbibition, viability and germination tests were performed.

4.2.3.3. Disruption of the Seed Coat

As the cavitation can damage the seed coat or create fissures and pores, the percentage of disruption that the seed coat presented after the ultrasound treatments was determined as the percentage of the damaged area in relation to the total seed coat area. These areas were determined from a photographic record taken by a photomicroscope (U500X Digital Microscope; Cooling Tech, Shenzhen, China), and the ImageJ program [40] was used to perform the surface measurements. It was determined in four repetitions of ten seeds each for each treatment.

4.2.3.4. Imbibition

The imbibition test was performed according to Ma et al. [41] and Juan [7]. After the ultrasound treatments, the seeds were weighed, and their moisture content determined, and then they were soaked in water (10 mL) in glass test tubes at laboratory room conditions. Four replications of ten seeds per treatment were considered. The moisture content of the seeds was determined according to the International Rules for Seed Analysis [42]. Seed weight was determined every 2 h during the first 8 h of imbibition and then every 24 h for 8 days. Seeds were blotted with a paper towel, immediately weighed on a precision balance (Sartorius, model B 120S, Barcelona, Spain) and returned to the test tubes immediately after. Dry weight was determined at each stablished period, by drying the seeds (four samples per treatment) for 48 h at 103 ºC in a forced-air oven (Selecta 297; Selecta, Barcelona, Spain). The daily seed moisture content was calculated (Equation (1)) on a fresh mass basis [42]:

$$\text{Seed moisture (\%)} = 100 * (\text{Fresh weight}_i - \text{Dry weight})/\text{Fresh weight}_i \quad (1)$$

Since water is colorless, the advance waterfront within the seeds was determined parallel to the imbibition test, by soaking the seeds in 5 mL of a methylene blue solution for microscopy (Scharlau, Sentmenat, Spain) in test tubes. At each stablished period, seeds were cut in half to observe the entry of the dye inside them, and a photographic record was taken by a photomicroscope (U500X Digital Microscope; Cooling Tech, Shenzhen, China). Four replications of ten seeds per treatment were considered.

4.2.3.5. Seed Viability

The tetrazolium topographic test was used to determine the seed viability [43]. For preconditioning, seeds were soaked in water at room temperature for 18 h; then, they were pierced opposite the micropyle and immersed in a 1% tetrazolium solution (Tetrazolium Red. 2,3,5-Triphenyltetrazolium chloride; Sigma, Barcelona, Spain) in the dark, at 30 ± 1 ºC, inside a growth chamber (model Zimbueze, Seville, Spain) for another 18 h [43]. After this period, seeds were cut longitudinally to evaluate the staining in the tissues, taking a photographic record with the already mentioned photomicroscope. Four replications of fifty seeds each were performed. A seed was considered viable when the maximum area of unstained tissue was the radicle tip and when it is expected to germinate under normal conditions [44]. Seeds with sound tissues (gradual and uniform spots on exposed surfaces) and weak but viable tissues (stain grayish red or brighter red than normal) are considered viable [44].

4.2.3.6. Seed Germination

Germination tests were carried out following the Between Paper (BP) method [42], within Petri dishes (9 cm). The samples consisted of 400 seeds (4 replications of 100 seeds). Two solutions

were used to moisten the paper: a GA_3 solution (500 mg L^{-1}; Semefil L; Nufarm, Melbourne, Australia) and water. To avoid fungal contamination, 2 g L^{-1} of Captan (Captan 50; Bayer, Leverkusen, Germany) was added to the solutions. Germination took place for a maximum of 120 days in a growth chamber (model Zimbueze, Seville, Spain) which conditions were 30 ± 1/20 ± 1 ºC, 85 ± 1% relative humidity, and a photoperiod of 12 h (81.1 µmol m^{-2} s^{-1}).

A seed was considered germinated when the emerged radicle reached 2 mm long. The difference between the maximum and minimum germination percentages of the four replications did never exceed the tolerance level established [42]. For analyzing the germination curves, the model applied to each repetition is that of the logistic function (Equation (2)) [45]:

$$G = \frac{A}{1 + e^{(\beta - kt)}} \quad (2)$$

with G being the accumulated germination (%), A being the maximum germination (%), t being the germination period (d), β being a parameter referring to the position of the curve relative to the time axis and k being a velocity parameter. The last two values are used to calculate the number of days needed to reach 50% A ($\beta/k = Gt_{50}$) and the average relative rate of cumulative germination ($k/2$, d^{-1}).

4.2.3.7. Statistical Analysis

Analysis of variance (ANOVA) was used to analyze the results with Statgraphics Centurion 18 software [46]. The differences were considered significant for a probability of $p \leq 0.05$%. The means were separated by the Fisher's minimum significant differences test (LSD test) at $p \leq 0.05$. The percentage data were arcsin √x transformed before analysis to accomplish the normality assumption. The normality distribution was analyzed by verifying the residuals' normal distribution [47] by the Shapiro–Wilk test [46].

4.2.4. Results and Discussion

4.2.4.1. Ultrasonication

The calibration resulted in the corresponding output powers for 25%, 50% and 75% amplitudes to be 20, 50 and 100 W, respectively. Regarding the temperature, the starting point was the ambient temperature (20 ºC) increasing as both the output power and the holding time (exposure time) increased, reaching over 80 ºC at maximum amplitude and time (75% (100 W) for 300 s). Similarly, the electrical conductivity (EC) increased as the output power and the holding time increased. At the same time, the water used in the ultrasonication acquired a brown hue, becoming darker as the output power and holding time increased (Figure S1), probably due to partial degradation of the seed coat.

Figure 1 shows the effect of the ultrasound treatment on the viability of the seeds for the different output powers and holding times applied in the calibration test. The viability of the seeds stimulated for 300 s, with any of the three amplitudes tested, was statistically lower ($p \leq 0.05$) than those of the control seeds, showing a tendency to decrease with increasing output power (although not statistically significant). Regarding the other holding times, only the application of the 100 W during 120 s statistically reduced the seed viability ($p \leq 0.05$) compared to the non-treated seeds.

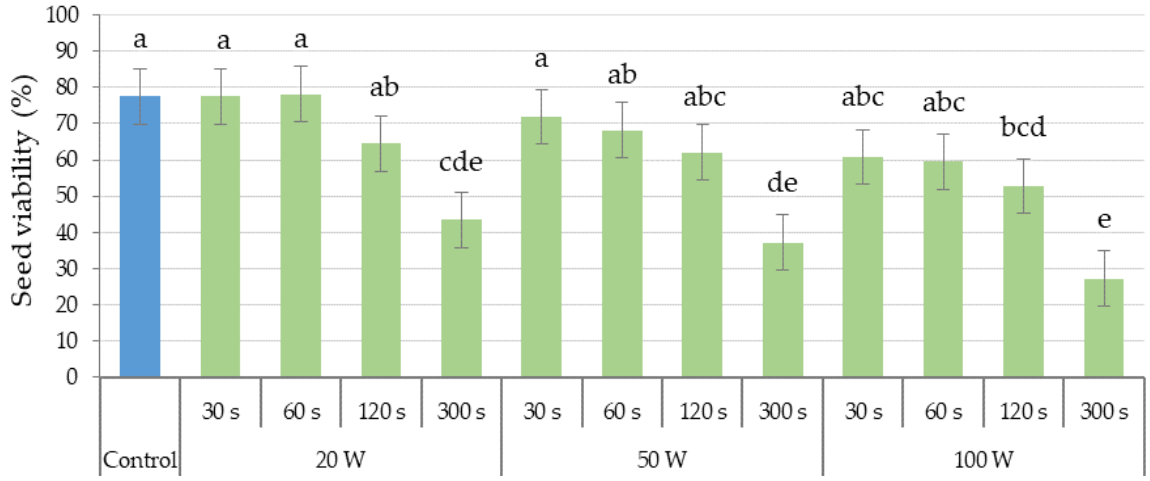

Figure 1. Effect of ultrasound treatments at different output powers (20, 50 and 100 W) and holding times (30, 60, 120 and 300 s) in the seed viability. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$), which are represented by the error bars.

Given the low viability of seeds stimulated during 300 s, probably related to the high temperature reached and possibly the damage produced in the seed coat, it was decided to discard this holding time, trying to keep the temperature below 60 ºC. Thus, to carry out the experiment, the seeds were ultrasonically stimulated using the sonicator with holding times of 60, 120 and 180 s and with the three already assayed output powers (20, 50 and 100 W). These powers are lower than those used in seed hydration and germination studies such as chickpea and rice, in which powers up to 400 W were used [16,24]. Figure 2 presents the temperature and EC registered in the water after applying the ultrasounds at different output powers and holding times.

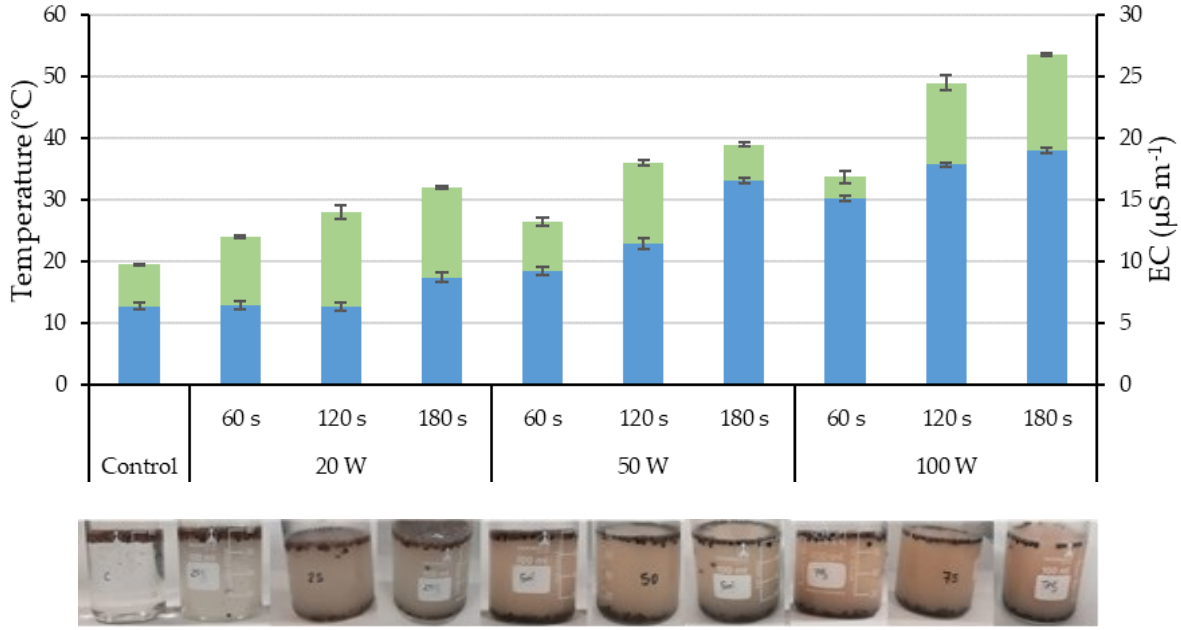

Figure 2. Temperature (green) and electrical conductivity (EC; blue) of the water after ultrasonication of the seeds with different output powers (20, 50 and 100 W) and holding times (60, 120 and 180 s). Error bars represent the standard error (**above**). Appearance of the water used in the corresponding ultrasonication (**bass**).

The initial water temperature (20 ºC) increased with both the increasing output power and holding time, ranging from 24 ºC (20 W, 60 s) to 53.5 ºC (100 W, 180 s). The ultrasonication treatments of 20 W both during 60 s and 120 s did not alter the water EC (6 µS m^{-1}), but it increased by increasing both the output power and the holding time, up to 19 µS m^{-1} at 100 W for 180 s (Figure 2). At the same time, the water used in the ultrasonication acquired a brown tone, becoming darker as both the output power and the holding time increased (Figure 2). It should be noted that by increasing the holding time and/or the output power of the ultrasonic treatment, a higher percentage of seeds moved to the bottom of the beaker, indicating that the density of these seeds had increased during the treatment because during this short period, further imbibition had occurred in them.

4.2.4.2. Disruption of the Seed Coat

Figure 3 shows the damage produced in the seed coat (expressed as a percentage of the affected surface compared to the seed surface) due to the different treatments (output power and holding time). The scarification of the testa is observed, while the tegmen is visible in the scarified area. The highest percentage of the affected area ($p \leq 0.05$) corresponded to the treatments with the greatest output power (100 W) and longer holding times (120 and 180 s), representing the affected surface around 15% of the testa. As the output power and holding time were lower, the damage statistically decreased, so the treatments with 20 W applied during 60 and 120 s did not present differences ($p \leq 0.05$) compared to the control. The brown tone of the water (of increasing darkness with the increasing output powers and/or holding times; Figure 2) indicates that it is related to the corresponding degradation of the testa.

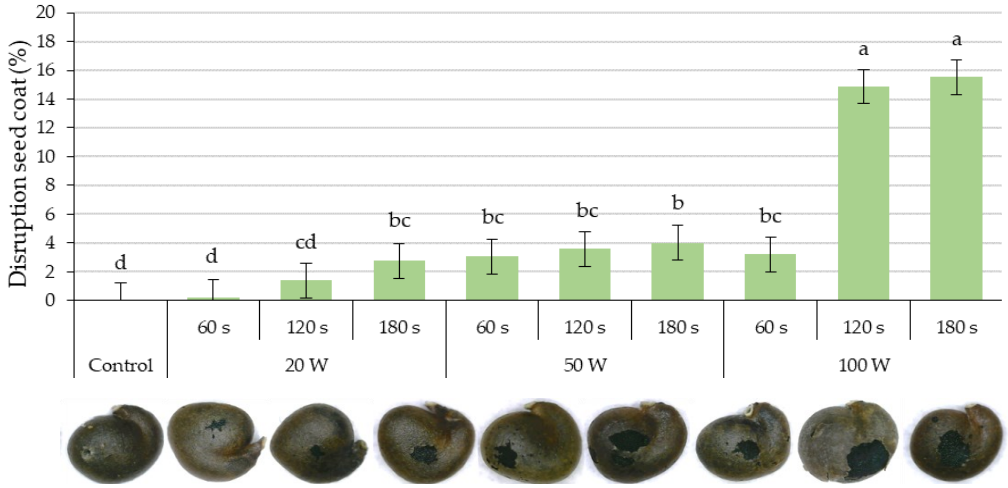

Figure 3. Disruption of the seed coat. Percentage of scarified area in seeds treated with the sonicator at different output powers (20, 50 and 100 W) for 60, 120 and 180 s. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$), which are represented by the error bars (**above**). Seed coat appearance after ultrasonication (**bass**).

4.2.4.3. Imbibition

The initial seed moisture content was 7.9 ± 0.05%, similar to that of the other lots of seeds used in the other studies carried out by our research team [11,25], and they are within the range usually recommended (5 and 8%, depending on the species) for adequate conservation of the seeds [26–28].

The seed moisture content along the soaking period (Figure 4) followed the first two phases of the typical triphasic water uptake model in seed germination [11]. First (phase I of germination; imbibition itself), the water uptake was initially rapid, followed by a slower wetting step. At the end of phase I (from the first to the second day, depending on the seed treatment), the water uptake stopped as the seed entered the lag phase of germination (phase II), in which the metabolism was already supposedly active.

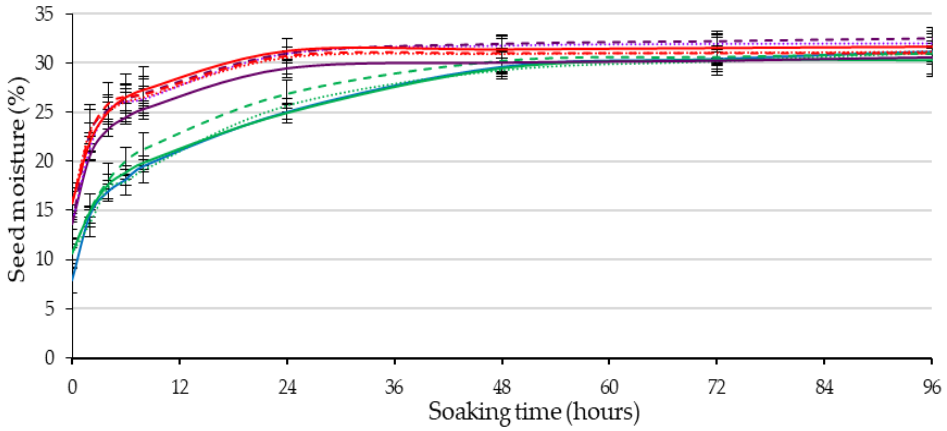

Figure 4. Time course of seed moisture content along the soaking period, considering the output power (control (blue), 20 W (green), 50 W (violet) and 100 W (red)) and the holding time of ultrasound stimulation (60 s (solid), 120 s (dashed) and 180 s min (dotted lines)). Error bars represent the standard error.

The seed moisture content increased quickly during the first 24 h of soaking, stabilizing in the seeds stimulated with 50 and 100 W (Figure 4). In contrast, water uptake continued until the second day in control (intact) and in seeds subjected to 20 W. The moisture content of the seeds stimulated with 50 and 100 W was statistically higher ($p \leq 0.05$; Table 1) than that of the seeds stimulated with 20 W and the control seeds after soaking for 8 and 24 h. When the seed moisture is compared at 48 h of soaking, there were no statistical differences ($p \leq 0.05$; Table 1) between treatments, reaching a seed moisture content of approximately 31% in all cases. This moisture content is sufficient for the efficient germination of caper seeds [11,25].

Table 1. Effect of the output power (W) and holding time (s) of ultrasonic treatments on seed moisture content during the seed soaking in water.

	0 h	**8 h**	**24 h**	**96 h**
Output power (P)				
Control	7.9 c	19.6 b	25.0 b	31.2
20	10.6 b	20.4 b	25.8 b	30.7
50	13.5 a	26.2 a	30.9 a	31.7
100	15.8 a	26.9 a	30.8 a	31.3
Holding time (T)				
60	11.3	23.0	27.7	30.9
120	12.0	23.1	28.2	31.4
180	12.6	23.6	28.6	31.4
Anaysis of variance				
Factors (degrees of freedom)	% Sum of squares			
P (3)	54.6 **	68.6 **	68.6 **	2.6 NS
T (2)	1.6 NS	0.5 NS	1.3 NS	1.4 NS
P × T (6)	1.0 NS	0.9 NS	2.8 NS	3.9 NS
Residual (36)	42.8	30.0	27.2	92.0
Standard deviation (+)	3.0	2.5	2.0	2.4

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$, NS: not significant. (+) The standard deviation was calculated as the square root of the residual mean square.

These results are in agreement with those reported in the literature. Ranjbari et al. [29] stated that in the initial stages of soaking, the treated seeds absorbed water faster than the control seeds and related this phenomenon to the cavitation process produced by the sonicator. Lo Porto et al. [18] indicated that when soybean seeds were treated with ultrasounds, their water uptake increased without modifying the seed coat's morphology and wettability, but inducing minor chemical changes of the outer part of the seed coat. Ding et al. [30] indicated that power ultrasound produced micro-openings on the surface of rice grains, which provided new pathways for water to enter, thus enhancing the hydration process. Miano et al. [31] found changes in the micro-structure of the seeds, increasing the seed porosity and improving the mass transfer among the seed tissues.

In general, water uptake in seeds occurs through the seed coat. However, in some seeds, the initial water uptake occurs in specific locations or through inherent structural features in the surrounding tissues, such as the micropylar region in the tobacco seeds, corn and some legumes. In seeds of western white pine (*Pinus monticola* Douglas ex D. Don), the micropyle is impermeable to water, which enters the seed through the surrounding testa [32]. In all cases, during the imbibition process, the seed whole is not moistened simultaneously, and there is a sharp limit of water content between wet cells and those about to get wet [32].

Previous studies determined that water uptake in caper seeds occurs through the hilar region [11]. However, it remains to be determined whether, in seeds treated with ultrasounds, it occurred through the scarified surface of the testa or the hilar region, as in control seeds. The hilar region in the caper seed coat contains two scars, the hilum (scar corresponding to the funiculus of the ovule) and the micropyle (scar corresponding to the micropyle of the ovule).

Figure S2 shows that the moistening of both control and ultrasonicated seeds begins in the hilar region, with the extra hilar region being impermeable. The hilar region is comported as a "water channel", and according to Foschi et al. [11], caper seeds do not have a strictly waterproof coat and can absorb water without breaking the seed coat. On the other hand, although the ultrasonication has scarified a part of the testa, the tegmen has prevented the uptake of water through it; however, it seems that the tegmen (and the water channel) can become wet, which may be confirmed by the increase in seed water content caused by ultrasonication (Table 1).

While control seeds after 48 h of soaking do not show any coloration, the ultrasonicated seeds with 20 W show the hilar region already colored in blue by the methylene blue solution (Figure S2). On the other hand, the seeds treated with higher output powers show the hilar region colored after 8 h of soaking. Specifically, the seeds treated with higher output powers and holding times show an important part of the embryo colored after 48 h of soaking. This moistening pattern agrees with the previously analyzed moisture contents (Table 1) in the sense that the most potent treatments (in output power and holding time) produced faster imbibition.

4.2.4.4. Seed Viability

The initial viability of the seed lot used in this study was 77.5%, decreasing with ultrasonication as both output power and holding time increased (Table 2; Figure 5) so that the lowest viability ($p \leq 0.05$) corresponded to the higher power treatments (50 and 100 W) and to the longer holding times (120 and 180 s). Seed viability was also significantly influenced by the interaction of output power and holding time ($p \leq 0.05$; Table 2; Figure 5). Seed viability decreases with ultrasonication, just slightly (not significantly) with the lowest output power (20 W) applied for the shortest time (60 s), and to a greater extent with increasing both output power and holding time, so that the lowest viability ($p \leq 0.05$) corresponded to the highest power treatments (50 and 100 W) with the most extended sonication times (120 and 180 s). These results agree with the damage produced in the testa of the seeds (Figure 3) and with the water EC and temperature (Figure 2). Treatments with the highest output power and holding time were the ones that caused the greatest damage to the testa leading, in turn, to the greatest water EC and temperature, which could be related to the loss of seed viability. When analyzing only the sound seeds, both factors significantly affected it (output power and holding time, $p \leq 0.01$ and $p \leq 0.05$, respectively), but not

their interaction (Table 2). The percentage of sound seeds was statistically reduced compared to the control ($p \leq 0.05$) even by the lowest power applied.

Table 2. Effect of the output power (W) and the holding time (s) of the ultrasonication on the viability (%) of the seeds, considering both sound tissues and sound + weak but viable tissues.

	Sound	**Sound + weak but viable**
Output power (P)		
Control	55.00 a	77.50 a
20	42.50 b	70.42 a
50	27.50 c	56.67 b
100	24.17 c	45.83 c
Holding time (T)		
60	43.75 a	71.25 a
120	35.63 ab	60.00 b
180	32.50 b	56.56 b
Analysis of variance		
Factors (degrees of freedom)	% Sum of squares	
P (3)	53.93 **	53.29 **
T (2)	7.97 *	13.99 **
P × T (6)	3.97 NS	9.62 *
Residual (36)	34.14	23.10
Standard Deviation (+)	11.33	9.31

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$, *: significance level $p \leq 0.05$, NS: not significant. (+) The standard deviation was calculated as the square root of the residual mean square.

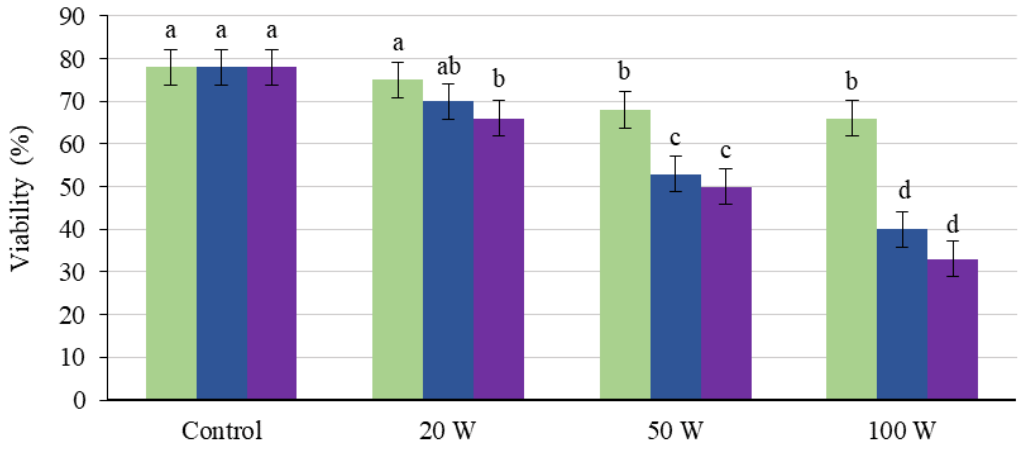

Figure 5. Analysis of the significant interaction of the analysis of variance presented in Table 2 between the output power (Control, 20, 50 and 100 W) and the holding time (60 (green), 120 (blue) and 180 s (violet)) of the ultrasonication effect on the viability (%; sound + weak but viable tissues) of the seeds. Average values of four replicates. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$), which are represented by the error bars.

4.2.4.5. Seed Germination

All the analyzed factors, output power and holding time of ultrasonication, the solu- tion used to moisten the substrate in the germination test and their interactions influenced the germination percentage ($p \leq 0.01$; Tables 3 and 4). The output power applied and the solution used to moisten the substrate and their interaction explain 21.2%, 49.0% and 23.6%, respectively, of the variability of the test, indicating the importance of both factors. It is observed that

germination statistically decreased as the output power and holding time increased, and its value was much higher when the GA_3 solution was used in the test than when water was applied. Regarding the output power and solution interaction, the significance is related to the low germination obtained with the 100 W treatment, regardless of whether the seeds were moistened with the GA_3 solution or with water, obtaining similar germination percentages in both cases. The positive results obtained by adding GA_3 to the substrate coincide with the previous studies by the research team [5,9,11,25], in which the addition of GA_3 increased germination.

Table 3. Effect of the output power (W) and the holding time (s) of the ultrasonication, and the use of the GA_3 solution to moisten the substrate of germination test, on the germination percentage (*G*, %) of the seeds.

	G (%)
Output power (P)	
Control	32.8 a
20 W	32.2 a
50 W	19.2 b
100 W	5.4 c
Holding time (T)	
60	26.4 a
120	22.8 b
180	17.9 c
Solution (S)	
Water	5.3 b
GA_3	39.4 a
Analysis of Variance	
Factors (degrees of freedom)	% Sum of squares
P (3)	21.2 **
T (2)	2.0 **
S (1)	49.0 **
P × T (6)	1.2 **
P × S (3)	23.6 **
T × S (2)	0.7 **
P × T x S (6)	1.0 **
Residual (72)	1.4
Standard deviation ($^+$)	3.3

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$. ($^+$) The standard deviation was calculated as the square root of the residual mean square.

On the other hand, the germination percentage obtained in all the combinations with 100 W of output power (applied during 60, 120 and 180 s and using both water and GA_3 solution to moisten the substrate in the germination test) was lower than 10%. The germination obtained was also lower than 10% when water was used to wet the substrate in any combination of output powers and holding times. This result agrees with those reported by the research team [33–35], who obtained high germination percentages with different scarification methods, but only when they were followed by the addition of a GA_3 solution. The low germination prevented the determination of the germination curves for these treatments; thus, the germination parameters were only analyzed for the seeds treated with 20 and 50 W and moistened with GA_3 during the germination test.

The coefficients of determination (R^2) obtained for the germination data fitted to the logistic function ($p \leq 0.01$) were greater than 0.99, for all of the four replicates from the different combinations of variation sources, allowing the use of the variable *A* (final germination

percentage; instead of *G*), as well as other variables, such as Gt_{50} (number of days needed to reach 50% of the final germination) and k/2 (average germination rate; d^{-1}), as performed in previous studies of caper seed germination [7,33].

Table 4. Analysis of the significant interactions of the analysis of variance in Table 3: output power (W) and holding time (s); output power and the solution used to moisten the substrate of germination test; holding time and solution on the germination percentage (*G*) of the seeds. Average values of four replicates.

Power	Time	*G (%)*
Control	-	32.8 b
20	60	36.8 a
20	120	32.3 b
20	180	27.5 c
50	60	23.0 d
50	120	23.5 d
50	180	11.0 e
100	60	13.0 e
100	120	2.8 f
100	180	0.5 f
LSD ($p \leq 0.05$)		3.3

Power	Solution	*G (%)*
Control	GA_3	62.0 a
20	GA_3	59.7 a
50	GA_3	29.5 b
100	GA_3	6.5 cd
Control	Water	3.5 e
20	Water	4.7 de
50	Water	8.8 c
100	Water	4.3 de
LSD ($p \leq 0.05$)		2.69

Time	Solution	*G (%)*
60	GA_3	46.0 a
120	GA_3	39.5 b
180	GA_3	32.8 c
60	Water	6.8 d
120	Water	6.1 d
180	Water	3.1 e
LSD ($p \leq 0.05$)		2.3

Different letters in the same column indicate significant differences ($p \leq 0.05$) according to the LSD test.

Significant differences were obtained for the analyzed factors, output power and holding time and their interaction ($p \leq 0.01$; Table 5), representing 76.6%, 8.5% and 8.6%, respectively, of the variability of *A* in the germination test. This indicates that output power is the factor causing a greater effect. Given the statistical significance ($p \leq 0.01$) of the interaction, it is presented in Figure 6.

Table 5. Effect of the output power (W) and the holding time (s) of the ultrasonication on the germination variables: final germination (*A*), number of days needed to reach 50% of the final germination (Gt_{50}), and average germination rate ($k/2$; d^{-1}). Average values of four replicates.

	A (%)	Gt_{50}	*k/2*
Output power (P)			
Control	61.1 a	27.2 a	0.06 c
20 W	59.3 a	23.9 b	0.09 a
50 W	29.1 b	27.3 a	0.08 b
Holding time (T)			
60	54.8 a	24.2 b	0.08
120	51.4 a	28.0 a	0.07
180	43.2 b	26.2 ab	0.08
Analysis of variance			
Factors (degrees of freedom)	% Sum of squares		
P (2)	76.6 **	18.8 *	41.6 **
T(2)	8.5 **	18.1 *	1.9 NS
P × T (4)	8.6 **	16.4 NS	6.4 NS
Residual (27)	6.3	46.7	50.0
Standard deviation(+)	4.9	2.9	0.01

Different letters in the same column within each factor indicate significant differences ($p \leq 0.05$) according to the LSD test. **: significance level $p \leq 0.01$, *: significance level $p \leq 0.05$, NS: not significant. (+) The standard deviation was calculated as the square root of the residual mean square.

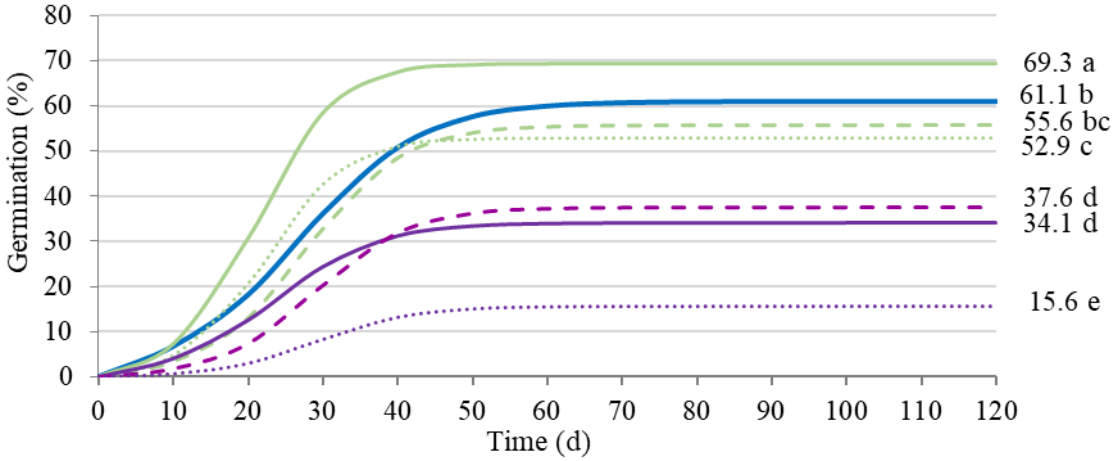

Figure 6. Analysis of the significant interaction of the analysis of variance in Table 5 between the output power (control (blue), 20 W (green) and 50 W (violet)) and the holding time of ultrasounds stimulation (60 s (solid), 120 s (dashed) and 180 s (dotted lines)) on the final germination percentage (*A*). Average values of four replicates. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$).

Seeds ultrasonicated for 60 s at 20 W power obtained a statistically higher *A* ($p \leq 0.05$) than control seeds, also reducing Gt_{50} (from 27.2 to 21.6 d). *A* was significantly affected ($p \leq 0.01$) by the interaction between the power and the holding time since ultrasonication of 20 W for 60 s was the only treatment that increased *A*, so when the treatment was extended to 120 and 180 s, *A* decreased, being lower than that of the control seeds ($p \leq 0.05$). *k*/2 was only affected by the output power ($p \leq 0.01$), resulting in an acceleration of germination when the seeds were treated with ultrasounds compared to the control.

These results coincide with those obtained by Pascual et al. [5], who applying ultrasonication in a bath (for 30 min, using an ultrasonic apparatus P. Selecta Ultrasons, model 513, 150 W, 40 kHz) obtained an increase in germination (up to 71.7%, on average) in caper seeds that presented (on average) a germination percentage of 48%, when they were not treated. Foschi et al. [34] reported that irradiation of caper seeds with a He-Ne laser for short exposure times improved the germination percentage (about 13%) of caper seeds previously soaked in water, with the application of a GA_3 solution to the germination substrate. This irradiation is a time demanding treatment, as the seeds have to be irradiated one by one, since this He-Ne laser has a circular beam of 0.7 mm in diameter. Juan et al. [35] obtained that exposing caper seeds to magnetic fields increased their imbibition compared to control seeds, and they reported a positive (although not statistically significant) effect of magnetic field exposure on seed germination, which only occurred with the addition of a GA_3 solution to the substrate. Thus, it can be stated that the herein presented results coincide with those obtained by this team in previous studies, using chemical scarification [33], irradiation with a He-Ne laser [34], and the exposure to magnetic fields [35]. In all these studies, a slight increase in the germination percentage has been obtained when any of these scarification treatments have been carried out together with the addition of GA_3 compared to those obtained with the sole addition of the GA_3 solution. However, neither of these treatments replace the action of GA_3 but rather complemented it.

The germination results also coincide with those reported by López-Ribera and Vicient [14] in the sense that short ultrasonic stimulation (for < 1 min) generated with a 45 kHz ultrasonic bath significantly increased the germination of *A. thaliana* seeds; however, they did not observe differences in the germination rate when using recently collected seeds that shown a germination percentage close to 90%. On the other hand, longer sonication treatments led to a decline in germination, as in the present study. Babaei-Ghaghelestany et al. [15], in order to break seed dormancy of *Chenopodium album*, applied ultrasonic waves with a frequency of 35 kHz for 5, 10, 15 and 30 min, and they observed that ultrasound waves enhanced the germination percentage, obtaining the greatest germination percentage when seeds were sonicated for 15 min.

The scientific literature relates the effects of ultrasonication on seeds to some mecha- nisms such as (i) the increase in the seed coat porosity that results in greater absorption of water during hydration, (ii) a slight oscillation of particles due to the movement of waves through tissues, which can lead to a rupture of the cell walls of the seeds, which increases the absorption of water by the seed, and (iii) the intensification of the mass transfer that improves the mobilization of nutrients from the endosperm by breaking the cell membrane [31,36]. Yaldagard et al. [13] stated that the ultrasonication (at 20 kHz on the ultrasonic generator in three different ultrasonic output powers and three holding times) resulted in better and faster germination on barley seeds ($p \leq 0.01$) due to both the fragmentation of the seed coat and the enlargement of its pore size, improving their hydration, which increased alpha-amylase activity causing their faster germination.

As previously mentioned, the water used for ultrasonication acquired a brown hue, their EC increased, and the testa of the seeds was scarified up to around 15% of their surface (Figure 4). Initially (approximately up to 24 h), ultrasonication stimulated the imbibition process (Figure 5), but there were no differences in the statistical analyzes carried out after 48 h of seed soaking (Table 1). Imbibition in both control and ultrasonicated seeds occurred through the hilar region and not through the tegmen (Figure S2), even though a fraction of the testa had been scarified (Figure 3). Therefore, it seems that ultrasound waves may fragment the hilar region of the seed coat and/or increase its pore size, as indicated by Yaldagard et al. [13] for barley seeds, which led to faster imbibition.

During the seed imbibition, if the hydration occurs slowly, the membranes can reorganize and reach their original structure, increasing the integrity of the membrane. However, if the hydration is too fast, it can cause the leakage of sugars, amino acids and minerals [37]. In the present study, the ultrasound treatment has caused hydration to be faster, to a greater extent, at 50 and 100 W, anticipating the maximum moisture level 24 h compared to the control seeds and those ultrasonicated at 20 W. The water used in the ultrasonication took on a brown hue (Figure 2) of increasing darkness coinciding with the increase in treatment output power and/or holding time, and at the same time, both its EC and its temperature increased (Figure 2). These effects are related to the evident degradation of part of the testa, but they could also be due to the leaching of sugars, amino acids and minerals and to the metabolism caused by a different enzymatic activity, but this should be verified in subsequent studies. It does not seem probable that the acceleration of the hydration process causes the leaching of nutrients, although the high temperatures could affect the enzymatic activity and, therefore, the metabolism of the reserves, which, together with the rupture of the cell walls, could contribute to the decrease of the viability and the percentage of germination of the seeds obtained with the higher output powers and/or holding times (Tables 2–5).

Wang et al. [38] studied the effects of ultrasonication on germination and seedling growth in switchgrass (*Panicum virgatum*) using an orthogonal matrix design. Among the studied factors (sonication time, temperature and output power), sonication temperature had the largest effect on germination, and temperatures around 40 ºC provided the greatest germination percentage, decreasing with increasing temperature. These authors concluded that ultrasonic treatments could have both positive and negative effects on seeds and that, when the output powers, holding times and consequently temperatures used are higher than the corresponding optimal levels, physical or chemical damage may occur due to ultrasonic waves. In the present study, the ultrasound output power of 20 W and the holding time of 60 s provided the greatest germination percentage, reaching 24 ºC during the ultrasound application. When the output power and/or holding time were higher, the temperature increased (up to 53.5 ºC), and the germination percentage decreased (up to 16%), probably as consequence of the physical or chemical damage produced by the bubble collapse.

Figure 7 shows a high lineal and negative correlation between both viability and germination (G when GA_3 is applied) with the temperature reached during the sonication treatment. In both cases, the correlation is statistically significant ($p \leq 0.01$), and the

corresponding correlation coefficients are −0.98 and −0.89. As seen in the figure, temperatures above 40 ºC considerably reduce the seed viability and, in a greater measure, the germination, practically nullifying it.

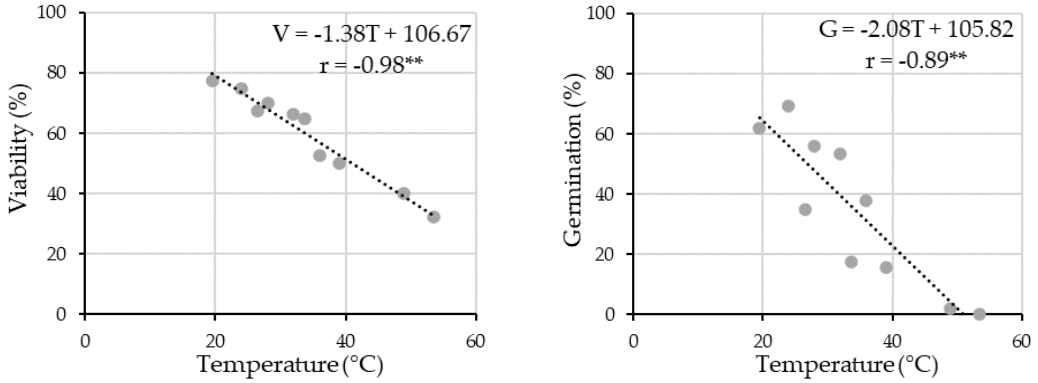

Figure 7. Viability–temperature relationship (**left**) and germination (G with GA_3 application; (**right**))–temperature relationship. **: significance level $p \leq 0.01$.

Analyzing the viability of the seeds that had not germinated after 120 days, it can be stated that those treated with 50 W are all not viable. For control seeds and those subjected to 20 W for 120 and 180 s, the viability of the non-germinated seeds ranged from 2.5% to 5.0%, indicating that slightly higher percentages of seeds could have germinated with more extended germination periods. However, when the seeds were treated with 20 W for 60 s, there were no viable seeds after 120 d, indicating that all the viable seeds germinated. Thus, it can be stated that subjecting the seeds to 20 W for 60 s encourages the germination of all viable seeds.

Foschi et al. [4] stated that adding gibberellins to the substrate in the caper seed germination tests increases the embryonic growth potential so that it can overcome the mechanical resistance imposed by the testa and therefore crack it without weakening it. Probably, according to Yaldagard et al. [13], the fragmentation of the seed coat and/or the enlargement of its pore size in the hilar region caused by ultrasonication led to faster imbibition. However, it could also have weakened the seminal coat in the hilar region and, consequently, improved the germination process, increasing *G*. This is what seems to have happened in the case of ultrasonication with 20 W for 1 min, in which ultrasonication has not replaced the action of GA_3 but rather has complemented it. Yaldagard et al. [13] also related better seed hydration with increased alpha-amylase activity, which led to a faster germination of barley seeds, but it should be tested for caper seeds in future studies.

4.2.5. Conclusions

Ultrasonic treatments can have both positive and negative effects on caper seeds; when the output power and sonication holding times are higher than the corresponding optimal levels, damage may occur due to ultrasonic waves. Ultrasonication produces faster initial imbibition, but after 48 h of soaking in water, seed moisture does not present differences compared to non-sonicated seeds. Ultrasonication leads to scarification of the testa but does not affect the tegmen, so moistening occurs through the hilar region, as in control seeds. A high linear and negative correlation between the germination of the seeds with the temperature reached during the sonication treatment was obtained, so that temperatures above 40 ºC practically annulled the germination. In the present study, the ultrasound output power of 20 W for 60 s provided the greatest germination percentage, being the only treatment that statistically improves germination in relation to control seeds. When the output power and/or holding time were higher, the temperature increased, and the germination percentage statistically decreased.

4.2.6. References

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Supplementary Materials:

Figure S1. Appearance of 100 mL glass beakers with 80 mL of water and 50 seeds after ultrasonic treatments at powers of 0, 20, 50 and 100 W (from left to right) and a holding time of 60 s.

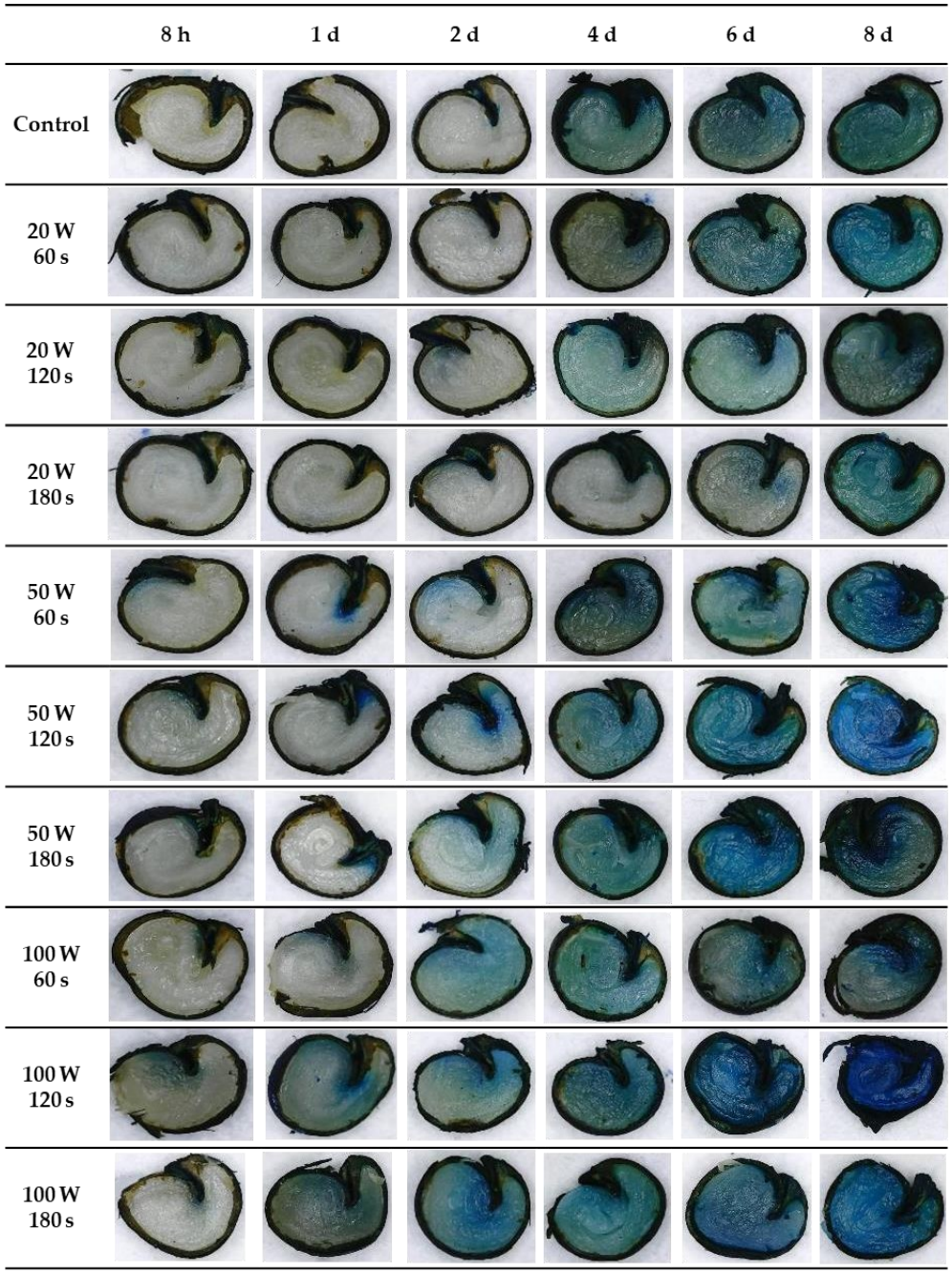

Figure S2. Time course of the staining with the methylene blue solution of the ultrasonicated seeds for 60, 120 and 180 s with the output powers of 20,50 and 100 W.

Capítulo 5. Discusión General

5.1. Discusión

La deficiente germinación (bajo porcentaje y elevado Gt_{50}) de las semillas de alcaparra tradicionalmente se ha relacionado con la latencia de estas (Sozzi y Chiesa, 1995; Pascual et al., 2009; Juan, 2017). De igual manera se ha considerado que se trata de una latencia primaria, y que de acuerdo con la clasificación de Baskin y Baskin (2014), confluían dos tipos de latencia: por una parte, una latencia exógena, concretamente física, debida a la impermeabilidad de su cubierta y, por otra parte, una latencia endógena, fisiológica, impuesta por factores presentes en el embrión (Orphanos, 1983; Ölmez et al., 2004; Pascual et al., 2004; Soyler y Khawar, 2007; Bahrani et al., 2008; Khaninejad et al., 2012; Marković et al., 2019; Nowruzian y Aalami, 2022).

La única forma de determinar si la cubierta seminal es o no impermeable es analizando la imbibición de las semillas durante el proceso de germinación. En el artículo *Water uptake and germination of caper (Capparis spinosa L.) seeds* (*Agronomy* 2020, 10, 838; https://doi.org/10.3390/agronomy10060838) se analizaron la imbibición, la viabilidad y la germinación de semillas de diferentes edades, procedencias (producción propia y comerciales) y el nivel de deterioro de su cubierta. Se comprobó que la hidratación de las semillas comienza por la región hilo-micropilar y en las primeras 24 h de remojo se alcanza aproximadamente el 85% del total de humedad, hasta estabilizarse el cuarto día de remojo, con valores de 34% de humedad en semillas propias y del 39% en semillas comerciales; en estas semillas cuando la cubierta está dañada, la hidratación se inicia por el área dañada, como ocurre en las semillas de otras especies, como la soja (Ma et al., 2004).

La evolución del contenido de humedad de las semillas, tanto de producción propia como comerciales, a lo largo del período de remojo sigue las dos primeras fases del modelo trifásico típico de absorción de agua en la germinación de las semillas: Fase I (imbibición propiamente dicha) y Fase II (*lag phase* o fase de meseta) de la germinación. En la Fase I, la absorción inicial de agua es principalmente un proceso físico y la actividad fisiológica podría comenzar a los pocos minutos de que las células se hidraten, mucho antes de que se complete la imbibición de todos los tejidos de la semilla (Bewley et al., 2013). Durante la Fase II, el contenido de agua de la semilla es bastante constante y la actividad metabólica aumenta con la transcripción de nuevos genes (Davies et al., 2018). La emergencia de la radícula al final de esta fase marca el fin de la germinación.

Las semillas de producción propia alcanzaron el 90% tanto en viabilidad como en germinación, lo que significa que todas las semillas de producción propia viables germinaron, y que el contenido de humedad alcanzado por las semillas era suficiente para permitir la germinación, es decir que las semillas de alcaparra no tienen una cubierta impermeable al agua *sensu stricto*, es decir, no muestran latencia física; sin embargo, la necesidad de utilizar ácido giberélico (AG) para obtener elevados porcentajes de germinación, demuestra la presencia de latencia fisiológica (LFg). Ninguno de los lotes comerciales de semillas estándar ensayados superó el 6% de germinación, ni el 35% de viabilidad. Una de las causas de esta baja viabilidad y germinación es la presencia de semillas con la cubierta agrietada o rota; este deterioro, visible a simple vista o no, producido en los procesos de extracción de los frutos, limpieza, secado y almacenamiento, disminuyó la viabilidad y el vigor de estas semillas, y consecuentemente su poder germinativo.

En el artículo titulado *Influence of seed-covering layers on caper seed germination* (*Plants* 2023, 12, 439; https://doi.org/10.3390/plants12030439) se ha estudiado la influencia de la cubierta de la semilla (testa y tegmen) y del endospermo en la germinación de las semillas de alcaparra, así como el efecto de la aplicación de AG en la superación de la LFg, permitiendo la germinación. Se ha medido la resistencia mecánica que la cubierta y el endospermo ejercen frente al crecimiento de la radícula. Con la adición de AG al sustrato de germinación de las semillas intactas se han obtenido porcentajes de germinación idénticos a los obtenidos con las semillas desprovistas de la cubierta sin adición de AG, dando valores de germinación superiores al 80%.

La adición de AG desencadena un aumento en el contenido de giberelinas activas endógenas, particularmente giberelina A_1 (A_1), una disminución en el contenido de ABA y, consecuentemente, el incremento de la relación [giberelinas activas endógenas]/[ácido abscísico]. El contenido creciente de giberelinas endógenas (particularmente A_1) probablemente se debe a la biosíntesis *de novo* más que a una reconversión de AG a A_1. Además del contenido hormonal, la transición del estado de latencia fisiológica no profunda a la germinación se acompaña de una disminución de la sensibilidad al ABA, concomitante con un aumento de la sensibilidad a las giberelinas (Matilla, 2013; Yuxi et al., 2021).

La germinación de las semillas de alcaparra consta de dos procesos separados temporalmente: primero se produce el rajado (*cracking*) de la cubierta, y después tiene lugar la perforación del endospermo, al igual que ocurre en semillas de otras especies como *Nicotiana tabacum* (Finch-Savage y Leubner-Metzger, 2006), *Lepidium sativum* y *Arabidopsis thaliana* (Müller et al., 2006). El rajado de la cubierta (testa y tegmen) comienza en el área hilo-micropilar e implica una señal del embrión, que puede ser reemplazada por las giberelinas, probablemente aumentando el potencial de crecimiento del embrión.

Después del rajado de la cubierta, la radícula emerge a través de un orificio en el endospermo micropilar (EM). La fuerza de punción necesaria para perforar el EM disminuyó drásticamente durante el primer día de imbibición (de 0,35 a 0,09 N), tanto con el uso de agua como de AG, manteniéndose prácticamente constante hasta el rajado de la cubierta, disminuyendo posteriormente, independientemente de la adición o no de giberelinas. Según Steinbrecher y Leubner-Metzger (2017), el debilitamiento del EM implica el aflojamiento de la pared celular, la separación celular y la muerte celular programada para proporcionar una disminución en la resistencia del tejido localizado en el EM, la autolisis y, finalmente, la formación del orificio requerido para la emergencia de la radícula.

Con el objetivo de proponer pautas prácticas para la utilización de giberelinas para mejorar la germinación de las semillas de la alcaparra, se realizaron tres experimentos incluidos en el artículo *Gibberellins improve caper seeds germination: guidelines for their application* (*Acta Horticulturae* 1365. ISHS 2023. DOI 10.17660/ActaHortic.2023.1365.7). Con una solución de 500 mg L^{-1} de AG aplicada al sustrato de germinación se obtuvo la máxima germinación (76%). Concentraciones más elevadas de AG aplicadas al sustrato disminuyeron la germinación, probablemente debido a una sobreexposición a tan alta concentración, ya que al final de las pruebas más del 70% de las semillas no germinadas estaban muertas. El remojo de las semillas durante 72 h con la concentración de 2000 mg L^{-1} de AG incrementó la germinación en relación con el control (8,6%), pero no superó el 31%.

Cuando se utilizó la concentración con la que se lograron los mejores resultados, que fue la solución de 500 mg L^{-1} de AG para humedecer y mantener el sustrato húmedo, únicamente durante los 30 primeros días (y después la humedad del sustrato se mantuvo con el aporte de agua) o se cambió el papel de filtro con una frecuencia quincenal, el porcentaje de germinación se incrementó significativamente (a 82,5% y 85,9% respectivamente), comparado con 76,3% cuando se humedeció continuamente con la solución de AG.

En cuanto a los diferentes tipos de giberelinas (A_3, A_4 y AG), con el uso de A_4, con concentraciones $\geq$ 5 mg L^{-1}, se obtuvieron porcentajes de germinación $\geq$ 90%. Mientras que con esas concentraciones de A_3 y AG la germinación no superó el 19%. La giberelina A_4 se ha comportado de 10 a 100 veces más activa que la giberelina A_3 y que el AG en la mejora de la germinación de las semillas de alcaparra, al igual que se ha demostrado en semillas de otras especies (Groot y Karssen, 1987). Sin duda alguna, el motivo de su infrautilización en la germinación de las semillas es su elevado precio en relación al AG.

En estudios previos realizados en el seno del equipo de investigación se había constatado una gran variabilidad en la germinación de semillas de alcaparra procedentes de lotes diferentes, así como la prácticamente nula germinación obtenida en varios lotes comerciales de semillas. Con el objetivo de proponer pautas de recolección para lograr una propagación comercial viable de la alcaparra a través de sus semillas, en el artículo titulado *Collection guidelines to achieve a viable caper commercial propagation* (*Agronomy* 2022, 12, 74. https://doi.org/10.3390/agronomy12010074), se

presenta el estudio realizado para determinar la viabilidad y la germinación de diferentes lotes de semillas. Las semillas de producción propia recolectadas en la dehiscencia del fruto (incluyendo los frutos de las posiciones anterior y posterior), presentaron mayores valores de viabilidad y de germinación final (humedeciendo el sustrato de germinación con la solución de 500 mg L^{-1} de AG), que las semillas de producción propia procedentes de frutos recolectados con la pulpa seca, que a su vez superaron a los de las semillas comerciales, con valores de germinación de 71,2%, 30,9% y < 2% respectivamente. Además, se constata que paralelamente a la disminución de la humedad de las semillas cuando su recolección se retrasa a partir del día de la dehiscencia del fruto, disminuye el porcentaje de germinación. Esto difiere de lo que ocurre en semillas de otras familias como las Asteraceae y Poaceae (Baskin y Baskin, 2020), que presentan una latencia fisiológica no profunda y presentan una postmaduración, es decir, una ruptura de la latencia durante el almacenamiento en seco. Se concluye que las semillas de alcaparra son sensibles a la desecación y que como regla general para la producción de semilla comercial se aconseja recolectar los frutos al menos una vez por semana, e inmediatamente extraer las semillas y ponerlas a germinar.

Conscientes del bajo porcentaje de germinación de las semillas de alcaparra, el equipo de investigación siempre ha separado y seleccionado las semillas a utilizar mediante el método de flotación (Davies et al., 2018). Por otra parte, también ha sido consciente de que con esta selección pueden desecharse algunas semillas viables. En estudios previos realizados con semillas procedentes de lotes comerciales se constató por una parte el bajísimo porcentaje de germinación obtenido y, por otra parte, la presencia de semillas con diferente color de la testa, relacionado con el grado de madurez de la semilla (Juan, 2017); concretamente en orden de madurez creciente aparecen semillas de color marrón claro (color Munsell 7.5 YR 5/8), marrón (7.5 YR,4/4) y marrón oscuro (7.5 YR 3/3). En el artículo *Criteria for the caper seeds collection and selection for commercial use* (*Acta Horticulturae* 1365. ISHS 2023. DOI 10.17660/ActaHortic.2023.1365.8), se analiza la respuesta germinativa de las semillas que flotan en el método de flotación (cuya testa puede ser de uno de los tres colores indicados), previamente extraídas de frutos, aparentemente maduros, recolectados con cuatro criterios de recolección: antes de la dehiscencia; inmediatamente después de la dehiscencia; 2-3 días después de la dehiscencia; y con la pulpa seca (incluyendo frutos recolectados de 4 a 10 días después de la dehiscencia).

El porcentaje de semillas que flotan en el test de flotación aumenta con el retraso de la recolección, alcanzando el 100% cuando el fruto se recolecta con la pulpa seca, debido a que tras la dehiscencia del fruto, como ya se ha comentado, las semillas se deshidratan, disminuyendo su densidad, de modo que todas flotan, tanto maduras como inmaduras. No obstante, el 95% de las semillas de los frutos recolectados con pulpa seca eran de color marrón oscuro, lo que significa que prácticamente todas las semillas estaban maduras.

La germinación de las semillas que flotan se ve afectada por el tipo de fruto y por el color de la testa, así como por su interacción, de manera que la máxima germinación, 22%, se obtuvo con semillas de color marrón oscuro extraídas de frutos con la pulpa seca, mientras que en el resto de las semillas la germinación fue inferior al 7%. Los bajos valores de germinación obtenidos podrían justificarse por el origen de las semillas, ya que son la fracción de semillas que flotaron en la prueba de flotación, y que normalmente se consideran de mala calidad y se descartan. En promedio, las semillas que flotan extraídas de frutos maduros, recolectados antes o en la dehiscencia representan el 8% del total, con un porcentaje de germinación del 3%, es decir que la pérdida de las semillas con capacidad germinativa que se produce en el test de flotación supone únicamente el 0,24%. Por tanto, se recomienda recolectar los frutos de alcaparra justo antes o después de su dehiscencia y desechar las semillas flotantes, independientemente de su apariencia.

En otros estudios realizados anteriormente, Pascual et al. (2006), determinaron 3,85 años para la longevidad de las semillas de alcaparra (almacenadas a 7 °C), recomendando un período de almacenamiento no mayor de dos años. En el artículo *The imbibition, viability, and germination of caper seeds (Capparis spinosa L.) in the first year of storage* (*Plants* 2022, 11, 202. https://doi.org/10.3390/plants11020202), se estudia la pérdida de poder germinativo de las semillas

durante el primer año de almacenamiento, analizando la imbibición, viabilidad y germinación de dos lotes de semillas, obtenidos en diferentes años, realizando las evaluaciones inmediatamente después de su recolección (semillas sin secar) y tras el almacenamiento (a 7º C) durante un mes y durante un año. La humedad inicial de las semillas recién extraídas del fruto fue mayor (25,1%) que las almacenadas durante un mes y un año (9,8% y 9,5% respectivamente). Se constata que al igual que en otros estudios, el contenido de humedad de la semilla se estabiliza a los pocos días del inicio del ensayo de germinación (en este caso el cuarto día), superando en todos los casos el 31%. Este nivel de humedad permite la germinación de todas las semillas viables, necesitando únicamente la adición de AG al sustrato de germinación, sin necesidad de escarificación, por lo que en este estudio también se confirma que las semillas de alcaparra presentan exclusivamente latencia fisiológica (*nondeep physiological dormancy*), concretamente una latencia impuesta por la cubierta de las semillas, debida a sus características mecánicas, que puede superarse añadiendo AG al sustrato de germinación. Con la adición de AG se obtuvieron valores de germinación muy altos (95% en las semillas recién recolectadas), disminuyendo durante el almacenamiento (88% en las semillas almacenadas un mes y 87% en las semillas almacenadas durante un año).

Paralelamente a los estudios presentados se han ensayado diversos tratamientos con el objetivo de mejorar la germinación, disminuyendo la fuerza que necesita el embrión para producir inicialmente el *cracking* de la cubierta y posteriormente la perforación del endospermo micropilar por parte de la radícula, y/o aumentando el potencial de crecimiento del embrión. Concretamente se han ensayado la iluminación con luces LED de diferentes longitudes de onda durante el ensayo de germinación, la irradiación de las semillas con luz láser, y la aplicación ultrasónica mediante un procesador de sonda.

En el artículo titulado *Influence of lighting and laser irradiation on the germination of caper sedes* (*Agriculture* 2022, 12, 1612. https://doi.org/10.3390/agriculture12101612) se analiza el efecto de la exposición de las semillas de alcaparra a la luz durante el ensayo de germinación, para determinar si se puede romper la latencia de las semillas mediante la activación de diferentes pigmentos fotosensibles por parte de fotones de diferentes longitudes de onda (Meisel et al., 2011). Concretamente se estudia la respuesta a la iluminación con diferentes longitudes de onda, correspondientes a las luces blanca, roja, azul, roja + azul, y por otra parte a la luz del láser He-Ne, con relación a la oscuridad. De los resultados se concluye que durante el proceso de germinación las semillas de alcaparra son insensibles a la luz, por lo que la germinación se podría realizar tanto con luz como en oscuridad, es decir que en los semilleros comerciales la germinación podría realizarse en la oscuridad, lo que supondría un importante ahorro energético. Germanà y Chiancone (2009), tampoco obtuvieron una diferencia significativa al incubar semillas de alcaparras escarificadas mecánicamente en luz blanca y oscuridad.

La irradiación de semillas de alcaparra con un láser He-Ne durante tiempos cortos de exposición (1 y 15 s), mejoró el porcentaje de germinación de las semillas previamente humedecidas, germinando todas las semillas viables. No obstante, en este trabajo la exposición de las semillas al láser He-Ne no sustituyó a la adición de AG al sustrato, sino que complementó su efecto.

En el artículo titulado *Effects of high intensity ultrasound stimulation on the germination performance of caper seeds* (*Plants* 2023, 12, 2379. https://doi.org/10.3390/plants12122379) se estudia el efecto de la aplicación ultrasónica mediante un procesador de sonda (que actúa por cavitación; Ranade et al., 2013), en la disrupción de la cubierta de la semilla, la imbibición, la viabilidad y la germinación de semillas de alcaparra. La ultrasonicación acelera la imbibición inicial, pero después de 48 h de remojo, la humedad de la semilla es la misma que la de las semillas no ultrasonicadas. En estas semillas se escarifica la testa pero no afecta el tegmen, por lo que la humectación se produce por la región hilo-micropilar, igual que en las semillas testigo, ya que el daño que se produce en la testa es mínimo, mientras que en los tratamientos con mayores potencias y tiempos se produce un daño significativamente mayor en la testa y se alcanzan mayores temperaturas lo que podría afectar la viabilidad y germinación. Existe una correlación significativa, lineal y negativa, entre la germinación de las semillas y la temperatura alcanzada durante el tratamiento de

ultrasonicación, de modo que con temperaturas superiores a 40 °C se reduce considerablemente la germinación, llegando a anularse. La ultrasonicación de las semillas durante 60 s con una potencia de 20 W proporcionó el mayor porcentaje de germinación, superando al tratamiento considerado control (con adición de AG al sustrato). Los efectos de la ultrasonicación se relacionan con algunos mecanismos como el aumento de la porosidad de la cubierta seminal, lo que lleva en una mayor absorción de agua y una mejora en la movilización de nutrientes del endospermo al romper la membrana celular (Miano et al., 2016; Andriamparany y Buerkert, 2019), lo que no ha ocurrido en las semillas sometidas al tratamiento con el que se ha obtenido la mejor germinación.

5.2. Perspectivas futuras

A pesar del enorme trabajo realizado en esta tesis doctoral, dada la limitación temporal ha sido imposible abordar algunos apartados, por lo que, continuando la línea de investigación, se recomienda realizar a corto plazo, los siguientes estudios:

- Evaluar tratamientos para superar la latencia y/o mejorar la germinación, no ensayados en semillas de alcaparra, como la utilización de pulsos eléctricos, rayos UV o de compuestos que actúan como reguladores de crecimiento de las plantas, como las karrikinas (presentes en el humo de la quema de material vegetal) y los brasinoesteroides. Asimismo, convendría estudiar el efecto de tratamientos como el *priming* biológico con bacterias, por ejemplo, *Bacillus megaterium*, muy poco estudiadas en la germinación de las semillas de alcaparra.
- Analizar el vigor de las plántulas y la supervivencia de las mismas tras el trasplante, para trasladar este conocimiento al proceso de obtención de plantas en vivero.
- Analizar la actividad enzimática (lipasas, amilasas y proteasas) y los niveles hormonales en el proceso de la germinación, particularmente en semillas irradiadas con luz láser o ultrasonicadas.
- Estudiar las familias de fotorreceptores presentes en las semillas de alcaparras.
- Aunque se sale del objetivo de esta tesis doctoral, sería interesante determinar los mecanismos moleculares y la expresión génica conducentes a la germinación de las semillas de alcaparra.
- Trabajar en el desarrollo de maquinaria específica para la recolección de las alcaparras, debido a que es una tarea que demanda mucha mano de obra temporal, es una labor lenta y en algunos casos las espinas hacen que sea muy dificultosa, por lo que esta labor se convierte en un problema a la hora de manejar el cultivo, siendo uno de los principales motivos de la falta de interés de la industria en su expansión en España.

5.3. Referencias

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Capítulo 6. Conclusiones Finales

- La germinación de las semillas de alcaparra consta de dos procesos separados temporalmente: primero se produce el rajado de la cubierta, y después la perforación del endospermo.
- Durante la primera fase de la germinación, la hidratación de la semilla comienza por la región hilo-micropilar.
- El rajado de la cubierta comienza en el área hilo-micropilar. Tras el rajado de la cubierta, la radícula emerge a través de un orificio que se produce en el endospermo micropilar.
- El contenido de humedad de las semillas sigue las dos primeras fases del modelo trifásico típico de absorción de agua en la germinación de las semillas: Fase I (imbibición propiamente dicha) y Fase II (*lag pase* o fase de meseta) de la germinación.
- El contenido de humedad alcanzado por las semillas se estabiliza a los pocos días del inicio del ensayo de germinación (el cuarto día de remojo, alcanzando valores del 32% de humedad), superando en todos los casos el nivel de humedad que permite la germinación de todas las semillas viables. Por este motivo, las semillas de alcaparra no tienen una cubierta impermeable al agua *sensu stricto* y, por tanto, no presentan latencia física.
- Para conseguir la germinación de todas las semillas, solo es necesaria la adición de AG al sustrato de germinación, sin necesidad de escarificación, por lo que las semillas de alcaparra presentan exclusivamente latencia fisiológica (*nondeep physiological dormancy*).
- Se trata de una latencia impuesta por la cubierta de las semillas, debida a sus características mecánicas, que puede superarse añadiendo una solución de AG al sustrato de germinación.
- La adición de AG desencadena un aumento en el contenido de giberelinas activas endógenas, una disminución en el contenido de ABA y un aumento de la relación [giberelinas activas endógenas]/[ABA].
- Las giberelinas activas no disminuyen la fuerza de punción necesaria para perforar el endospermo, sino que aumentan el potencial de crecimiento del embrión.
- En cuanto a la utilización del AG los mejores resultados se obtienen humedeciendo el sustrato con la solución de 500 mg L^{-1}, cambiando quincenalmente el sustrato o utilizando la solución únicamente durante el primer mes y luego humedeciendo el sustrato solo con agua.
- La giberelina A_4 es hasta 100 veces más activa que la giberelina A_3 y que el AG en la mejora de la germinación de las semillas de alcaparra. Se recomienda el uso de soluciones con concentraciones entre 5 a 50 mg L^{-1} de A_4 o 500 mg L^{-1} de AG para humedecer el sustrato de germinación.
- La viabilidad y el porcentaje de germinación de las semillas de alcaparra recién extraídas de frutos recolectados en su dehiscencia alcanzan valores muy elevados.
- Si la recolección de los frutos se retrasa y la pulpa se seca, la viabilidad y el porcentaje de germinación disminuyen, por lo que, como regla general, para la producción de semilla comercial se aconseja recolectar los frutos al menos una vez por semana, e inmediatamente extraer las semillas y ponerlas a germinar.
- Las semillas que flotan con el método de extracción por flotación y con capacidad germinativa supone únicamente el 0,24%, por lo que se recomienda desechar las semillas que flotan, independientemente de su apariencia.
- La iluminación, de las semillas de alcaparra durante el proceso de germinación con longitudes de onda correspondientes a las luces blanca, roja, azul, roja + azul, no afecta a su germinación. En los semilleros comerciales la germinación puede realizarse en la oscuridad, lo que puede suponer un importante ahorro económico y energético.

- La irradiación de semillas de alcaparra con láser He-Ne durante tiempos cortos de exposición mejora el porcentaje de germinación de las semillas previamente hidratadas. No obstante, la exposición de las semillas al láser He-Ne no sustituye a la adición de AG al sustrato, sino que complementa su efecto.
- La ultrasonicación acelera la imbibición inicial, pero después de 48 h de remojo, la humedad de las semillas es la misma que la de las semillas no sonicadas. Con ésta, se escarifica la testa sin afectar al tegmen, de modo que la humectación se produce por la región hilo-micropilar, igual que en las semillas testigo.
- Existe una correlación significativa, lineal y negativa, entre la germinación de las semillas y la temperatura alcanzada durante el tratamiento de ultrasonicación. Entre los tratamientos ensayados, el único que mejora la germinación respecto al control (en ambos casos con adición de AG al sustrato), es la exposición de las semillas durante 60 s a la potencia de 20 W.