UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE ESTUDIOS SUPERIORES IZTACALA

Papel de la temperatura en la germinación y en el perfil de ácidos grasos de semillas de chía (*Salvia hispanica* L.)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

Daniel Cabrera Santos

TUTOR PRINCIPAL DE TESIS: Dr. César M. Flores Ortiz. FES-I, UNAM.
COMITÉ TUTOR: Dra. Alma Delfina Lucía Orozco Segovia. Instituto de Ecología, UNAM.
Dr. Jorge E. Campos Contreras. FES-I, UNAM.

Los Reyes Iztacala, Estado de México, Febrero, 2022

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Los Reyes Iztacala, Tlalnepantla, Estado de México 2022

OFICIO CPCB/136/2022

ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **08 de noviembre de 2021** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **CABRERA SANTOS DANIEL** con número de cuenta **302065101** con la tesis titulada **"Papel de la temperatura en la germinación y en el perfil de ácidos grasos de semillas de chía (*Salvia hispánica* L.).",** realizada bajo la dirección del **DR. CÉSAR MATEO FLORES ORTÍZ,** quedando integrado de la siguiente manera:

Presidente:	DRA. MARÍA DEL ROCÍO CRUZ ORTEGA
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Vocal:	DR. VICTOR MANUEL CHÁVEZ ÁVILA
Secretario:	DRA. ALMA DELFINA LUCÍA OROZCO SEGOVIA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 03 de FEBRERO de 2022

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

AGRADECIMIENTOS

AGRADECIMIENTOS

DEDICATORIA

A mi madre:

Eres mi testigo, mis ojos, mi evidencia
Irene, mi incondicional

A la memoria de mi Padre y hermano, a mis hermanos y al resto de mi familia.

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El mundo es incomprensible. No vamos a entenderlo nunca, no vamos a desentrañar sus secretos nunca. Por lo tanto, debemos tratar al mundo tal como es: un gran misterio.
Don Juan Matus

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Resumen

La chía (*Salvia hispanica* L.) o "aceitosa" para las culturas azteca y maya, es una planta herbácea y oleaginosa anual de verano, incluida dentro de la familia de las mentas (Lamiaceae). Algunos de los beneficios de esta oleaginosa en la nutrición y la salud se relacionan con su cantidad sustancial de aceite. Por lo tanto, es importante investigar los aspectos moleculares, bioquímicos, fisiológicos y agronómicos de las semillas de chía; áreas en las que, hasta ahora, eventos como la germinación temprana y el posterior establecimiento de las plántulas han sido poco estudiados.

Durante nuestros ensayos de imbibición llevados a cabo a 10, 20 y 30 °C, se observaron tres diferentes fases de imbibición; a estas fases se les denomino FI, FII y FII$_{end}$, de acuerdo con la denominación aceptada en la actualidad para describir las diferentes etapas de imbibición que acontecen en las semillas. FI se caracteriza por una absorción de agua inicial y significativa, luego una fase de meseta sin cambios significativos en el peso fresco de la semilla (FII), que representa la etapa final para las semillas muertas y latentes, y finalmente, una nueva absorción de agua significativa correspondiente a la etapa de germinación (FIII). En chia, sin embargo, después de 3 a 4 h de imbibición, observamos una pérdida de peso relacionada con la pérdida de mucílago que ocurrió al comienzo de FIII, esta pérdida de peso enmascaró el inicio de FIII. Debido a que el FIII no fue observada con claridad por la pérdida de mucílago, el lapso entre el último aumento de peso y el final de la pérdida de peso se consideró como la extensión de FII y se denominó FII$_{end}$.

En nuestros ensayos observamos que FI ocurre dentro de la primera hora en las tres temperaturas ensayadas; mientras que FII y FII$_{end}$ transcurren una hora antes a 20 °C y 30 °C con respecto a 10 °C. Las mayores tasas de viabilidad y germinación se observaron a 30 °C;

mientras que las concentraciones más altas de todos los ácidos grasos, excepto el ácido oleico, se observaron a 20 °C. Las concentraciones máximas de ácidos grasos se detectaron durante FI y FII_{end}; mientras que a 30 °C se observaron patrones diferentes para los ácidos grasos saturados e insaturados y tres isómeros en *trans* del ácido linolénico. Nuestros resultados muestran que una FII con menor duración se puede asociar con una germinación más temprana; mientras que el aumento en la concentración de ácidos grasos después de 3 horas de imbibición y una correlación negativa entre las concentraciones de los ácidos linoleico y linolénico observada a 20 °C, se relacionaron con una mayor eficiencia de germinación. A 30 °C, la formación de isómeros está relacionada con la adaptación homeoviscosa de la membrana celular.

Durante los ensayos llevados a cabo con el gradiente de temperatura de los 10–45 °C, se observó que la germinación de semillas de chia ocurre en temperaturas frías a moderadas-altas (10–30 °C), con un rango óptimo entre los 25–35 °C, con una mayor velocidad de germinación (GR) y tiempo medio de germinación (t_{50}) a 30 °C; mientras que las temperaturas superiores a 35 °C reducen significativamente la germinación. Los resultados concuerdan con las condiciones ambientales bajo los que la especie crece, es decir, ambientes tropicales y subtropicales, elevaciones de 400 a 2500 m s. n. m. y temperaturas moderadas.

Los parámetros de los seis diferentes modelos de regresión no lineal para estimar las temperaturas cardinales de semillas de chía mostraron que la respuesta de la germinación en función de la temperatura se explica mejor mediante modelos de tipo beta (B). Las temperaturas cardinales calculadas por el modelo B1 para la germinación de chía fueron: 2.52 ± 6.82 °C para la temperatura base, 30.45 ± 0.32 °C para la temperatura óptima y 48.58 ± 2.93 °C para la temperatura tope.

Abstract

Chia (*Salvia hispa*nica L.) or "oily" for the Aztec and Mayan cultures, is an annual summer herbaceous and oleaginous plant, included within the mint family (Lamiaceae). Some of the benefits of this oilseed in nutrition and health are related to its substantial amount of oil. Therefore, it is important to investigate the molecular, biochemical, physiological, and agronomic aspects of chia seeds areas in which, until now, early germination events that are directly related to the subsequent establishment of seedlings have been little studied.

During our imbibition assays performed at 10, 20 and 30 °C, three different imbibition phases were observed; these phases were called FI, FII and FII$_{end}$, according to the denomination currently accepted to describe the different imbibition phases that occur in the seed. FI is characterized by an initial and significant water absorption, then a plateau phase without significant changes in the fresh weight of the seed (FII), which represents the final stage for dead and dormant seeds, and finally, significant new water uptake corresponding to the germination stage (FIII), this final restart of water uptake is only experienced by germinating seeds. In chia, however, after 3 to 4 h of imbibition, we observed a weight loss related to the loss of mucilage that occurred at the beginning of FIII, this weight loss masks the beginning of FIII. Because FIII is not clearly distinguished by mucilage loss, the period between the last weight gain and the end of weight loss was considered as the extension of FII and was designated FII$_{end}$.

Our imbibition assays showed that FI occurs within the first hour at the three temperatures tested; while FII and FII$_{end}$ take place one hour earlier at 20 °C and 30 °C compared to 10 °C. The highest rates of viability and germination rate were observed at 30 °C; while the highest concentrations of all fatty acids, except oleic acid, were observed at 20 °C. The

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maximum concentrations of fatty acids were detected at FI and FII_{end}; while at 30 °C different patterns were observed for saturated and unsaturated fatty acids and three trans isomers of linolenic acid. Our results showed that a shorter FII can be associated with earlier germination; while the increase in the concentration of fatty acids after 3 hours and a negative correlation between linoleic and linolenic acid concentrations observed at 20 °C, was related to a higher germination efficiency. At 30 °C, isomers formation is related to homeoviscous adaptation of the cell membrane.

During tests carried out with a temperature gradient of 10–45 °C, it was observed that chia seed germination occurs in cold to moderate-high temperatures (10–30 °C), having an optimal range between 25–35 °C, with higher germination rate (GR) and mean germination time (t_{50}) at 30 °C; while temperatures above 35 °C significantly reduce germination. The results agree with the environmental conditions under which the species grows, that is, tropical and subtropical environments, elevations of 400 to 2500 m. a. s. l. and mild temperatures.

The parameters of the six different nonlinear regression models to estimate the cardinal temperatures of chia seeds showed that the germination response as a function of temperature is best explained by beta-type models (B). The cardinal temperatures calculated by the B1 model for chia germination were: 2.52 ± 6.82 °C for the base temperature, 30.45 ± 0.32 °C for the optimum temperature and 48.58 ± 2.93 °C for the ceiling temperature.

Introducción general

Etapas de la germinación

La germinación es el proceso fisiológico que comienza con la toma de agua y culmina con la emergencia del embrión (radícula u otro tejido embrionario) a través de las estructuras que lo recubren. Este evento final es referido a menudo como "germinación visible" y constituye la prueba tangible de que la germinación *sensu stricto* se ha completado (Bewley *et al*., 2013). Debido a la alta vulnerabilidad a lesiones, enfermedades o estrés medioambiental, la germinación es considerada como el evento más decisivo durante el ciclo de vida de las plantas. La germinación excluye el crecimiento y/o establecimiento de la plántula, eventos que comienzan ulteriormente; por lo tanto, la *emergencia de la plántula* y el *establecimiento* de ésta, constituyen eventos posgerminativos.

El proceso de germinación implica que la semilla debe recuperarse rápidamente de la desecación intrínseca al proceso de maduración, el cual induce un estado de quiescencia; también es necesario reanudar el metabolismo, el completo desarrollo de eventos celulares que permiten la emergencia del embrión, así como la preparación del subsecuente desarrollo de la plántula (Bewley, 1997; Bewley *et al*., 2013; Bareke, 2018). Durante la germinación existen diferentes puntos de control (Figura 1b), el primero se encuentra gobernado por el ácido abscísico (ABA), previniendo la germinación bajo circunstancias desfavorables (Nambra *et al*., 2010). Además, la germinación es un proceso reversible donde se recapitula el programa previo de maduración (Lopez-Molina *et al*., 2002; 2004; Rajjou *et al*., 2004; 2006). La siguiente etapa también se encuentra mayoritariamente bajo el control del ABA y posteriormente, por la actividad del ácido giberélico (GA) cerca de la emergencia del embrión, cuya actividad continúa hasta el final de la germinación (Gallardo *et al*., 2002;

Ogawa *et al*., 2003). Antes de la emergencia de la radícula, es difícil o imposible predecir cuánto ha progresado una semilla hacia la culminación de la germinación. En este sentido, el avance durante la germinación se ha relacionado con la toma de agua o imbibición, la cual es requerida para reanudar el metabolismo y la iniciación de eventos celulares que conducen a la emergencia de la radícula (Bewley *et al*., 2013).

La toma de agua por parte de la semilla se ha descrito como una curva sigmoidea que exhibe un comportamiento trifásico (Figura 1a). Durante la Fase I, las semillas experimentan una absorción exponencial de agua; posteriormente, la absorción de agua disminuye y se hace constante dando lugar a una meseta, la cual constituye la Fase II, la culminación de esta fase da lugar a la emergencia de la radícula y solo las semillas que han completado la germinación en sentido estricto experimentan absorción de agua en la Fase III.

1. **Fase I**: Toma rápida de agua.

2. **Fase II**: Fase de meseta (*germinación visible* o en *sentido estricto*).

3. **Fase III**: Toma final de agua, la cual solo experimentan las semillas que han germinado.

Es importante mencionar que debido a que semillas dormantes o muertas también absorben agua, a menudo es difícil determinar el inicio y el final de FIII de semillas que han completado el proceso de germinación (Bewley, 1997). Dentro de estas dificultades también se encuentra el caso de chia, en donde la perdida de peso asociadas al mucilago también enmascaró el inicio de FIII y, por lo tanto, en este trabajo, se denominó FII$_{end}$ al final de la FII.
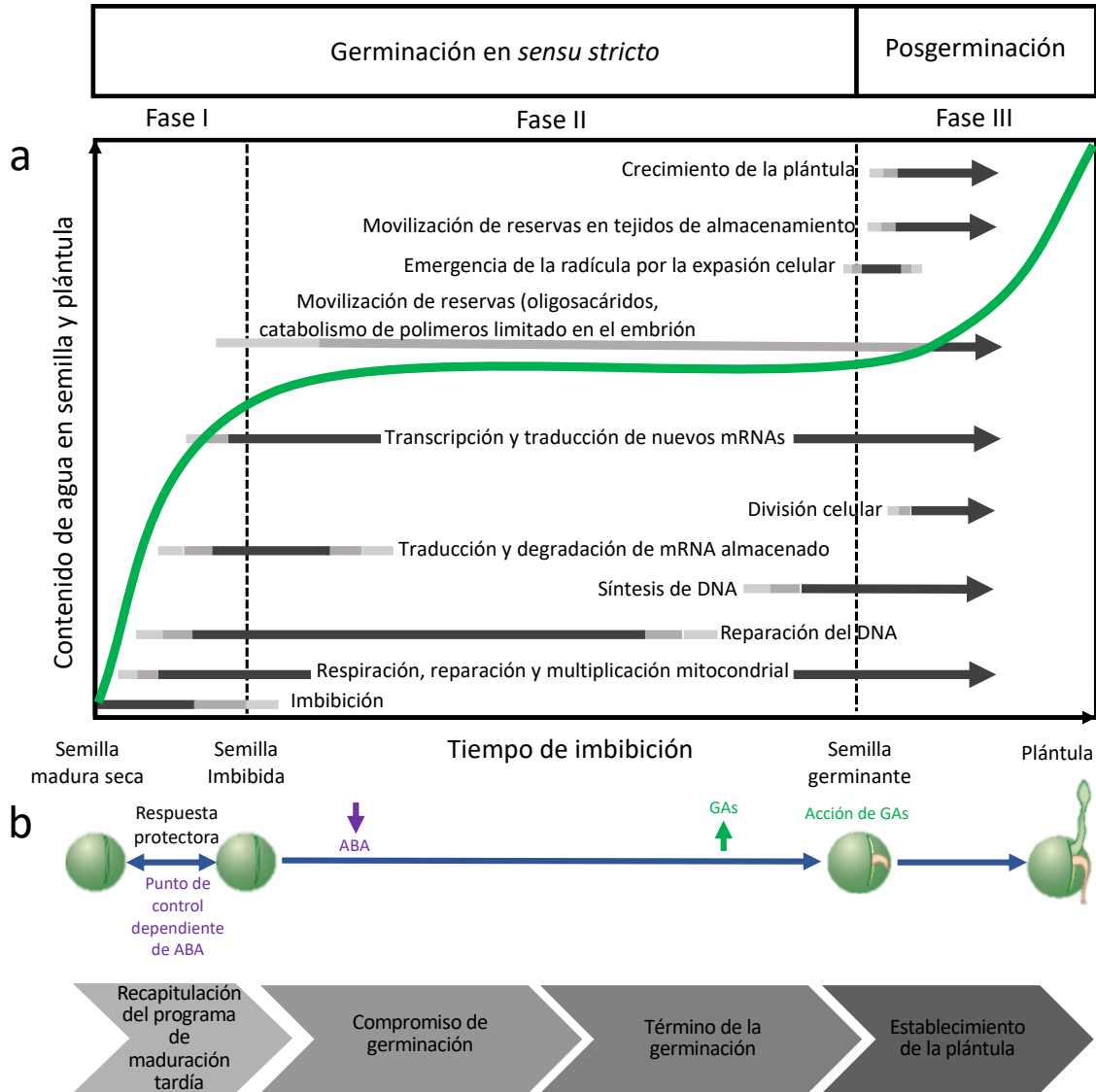
Figura 1: : **a) Absorción de agua durante las tres fases de la germinación y cambios importantes asociados a la germinación y al crecimiento temprano de la plántula**. Durante la absorción inicial de agua o Fase I de imbibición, el proceso es principalmente físico; las actividades fisiológicas pueden comenzar en cuestión de minutos después de que las células comienzan a hidratarse, mucho antes de que todos los tejidos de las semillas estén completamente imbibidos. Durante la Fase II la absorción de agua es constante y las actividades metabólicas incrementan con la transcripción sustancial de nuevos genes. La emergencia de la radícula a través de las estructuras que la rodean al final de esta fase, marca el final de la germinación; durante la Fase III hay una absorción adicional de agua a medida que se establece la plántula utilizando las principales reservas almacenadas. La curva sigmoidea representa el curso temporal estilizado para la absorción de agua. El tiempo para que se completen los eventos varía dependiendo de la especie y de las condiciones de germinación, a los que las semillas están sujetas. **b) Fenomenologia de la germinación.** En este panel se muestran los diferentes puntos de control hormonal ejercido por el ABA y GA durante cada fase de la germinación; el control hormonal es parte de los diferentes programas de desarrollo que atraviesa la semilla durante el tiempo de imbibición. Adaptado de Bewley *et al*., 2013 y Rajjou *et al*., 2012.

La absorción de agua por parte de las semillas se encuentra gobernada por las relaciones hídricas entre la semilla y el suelo, las cuales son conducidas por un gradiente de potencial hídrico de alrededor de 5 órdenes de magnitud entre la semilla (entre -50 a -350 MPa) y el suelo (-0.03 MPa) (Hadas, 1982; Bewley *et al.*, 2013). La cantidad de agua dentro de la semilla determina los procesos moleculares, metabólicos, celulares y fisiológicos que acontecen dentro de ella (Figura 1a). La Fase I de la germinación es conducida casi en su totalidad por el potencial mátrico (Ψ_m) o el agua que se encuentra unida a las superficies moleculares (proteínas, lípidos y otras macromoléculas).

Efecto de la temperatura sobre la germinación

Durante el ciclo de vida de las plantas, la germinación y el establecimiento de las plántulas son las etapas más críticas y de las cuales depende el éxito de la próxima generación. Específicamente, la germinación está sujeta a la selección natural antes de que ocurra el establecimiento de los individuos (Donohue *et al.*, 2010; Donohue *et al.*, 2015) y es regulada estrechamente por factores físicos como la temperatura, la disponibilidad del agua y el ambiente gaseoso (Bewley *et al.*, 2013). Cuando la disponibilidad de agua no es limitada, la temperatura es el elemento que afecta mayoritariamente la velocidad a la que ocurre la germinación, el nivel de latencia y la tasa de deterioro de las semillas. La temperatura afecta mayoritariamente la velocidad de germinación debido a que está relacionada directamente con la absorción de agua por parte de las semillas (Benech-Arnold *et al.*, 2000; Batlla & Benech-Arnold, 2015), así como con las reacciones bioquímicas que regulan el metabolismo durante el proceso de germinación (Marcos-Filho, 2015). Adicionalmente, el periodo de germinación puede alterarse completamente en respuesta a la temperatura (Bewley & Black, 1994).

Existen evidencias del efecto de la temperatura sobre la germinación, por ejemplo, en frijol se observó que semillas germinadas continuamente a 20°C tienen un umbral de humedad menor que semillas a 5 °C; debajo de este umbral de humedad, la germinación disminuye linealmente a medida que disminuye el contenido de humedad (Wolk *et al*., 1989) (Figura 2a). En este sentido, se ha planteado que, a temperaturas subóptimas, existen cambios en la configuración de las membranas, lo cual provoca cambios sobre la retención de solutos en el interior celular, incluyendo azúcares, ácidos orgánicos, iones, aminoácidos y proteínas hacia el medio circundante, provocando daño por imbibición. La temperatura parece determinar el contenido de humedad inicial, al cual inicia este daño. Por otra parte, en el girasol, se observó la disminución en la germinación de semillas imbibidas a 10°C con respecto a semillas a 20°C, alcanzado únicamente un 10% de la germinación total y manteniéndose así hasta el día 14 del experimento (Xia *et al*., 2018) (Figura 2b). En otro trabajo con semillas de papa, se observó que durante la germinación a 15°C existe un incremento en la velocidad de germinación alcanzando el 100% más temprano durante el tiempo de imbibición con respecto a semillas germinadas a 12.5 y 10°C (Alvarado & Bradford, 2005) (Figura 2c).
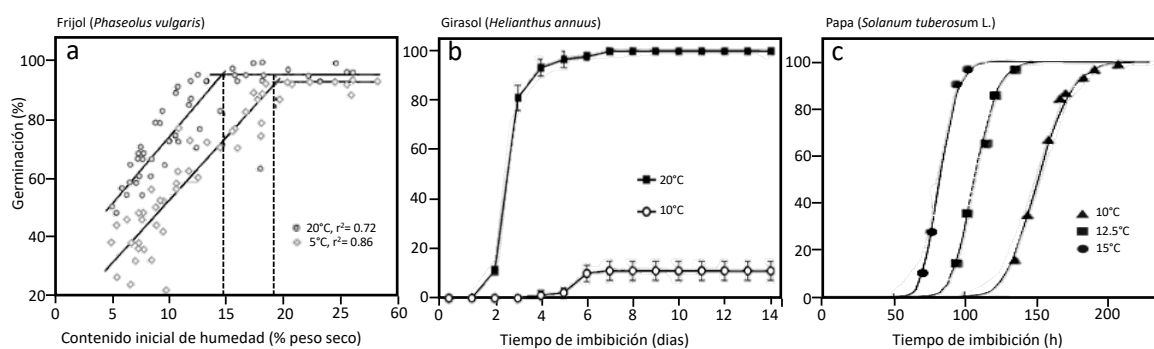
Figura 2: Efecto de la temperatura sobre la absorción de agua y germinación. **A) Efecto del contenido de humedad inicial y temperatura en el daño imbibicional en semillas de *Phaseolus* cv. Tendercrop.** Las semillas fueron germinadas continuamente a 20°C o a 5°C por 24 h y luego a 20°C. Debajo del umbral de humedad (línea punteada vertical), la germinación disminuye linealmente a medida que disminuye el contenido de humedad. El contenido de humedad umbral fue más alto (19%) a baja temperatura (5°C, gris claro, diamantes) en comparación con alta temperatura (15%) (20°C, gris

fuerte, círculos). Los coeficientes de determinación (r²) pertenecen a las pendientes debajo del umbral de contenido de humedad. Adaptado de Wolk *et al.*, (1989). **b) Curvas de germinación de semillas de girasol bajo diferentes temperaturas.** Los embriones latentes fueron imbidos con agua destilada a 10 °C y 20 °C en oscuridad. La tasa de germinación a 10 °C solo alcanzó un 10% de la germinación total después de 7 días de imbibición, manteniéndose así hasta el día 14 del experimento, mientras que a 20 °C cerca del 96% de germinación de las semillas dormantes fue alcanzada alrededor de los 7 días de imbibición. Adaptado de Xia *et al*. (2018). **c) Tiempos de germinación de semillas de papa.** Curvas de germinación a 10 (triángulos), 12.5 (cuadros) y 15 °C (círculos); a 15°C existe un incremento en la velocidad de germinación con respecto a semillas germinadas a 12.5 y 10°C. Adaptado de Alvarado & Bradford, 2005.

Caracterización de la germinación en función de la temperatura

Una forma de caracterizar la germinación en función de la temperatura es a través de las temperaturas cardinales o umbral. La importancia de determinar las temperaturas cardinales se basa en la identificación de los límites de temperatura inferior y superior de germinación. Su importancia también radica en la agricultura y en ecología, dándonos indicios del origen biogeográfico y del comportamiento de las especies bajo el escenarios de cambio climático.

Se reconocen tres temperaturas cardinales: temperatura base (T_b), debajo de la cual la germinación no ocurre; temperatura óptima (T_o), en la cual acontece la tasa de germinación más alta; y temperatura máxima o tope (T_c) por arriba de la cual la germinación cesa (Figura 3). De manera importante, se ha sugerido que estas tres temperaturas muestran variabilidad intra e interespecífica, llegando incluso a ser variables entre poblaciones. (Alvarado & Bradford 2002; Bewley *et al*., 2013; Batlla & Benech-Arnold, 2015; Dürr *et al*., 2015). La importancia de determinar las temperaturas cardinales se basa en la identificación de los límites de temperatura inferior y superior dentro del nicho de germinación identificado por Grubb (1977).

Dentro del rango subóptimo se puede emplear el concepto de tiempo térmico, integral térmica o suma de calor (θ_T), el cual se define como la temperatura acumulada en un intervalo de tiempo necesario para completar un proceso fisiológico; éste se expresa en unidades térmicas

grados centígrados/unidad de tiempo (°Ct) (Trudgill *et al*., 2000) (Figura 3a). La suma de calor, al ser diferente entre diferentes fracciones germinantes dentro de una población de semillas permite la predicción de la velocidad y tiempo de germinación de una determinada fracción de semillas a una temperatura dada dentro del intervalo subóptimo; por lo tanto, permite modelar la respuesta de germinación bajo diferentes escenarios ambientales (García-Huidobro *et al*., 1982; Steinmaus *et al*., 2000; Alvarado & Bradford, 2002; Bradford, 2002; Trudgill *et al*., 2000). El tiempo térmico está definido por la siguiente ecuación:

$$\theta_T(g) = (T - T_b)\, tg$$

Donde $\theta_T(g)$ es la *constante térmica* o *tiempo térmico* en el que la fracción **g** de la población completa su germinación; **T** es la *temperatura*, T_b es la *temperatura base*, y **tg** es el *tiempo para que la fracción* **g** *complete la germinación*. En este caso $\theta_T(g)$ se hace una constante para esa fracción en particular; diferentes fracciones requieren diferente tiempo térmico para completar la germinación y, por lo tanto, en un lote de semillas diferentes fracciones tienen una germinación escalonada en el tiempo.

La tasa de germinación (GR_g), puede definirse como el inverso del tiempo que necesita una determinada fracción de la población para completar la germinación:

$$GR_g = 1/tg = (T - T_b)/\theta_T(g)$$

Por lo tanto, la relación entre GR_g y **T** debería ser lineal con diferentes pendientes para diferentes porcentajes de germinación, con un punto en común, la T_b. De aquí se hace evidente que la velocidad de germinación GR_g es más rápida para porcentajes menores, resultando en diferentes pendientes de GR_g por arriba de la T_b. El inverso de estas pendientes

es igual al tiempo o constante térmica $\theta_T(g)$ para cada fracción en particular. Los valores de $\theta_T(g)$ se distribuyen generalmente de manera normal, caracterizada por $\theta_T(50)$ y su desviación estándar ($\sigma_{\theta T}$) (Figura 3a). La T_b y $\theta_T(50)$ son características de las **especies** y sirven como punto de partida para mejorar la comprensión de su emergencia en el campo.
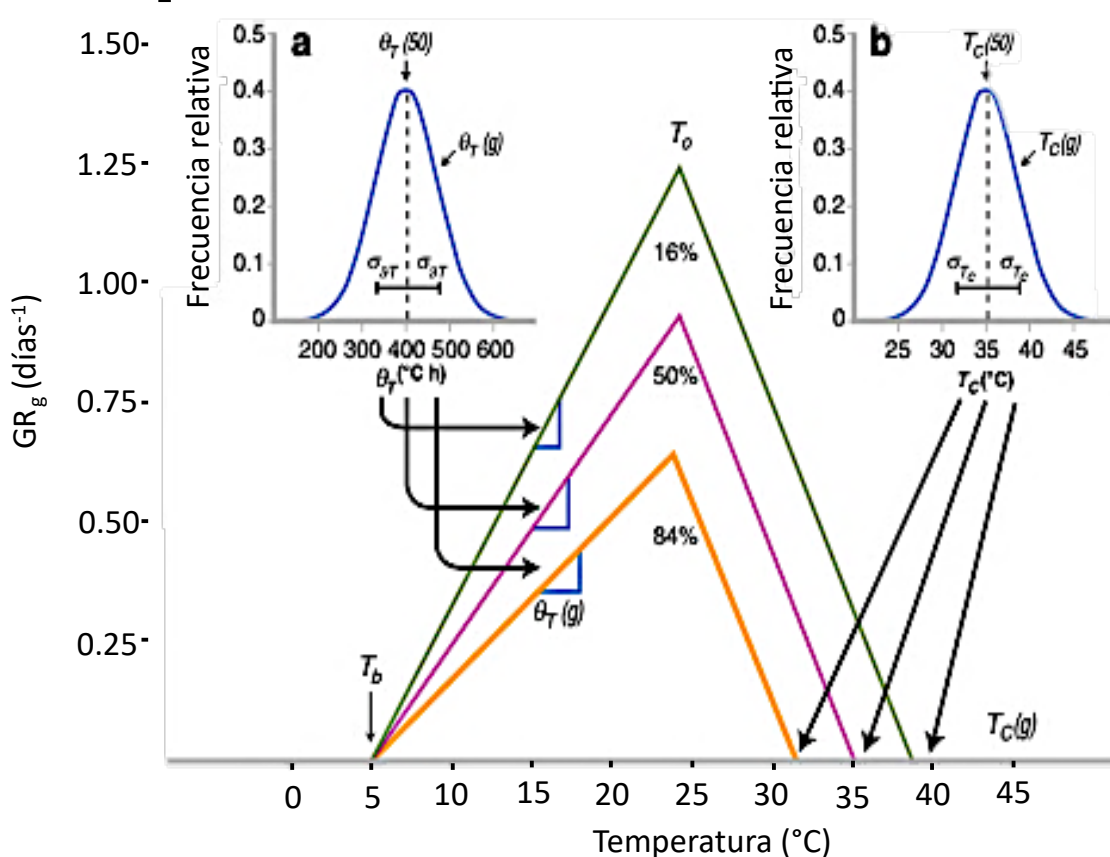
Figura 3: **Relación entre la tasa de germinación y la temperatura**. A bajas temperaturas, la tasa de germinación (GR$_g$ = 1/t_g) para diferentes porcentajes (g) de la población de semillas incrementa linealmente con la temperatura por arriba de una temperatura base comun (T_b). Las pendientes de las líneas son iguales al inverso de los tiempos térmicos para la germinación (1/$\theta_T(g)$), los cuales varian entre las semillas con respecto a una distribución normal (recuadro a). La GR$_g$ máxima ocurre a la temperatura óptima (T_o), y por arriba de esta temperatura la GR$_g$ disminuye linealmente. La temperatura tope de germinación ($T_c(g)$) varía en una distribución normal entre las semillas dentro de la población (recuadro b). Las tasas de germinación se muestran para los porcentajes 16, 50 y 84%, los cuales representan la media y la desviación estandar ± 1 de las respectivas distribuciones normales. Adaptado de Bewley *et al*., 2013.

En cuanto al rango supraóptimo (Figura 3b), también se han desarrollado modelos matemáticos, tomando en cuenta que, a diferencia de T_b, T_c no es la misma para todas las fracciones de la población, por lo tanto:

$\theta_2 = (T_c(g) - T)\, t_g$

12

y de la misma manera:

$$GR_g = 1/t_g = (T_c(g) - T) / \theta_2$$

Donde $T_c(g)$ indica que la T_c varía para cada fracción. En este intervalo de temperaturas, la diferencia en las GR_g es debida a las diferentes T_c y el tiempo térmico total es constante para todas las semillas de la población. De igual manera las T_c se distribuyen de manera normal (Figura 3b).

Los modelos umbral -como el de tiempo térmico- se caracterizan por predecir el tiempo en el que pueden ocurrir transiciones en estados de desarrollo o eventos fenológicos en función de una condición ambiental. A la fecha, se han desarrollado modelos bajo diferentes condiciones como la humedad (modelo de tiempo hídrico), fotoperiodo e incluso la combinación de dos condiciones (modelo del tiempo hidrotérmico, combinando temperatura y humedad) (Bradford, 2005; Donohue *et al.*, 2015). Adicionalmente, la caracterización del efecto de la temperatura sobre la germinación contribuye en la descripción del efecto eventual del cambio climático en la distribución de las especies y/o su eficiencia germinativa en programas de propagación y cultivo (Walck *et al*., 2011).

Características fisiológicas de la germinación

A partir de estudios a nivel transcripcional y proteómico en arroz (He & Yang, 2013) y a nivel proteómico y de actividad enzimática en girasol (Xia *et al*., 2018), podemos elucidar los cambios a distintos niveles que subyacen durante las distintas etapas de la germinación.

A partir de estas aproximaciones se ha determinado que durante la Fase I acontecen distintos procesos, tales como la reparación del material genético y de las proteínas que sufrieron

daños debido al proceso previo de desecación en la planta madre. Durante esta fase también ocurre la recapitulación del proceso de maduración, la síntesis de la maquinaria molecular encargada de la asimilación de sustancias de reserva a partir del mRNA y de proteínas remanentes (He *et al*., 2011a; He *et al*., 2011b; Kim *et al*., 2008; Kim *et al*., 2009). También acontece la degradación de macromoléculas implicadas en los procesos de maduración y desecación, en tanto que comienza la rediferenciación de las promitocondrias (Howell *et al*., 2006; Howell *et al*., 2007; Howell *et al*., 2009). Posteriormente, durante la Fase II se ha observado la síntesis de enzimas implicadas en el metabolismo central del carbono, una mayor eficiencia de la respiración celular, el ensamble de la maquinaria del ciclo de los TCA (Krishnan & Dayanandan, 2003; Yang *et al*., 2007; He *et al*., 2011a; Howell *et al*., 2006; Howell *et al*., 2007). Durante la Fase III se ven favorecidos los procesos implicados en la síntesis de componentes celulares que subyacen al crecimiento celular (Arc *et al*., 2011) (Figura 4a).

Por otra parte, en un estudio hecho en semillas de girasol, por medio de perfiles proteómicos se observó un incremento en proteínas involucradas en el metabolismo y energía durante las primeras horas de imbibición, seguido de un decremento en aquellas involucradas en el metabolismo proteico y de almacenamiento de reservas en semillas no dormantes. Los perfiles de actividad enzimática de semillas germinadas a 20 °C mostraron durante la Fase I una menor actividad de enzimas involucradas en la glucólisis y el ciclo de los TCA; en tanto que se observó un incremento en la actividad de enzimas implicadas en síntesis de sacarosa, lo que sugiere la regulación de la actividad del metabolismo central del carbono en semillas germinantes. Estos resultados sugieren que el control de la producción de energía durante la

imbibición está involucrado en redes moleculares que controlan la dormancia y la germinación de las semillas (Arc *et al*., 2012; Xia *et al*., 2018) (Figura 4b).
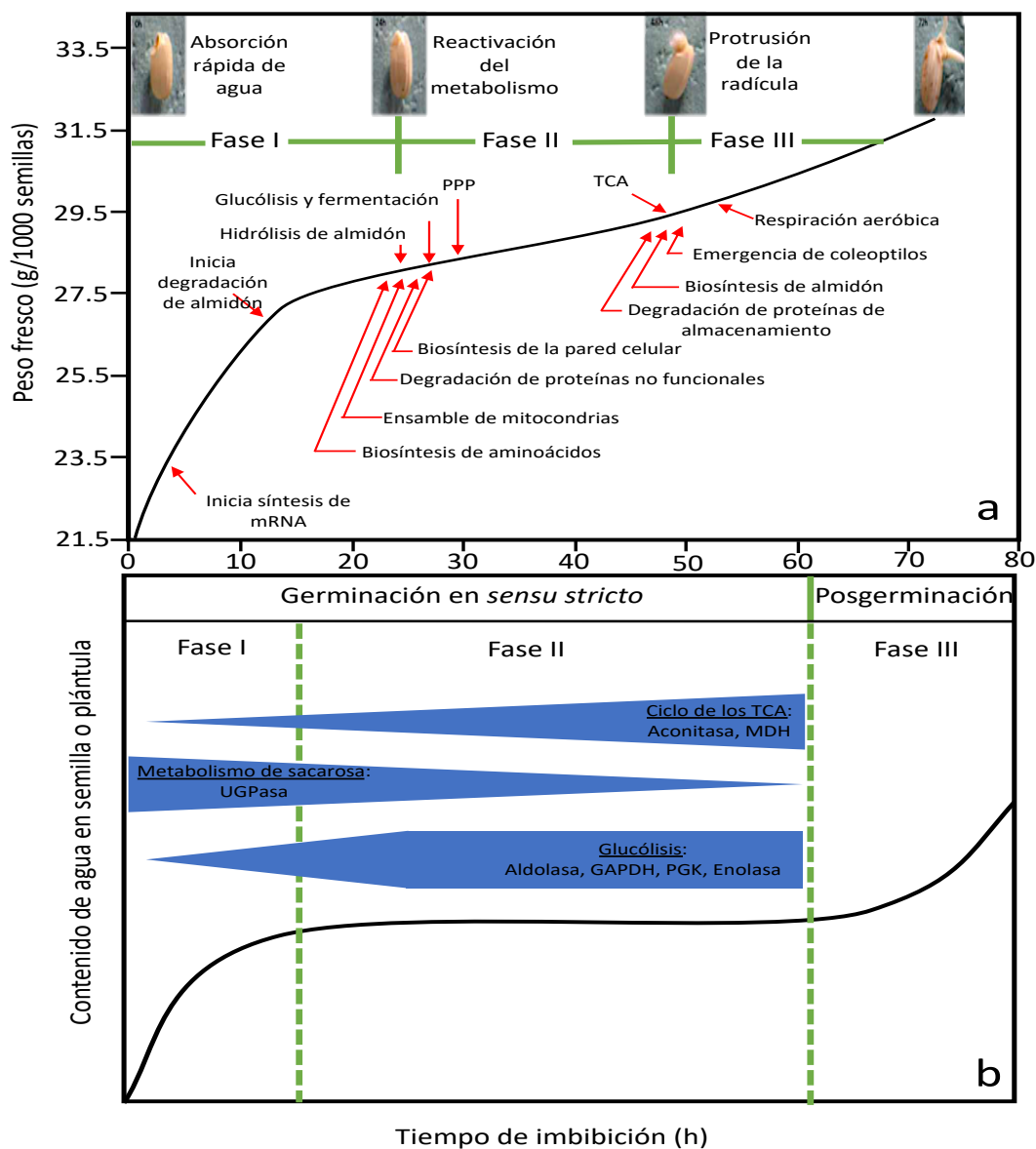
Figura 4: **a) Actividades ocurridas secuencialmente durante la germinación de semillas de arroz**. Durante la Fase I, la absorción rápida de agua se caracteriza por la puesta en marcha de la biosíntesis de mRNA. La Fase II, a menudo considerada como la fase más importante, se caracteriza por la reactivación del metabolismo, movilización de reservas, reparación de la estructura celular, relajación de la pared celular y la elongación del coleoptilo. La fase III, la cual involucra otra toma rápida de agua, se caracteriza por la recuperación de la actividad del ciclo de los TCA y de la respiración aeróbica, inicio de la división celular, protrusión de la radícula y el inicio de establecimiento de la plántula. En la figura se muestran fotografías de semillas de arroz a las 0, 24, 48 y 72 h durante la imbibición. Adaptado de He & Yang, 2013. **b) Regulación de metabolismo central del carbono durante la germinación en sensu stricto en semillas de girasol**. Los cambios en la actividad enzimática de semillas dormantes de girasol durante la germinación inducida a 20°C es representada por líneas cuya amplitud es proporcional al incremento en la actividad. UGPasa, UDP-glucosa pirofosforilasa; PGM, fosfoglucomutasa; FBP-Aldolasa, Fructosa-bisfosfato aldolasa; Enolasa; MDH, malato deshidrogenasa; Aconitasa; GAPDH, gliceraldehído 3-fosfato deshidrogenasa; PGK, fosfoglicerato quinasa. Adaptado de Xia *et al.*, 2018.

Metabolismo de ácidos grasos durante la germinación

Químicamente, los lípidos de almacenamiento son los triacilgliceroles o TAGs, la mayoría de los cuales son aceites (Bates *et al.*, 2013), es decir, se encuentran en estado líquido por encima de los 20 °C; algunas semillas pueden contener también cantidades significativas de fosfolípidos, glicolípidos y esteroles. Los TAGs son ésteres del glicerol y ácidos grasos, con estos últimos unidos al esqueleto del primero; los TAGs de reserva en semillas se localizan en organelos discretos llamados cuerpos de aceite, cuerpos de lípidos, oleosomas, esferosomas o, en algunas ocasiones, cuerpos de cera (Qu & Huang; 1990; Huang 1994). Predominantemente, los ácidos grasos en semillas son los insaturados (Gill & Valivety, 1997); de éstos, el oleico ($18:1\Delta9$; un doble enlace en la posición 9 de la cadena del ácido graso) y el linoleico ($18:2\Delta9,12$, con dos dobles enlaces en las posiciones 9 y 12) representan el 60% del peso total de los aceites en cultivos con semillas oleaginosas, siendo el ácido α-linolénico ($18:3\Delta 9,12,15$) el menos abundante (Baud & Lepiniec, 2010; Bewley *et al.*, 2013; Izquierdo *et al.*, 2017). Estos ácidos grasos son conocidos como esenciales, debido a que no pueden ser sintetizados por los humanos y deben de obtenerse de la dieta (Burdge, 2006). Los aceites que contienen estos ácidos grasos son importantes debido a que son empleados como materia prima en la elaboración de lubricantes, farmacéuticos, biodiesel, cosméticos, jabones, plásticos, revestimiento y pinturas (Izquierdo *et al.*, 2017).

Se conoce poco acerca del metabolismo de ácidos grasos durante la germinación; por ejemplo, durante el ensayo de la germinación de semillas de *Dioscorea tokoro* a 5, 20 y 30 °C; solo se observó crecimiento de semillas a 20°C (Figura 4a). De manera importante, se observó el uso selectivo de distintos triacilgliceroles o TAGs: de los 11 distintos tipos encontrados, redujeron su concentración los TAGs que contenían en su composición uno o

ningún ácido linoleico (OLO, OOO, PLO y POO; OLS y OLnO) en las tres temperaturas ensayadas. El decremento de OLO fue más marcado a 20°C, relacionándolo con el proceso de germinación. En tanto que se observaron cambios en la concentración de OOO en todas las temperaturas, sugiriendo su relación con el mantenimiento y viabilidad celular, incluyendo semillas dormantes (Okagami & Terui, 1996) (Figura 4b).
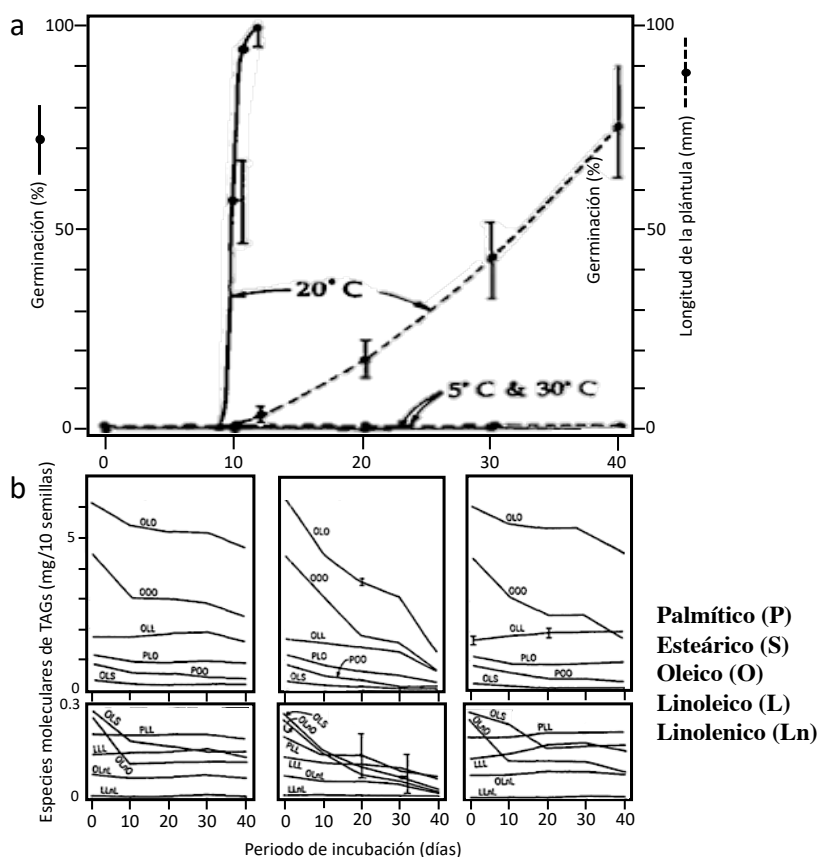
Figura 5: **a) Germinación de semillas y crecimiento de plántulas de *D. tokoro*.** Las semillas fueron incubadas en agua destilada a 5, 20 o 30 °C durante 40 días en oscuridad. No se observó germinación a 5 o a 30°C: barras verticales indican el límite de confianza del 90% para la germinación y los errores estándar para la longitud de la plántula. **b) Cambios en las concentraciones de los TAGs durante la incubación de semillas de *D. tokoro*.** Las semillas fueron incubadas con agua destilada a 5, 20 o 30 °C durante 40 días en oscuridad. Los TAGs que contaron para una concentración mayor de 0.3 mg/10 semillas, se muestran en el panel superior; mientras que aquellas especies moleculares que presentaron una concentración menor se muestran en el panel inferior. Se muestra la concentración de OLS en todos los paneles con fines comparativos. Nótese que la escala vertical difiere por un factor de 10 entre el panel superior e inferior. Las barras verticales indican las desviaciones estándar de tres mediciones distintas. Adaptado de Okagami & Terui, 1996.

Otra manera de evaluar el efecto combinado de la temperatura y humedad sobre la viabilidad y composición de semillas es por medio de experimentos de envejecimiento. En soya, por ejemplo, se ha observado que la viabilidad disminuye exponencialmente a medida que aumenta el contenido de humedad relativa (HR), este efecto se intensifica a mayor temperatura. Otro ejemplo surge de ensayos de envejecimiento en almendra (*Prunus dulcis*), donde las semillas fueron sometidas a una temperatura de 20°C y 80% de HR durante 40 días. Las concentraciones de proteínas y de lípidos totales disminuyeron y cambiaron también las concentraciones de ácido esteárico (disminuyó), oleico (aumentó), linoleico (disminuyó) y linolénico (disminuyó) (Zacheo *et al.*, 1998).

Muchos ácidos grasos polinsaturados encontrados en semillas sufren degradación peroxidativa. Los resultados de este proceso no solo se restringen a la degradación lipídica, sino también a una serie de reacciones que forman productos tóxicos. La composición de ácidos grasos es el factor más importante que determina la susceptibilidad de los aceites a la oxidación (Morello *et al*., 2004). Entre los lípidos susceptibles a la auto oxidación se encuentran el ácido oleico, linoleico y linolénico. Desde hace más de tres décadas se conoce que la oxidación de lípidos está asociada al envejecimiento de sistemas biológicos, incluyendo a las semillas (Wilson & McDonald, 1986). En este tema se ha observado que la reducción en la concentración de ácidos grasos insaturados está asociada a la pérdida de vigor en semillas de distintas especies (Harman & Mattick, 1976; Priestley & Leopold, 1983; Gidrol *et al*., 1989; Aiazzi *et al*., 1996).

Chía (*Salvia hispanica* L.)

La chía (*Salvia hispanica* L.) es una planta oleaginosa nativa de la región que comprende el centro-occidente de México al norte de Guatemala (Bueno *et al.*, 2010, Chicco *et al.*, 2009 Capitani *et al.*, 2015; Imram *et al.*, 2016), la cual ha sido empleada como cultivo para uso alimentario en México desde hace 5,500 años (Jamboonsri *et al.* 2012). Es importante mencionar que México cuenta con alrededor de 328 especies pertenecientes al género *Salvia*, siendo el género con el mayor número de especies vasculares del país (Villaseñor, 2016). Recientemente, las semillas de *S. hispanica* han recibido mayor atención debido a sus propiedades benéficas para la salud, como la regulación intestinal, reducción de enfermedades cardiovasculares, obesidad, colesterol y triglicéridos; así como la prevención de diabetes tipo II (Jin *et al.*, 2012; Poudyal *et al.*, 2012). Se conoce que las semillas de chía contienen del 25% al 40% de aceite y a la fecha son consideradas como una de las fuentes más importantes de ácidos grasos polinsaturados omega-3 (ω3), alcanzando altas concentraciones de ácido α-linolénico (50-57%) y ácido linoleico (17-26%) (Ixtaina *et al.*, 2011), los cuales son de importancia, ya que son esenciales para el ser humano y otras especies. Desde el punto de vista agronómico, las evidencias experimentales sobre *S. hispanica* siguen siendo escasas, especialmente con respecto a la tecnología de semillas. Por lo tanto, es necesario conocer los factores que limitan la germinación y el desarrollo de las plántulas, los cuales permitan el manejo de estrategias para el cultivo de la chía.

Se conocen distintos factores que afectan la concentración de algunos componentes bioquímicos incluyendo compuestos activos en las semillas de *S. hispanica* L. (Dubois *et al.*, 2007; Ayerza & Coates, 2009). Por ejemplo, se ha observado que, en plántulas de chía, la combinación de distintas condiciones de la luz y temperatura afectan el contenido de clorofila

a y b, carotenoides totales, azúcares y aminoácidos, en especial la prolina, (Pereira de Paiva *et al.*, 2018). Por otra parte, se ha observado una relación inversa entre la altitud y el contenido de ácidos grasos saturados; es decir, a baja elevación, se observó un aumento en la saturación de ácidos grasos en áreas donde la temperatura fue alta. De manera importante, se ha demostrado que la temperatura contribuye significativamente en el tipo de ácidos grasos encontrados en el aceite (Peiretti & Gai, 2009, Ayerza, 2010); encontrándose que, durante el desarrollo de las semillas, el incremento de la temperatura produce un decremento en el contenido de ácidos grasos polinsaturados (Ayerza, 1995). Otro factor que puede contribuir con las diferencias en la composición química de las semillas de chía es el estado del desarrollo de la planta. En este sentido, se ha observado un decremento del 23% en la concentración de α-linolénico desde la etapa inicial hasta la etapa de madurez de la semilla; esto a su vez, provoca un incremento de ácido linoleico y contenido de lignina (Peiretti & Gai, 2009).

En otro estudio realizado por Ayerza y colaboradores (2009), se observaron correlaciones positivas entre la altitud y el contenido de aceites de semillas de chía, así como entre la elevación y la extensión del ciclo de crecimiento de semillas de plantas cultivadas a diferentes altitudes; en tanto que se observó una correlación negativa entre la altitud y el contenido de proteína. Aparte de estas aproximaciones, existen pocos trabajos enfocados en la composición química de las semillas de chía, incluyendo durante su germinación.

Desde el punto de vista agronómico, las evidencias experimentales sobre *S. hispanica* siguen siendo escasas, especialmente con respecto a la tecnología de semillas, donde aún no se ha estudiado el efecto de la temperatura sobre su comportamiento germinativo. Por lo tanto, es

necesario conocer los factores que limitan la germinación y el desarrollo de las plántulas, los cuales permitan el manejo de estrategias para el cultivo de la chía.

En virtud de lo anteriormente expuesto, el objetivo de este trabajo fue contribuir con el conocimiento del papel de la temperatura en la germinación de semillas durante el proceso de germinación. Los resultados presentados en este trabajo tienen el potencial de establecer la base de futuras investigaciones sobre el metabolismo de los lípidos y ácidos grasos de las semillas de una especie de importancia agronómica y el potencial de establecerse como un modelo experimental para el estudio de los ácidos grasos durante la germinación de las semillas; así como la identificación las mejores fechas de siembra para este cultivo de semillas oleaginosas en una variedad de climas y regiones; y, lo que es más importante, su resistencia y distribución en el escenario del cambio climático.

El presente trabajo consta de dos capítulos estructurados con formato de articulo científico, cada uno de ellos bajo el formato requerido para las revistas en las cuales se publicaron y sometieron dichos artículos. Finalmente, como parte de esta tesis, se añadió una discusión en conjunto de ambos capítulos. El primer articulo aborda el papel de la temperatura (10, 20 y 30 °C) sobre la germinación de semillas de chia, así como en el comportamiento de los ácidos grasos más abundantes presentes en las semillas durante eventos anteriores a la germinación o eventos pre-germinativos; mientras que en el segundo capitulo aborda, además de la determinación de la respuesta germinativa en términos de Germinación final, t_{50} y Velocidad de germinación en un gradiente de los 10-45 °C, la elucidación de las temperaturas cardinales de la germinación de semillas de chía por medio de distintos modelos de regresión no lineal.

Chia (*Salvia hispanica* L.) Seed Soaking, Germination, and Fatty Acid Behavior at Different Temperatures

Artículo requisito, publicado bajo la siguiente referencia:

Chia (*Salvia hispanica* L.) Seed Soaking, Germination, and Fatty Acid Behavior at Different Temperatures

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Abstract: The temperature reduces the viability and seed vigor; however, the effect of temperature on imbibition and fatty acid profile has not been studied. Chia (*Salvia hispanica* L.) seeds have a substantial quantity of oil, making them a potential study model for fatty acid metabolism. Therefore, we explore the effect of temperature (10, 20, and 30 °C) on chia seed imbibition, germination, and fatty acid profile by GC-MS. Imbibition FI occurs within the first hour in all the treatments; while FII and FII$_{end}$ elapse with an hour of difference at 20 °C and 30 °C. The highest viability and germination rate were observed at 30 °C; while the highest concentrations of all fatty acids, except oleic acid, were observed at 20 °C. Maximum fatty acid concentrations were detected at FI and FII$_{end}$; while at 30 °C, different patterns for saturated and unsaturated fatty acids and three linolenic acid isomers were observed. A shorter FII is associated with earlier germination; the increase in concentration in fatty acids after 3 h and a negative correlation between linoleic and linolenic acid observed at 20 °C were related to a higher germination efficiency. At 30 °C, isomer formation is related to homeoviscous cell membrane adaptation.

Keywords: fatty acid isomerization; germination phases; homeoviscous adaptation; linolenic acid; lipid metabolism; polyunsaturated fatty acids; *Salvia hispanica*; seed imbibition

1. Introduction

Chia (*Salvia hispanica* L.) or "oily" for Aztec and Maya cultures is a summer biannual herbaceous and oleaginous plant, included within the family of mints (Lamiaceae). Chia is a Cem Anahuac oilseed crop that has been cultivated for 5500 years in territories covering midwestern Mexico to northern Guatemala [1–7]. Currently, chia is cultivated in Australia, Bolivia, Colombia, Guatemala, Mexico, Peru, and Argentina [5].

Some of the benefits of this oilseed in nutrition and health are related to its substantial quantity of oil (around 25–40% total weight of the seed), 50–57% as linolenic, and 17–26% linoleic (ω-3 and ω-6 fatty acids, respectively), essential fatty acids for health, antioxidant and antimicrobial activity [8–16]. Seeds are also composed of 15–25% protein, 30–33% fat, 26–41% carbohydrates, 18–30% fiber, 4–5% ashes, and minerals, vitamins, and dry matter [14,17], fundamental components of the human diet.

Chia seeds are appreciated and requested in Europe, the United States of America, Canada, China, Malaysia, Singapore, and the Philippines, due to the nutraceutical properties that characterize

them [8,14,18–20]. In 2018 chia seeds worldwide market was valued at USD 66.5 million and by 2024 is projected to reach a value of USD 88.1 million [21]. Therefore, it is important to investigate the molecular, biochemical, physiological, and agronomical aspects of chia seeds. So far, early seed germination events that are directly related to the subsequent success of the seedling establishment, have been little studied.

During the life cycle of plants, germination is often considered a critical stage due to its high sensitivity to environmental factors such as water, temperature, light, and gaseous environment [22–25]; when water availability is not a limitation, the temperature is the main factor controlling germination [23,24]. The influence of temperature on germination is related to water absorption by seeds; latency level and seed deterioration rate are also affected by temperature [26,27]. Additionally, the length of time at which germination occurs could be affected by temperature [23,28].

As soon as the dry seeds begin their imbibition, a precise temporal dynamic of events leads to metabolism resumption [23,29]. Membrane organization is an initial event that precedes subsequent physiological events; the proper membrane reorganization during imbibition is affected by temperature, modifying permeability and fluidity properties, contributing, or limiting the leakage of cellular components. Underlaying structural and domain membrane reorganization, lipid metabolism, and lipid biochemical properties carry out essential roles, for example, it has been observed that the increase in chain length, unsaturation number, and isomerization in a certain cohort of lipids supports membrane reorganization [30,31].

Although seed lipid changes have been investigated under scenarios of chilling imbibitional damage, cellular response to heat stress and seed aging [32–38], the precise nature of climatic influence on lipid and fatty acid composition is still unknown. For instance, during a global lipidomic study of chilling-imbibitional damage in maize seeds, it has been observed that germination ability under cold stress is related to phospholipid remodeling [39]; while at warmer temperatures, the key component of cellular tolerance to heat stress depends on membrane thermal stability combined with an efficient antioxidant response [40]. Global rise in temperature impacts negatively crop productivity, triggering a heat stress-mediated decay in germination rates [41]. Under this scenario, due to their susceptibility to oxidation, polyunsaturated fatty acids (PUFAs) are particularly related to reactive oxygen species and membrane damage [35,42], being directly linked with the decrease in seed quality. PUFAs isomerization is another event that has been implicated as seed stress-mediated mechanism, being *trans* fatty acid's structure more stable than *cis* fatty acids against thermodynamics [30,43]. Fatty acid isomer formation not only can be studied as a free radical-mediated chemical conversion but also as an important structural change associated with cellular stress or cellular signaling events.

Oilseeds arise as an alternative for the study of lipid metabolism during the early stages of germination and within these, chia is distinguished by the characteristics of its oil. Hence, in the present work, we explore fatty acid changes during chia seed imbibition at 10, 20, and 30 °C, to establish a correlation between fatty acids behavior, temperature, and germination. Those temperatures were chosen because they represent the minimum and maximum temperatures for chia growth, i.e., 11 and 36 °C, respectively; showing an optimum range between 16–26 °C [44]; however, cardinal temperatures for chia seed germination remains to be determined.

2. Materials and Methods

2.1. Seed Acquisition and Store

Medicinal variety of *S. hispanica* seeds were obtained without previous treatment, with 90% of germination and 99% of purity accordingly with the supplier (Okko super foods©; Jalisco, México; Lot/Batch: 130320/19). Seeds were stored in their shipping bag inside a cold and dry seed store chamber at 10 ± 5 °C and 20 ± 5% of relative humidity until imbibition assays were performed. No

previous disinfection treatment was applied in any of the experiments due to chia seeds' response at mucilage secretion level [45,46].

2.2. Imbibition Tests

Seed water uptake was evaluated in samples of 300 seeds placed inside mesh woven cotton bags (6.5 × 8 cm), 3 bags were placed inside Petri dishes (9 × 1.5 cm). Dishes were filled with distilled water (15 bags per treatment, 3 bags per Petri dish, 5 Petri dishes) and placed inside germination chambers same as Sampayo-Maldonado et al. [47], with 12 h photoperiod, using halogen lamps at a light intensity of 28.05 µmol m^{-2} s^{-1} (Quantum Meter Apogee Mod. QMSW-SS), programmed at 10 ± 2, 20 ± 2 and 30 ± 2 °C. The former lighting conditions were chosen because higher seedling growth and dry matter accumulation were observed in the presence of luminosity [48].

According to with literature, the first half-hour of imbibition is related to the complete mucilage secretion/hydration [44,45]; therefore, our weight measurements start after this time. Afterward, the bags were weighed every 30 min for the first 2 h and finally every hour for the next 3 h. Each time the wet seed bags were taken from Petri dishes, shaken for 5 s, weighted, and placed back under treatment. Changes in seed weight during imbibition time were calculated by subtracting the dry seed weight registered at the beginning of the experiments and the average weight of the hydrated empty bags from the total weight.

For the fatty acid analysis, additional seed bags were imbibed under the same conditions, bags ($n = 3$) were taken every hour, shaken, and weighed. The bagless seeds were stored individually at −70 °C until use in GC-MS fatty acid analysis.

2.3. Germination Tests

Five replicates of 25 seeds were sown randomly on agar medium (10 g L^{-1}) in Petri dishes (5.5 × 1.5 cm). Seeds were incubated at constant temperatures in germination chambers at 10 ± 2, 20 ± 2 and 30 ± 2 °C and with a 12 h photoperiod same as Sampayo-Maldonado et al. [47]. Seeds were considered germinated when radicle emerged ≥ 2 mm [49], after that, seedlings were removed from the Petri dish. Germination was recorded daily for 14 days, a time at which no more germination was observed.

2.4. Variables Evaluated

2.4.1. Total Germination

The daily number of germinated seeds in each Petri dish was recorded. **G(%)** was reported as the average cumulative percentage of germinated seeds in each treatment, calculated according to:

$$G(\%) = \frac{n}{N} \times 100 \tag{1}$$

where n is the number of seeds germinated and N the total number of seeds.

2.4.2. Median Germination Time (t_{50})

The total number of days between imbibition time and when 50% of the total germination was recorded. According to Ordoñez-Salanueva et al. [50], a sigmoid curve was fitted to the accumulated germination, allowing the median germination time to be determined by interpolation.

2.4.3. Germination Rate (GR)

Germination rate or the number of germinated seeds by day was obtained with the equation proposed by Maguire [51]:

$$GR = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \cdots + \frac{G_i}{N_i} + \frac{G_n}{N_n} = \sum_{i=1}^{n} \frac{G_i}{N_i} \qquad (2)$$

where G_i is the number of germinated seeds and N_i es the number of days after the beginning of the experiment.

2.5. Lipid Extraction and Fatty Acid Analysis by GC-MS

Total lipids were extracted from individual samples of additional frozen seeds stored every hour during the imbibition tests at 10, 20, and 30 °C. Seed samples in a range from 200 to 300 mg (n = 3) were grounded with nitrogen in presence of $CHCl_3:CH_3OH$ (2:1). Extraction was performed by organic phase separation with the same solvent mixture and adding NaCl 0.9%, according to Priestley et al., [52]. Fatty acid transesterification was done through evaporation of 100 µL of the chloroformic phase and reaction with 500 µL of BF_3-CH_3OH 12% *w/w*. After that, $C_6H_{14}:H_2O$ (2:1) was added to recover the methyl esters of fatty acids from the organic phase. Heptadecanoic acid was used as the internal standard for fatty acid quantification.

For the analysis of the methyl esters of fatty acids a gas chromatograph (Agilent Technologies 6850, Santa Clara, CA, USA) coupled with a mass spectrometer (Agilent Technologies 5975C VL MSD, Santa Clara, CA, USA) was used. A DB-1 (dimethylpolysiloxane) capillary column (30 m length × 0.32 mm i.d., 5.00 µm film thickness, part number: 123-1035E, Agilent Technologies 6850, Santa Clara, CA, USA) was used for the GC system. The oven temperature was programmed as follows: from 100 °C; ramp 1: To 250 °C with 5 °C/min. The injector temperature was 200 °C in split mode. Helium was used as carrier gas at a linear flow velocity of 35 cm s^{-1} o 1.4 mL min^{-1}. Mass detector conditions were: transfer line at 250 °C, range from 20 to 400 m/z, positive polarity, the ionization energy of 70 eV, and temperature of 200 °C, with an injection volume of 2 µL. The mass spectra were compared with the NIST/EPA/NIH Mass Spectral Library 2020 version [53]. Fatty acid analyses were performed by triplicate. Non imbibed (**NI**) seeds were the control for any of the treatments.

2.6. Statistical Analysis

Germination data did not fulfill the assumption of normality, therefore significant differences in final germination, median germination time (t_{50}), and germination rate (GR) were determined by Kruskal-Wallis and Dunn's test ($p < 0.001$). Differences in weight during imbibition at 10, 20, and 30 °C were determined by two-way ANOVAs and Tukey tests ($p < 0.001$), while differences in fatty acid concentrations were determined by two-way ANOVAs and Dunnett test ($p < 0.001$). Statistical analyses were carried out using the GraphPad Prism® software, version 8.4.0 for macOS, GraphPad Software, San Diego, CA, USA, www.graphpad.com (accessed on 10 January 2021).

3. Results

3.1. Imbibition

The weight gain of mature seeds during imbibition includes three different phases; the first comprises an initial and significant water uptake (FI), afterward a plateau phase without significant changes in seed fresh weight (FII), which represents the final stage for dead and dormant seeds, and finally, a newly significant water uptake corresponding to the germination stage (FIII), this final restart of water uptake is experienced only by germinating seeds [24,54] and is related to solutes formation, cell wall-loosening, and radicle tip weaken within embryonic tissues that leads to cell extension and visible germination [54]. Based on this criteria, *S. hispanica* seed weight changes were registered during imbibition at 10, 20, and 30 °C to relate the seed weight changes with the three imbibitional stages (Figure 1). During our imbibition assays, the last weight gain, related to water uptake, was observed after 3–4 h after that we observed a subsequent loss of weight-related to the

mucilage loss that occurred at the beginning of FIII, this weight loss masks the onset of FIII. Due to that FIII was not clearly distinguished by mucilage loss, the lapse between the last increase in weight and the end of loss of weight was considered as the extension of FII and called FII$_{end}$.

FI was characterized by a rapid and significant ($F_{7, 96}$ = 271.5; $p < 0.001$) increase in weight; this change occurs within the first hour of imbibition. At FII, no significant differences were observed, after 2–3 h imbibition weight increased at all temperatures (beginning of FII$_{end}$), but only at 30 °C, seed water uptake was faster and resulted in significantly different ($F_{2, 96}$ = 7.286; $p = 0.001$) in weight concerning the other two temperatures. After that, there was no significant difference in weight between all three temperatures (final of FII$_{end}$). Radicle protrusion was not distinguished during the observation period (5 h)

3.2. Germination

No significant differences were observed in final germination percentage between all treatments ($F_{2, 12}$ = 5.673; $p < 0.05$), final germination reached >80% (Figure 2). The lowest germination percentage was observed at 10 °C (80.8 ± 5.93%). The time required to reach 50% germination (t_{50}) was significantly different between treatments ($F_{2, 12}$ = 12.5; $p < 0.001$). t_{50} at 30 °C was 9.7-fold and 4.4-fold faster than 10 and 20 °C, respectively (Table 1).

GR was significantly different between treatments ($F_{2, 12}$ = 12.50; $p < 0.001$). It was highest at 30 °C, 18.5 seeds d^{-1}, and the lowest was at 10 °C, 4 seeds d^{-1} (Figure 3).

3.3. Fatty Acid Analysis

Fatty acid concentrations in frozen seeds stored every hour during the imbibition tests at 10, 20, and 30 °C were quantified by CG-MS (Figure 4). The highest concentrations of palmitic (**P**), stearic (**S**), linoleic (**L**), and linolenic (**Ln**) acids were observed at 20 °C. Oleic acid (**O**) was not detected in non-imbibed seeds (**NI**) nor in imbibed seeds at 10 °C. At 20 °C, **O** was not detected in all replicates and the concentrations were close to the concentration in **NI**. At 30 °C, **O** was detected in all replicates and the concentrations were the highest of the three treatments.

At 10 °C, maximum concentrations of **P**, **S**, **L**, and **Ln** were observed at 0 h and after 4 h of imbibition. At 20 °C maximum concentrations of **P**, **S** and **Ln** were observed after 1 h and 4 h of imbibition. After 4 h, at 30 °C maximum concentration was observed for **P** and after 3 h for **S**, **O**, **L**, and **Ln**. In all fatty acid maximum concentration occurred at FI and later occurred from the middle to the end of FII$_{end}$. At 10 °C maximum **P** concentration was 3-fold higher; **S** 3-fold higher; **L** 2.1-fold higher and **Ln** 2.7-fold higher than in **NI**. At 20 °C maximum **P** concentration was 3.2-fold higher; **S** 5.9-fold higher; **O** 0.5-fold higher; **L** 2.8-fold higher and **Ln** 4.7-fold higher than in **NI** seeds. Finally, at 30 °C maximum **P** concentration was 3.2-fold higher; **S** 2.9-fold higher; **O** 0.6-fold higher; **L** 1.1-fold higher and **Ln** 1-fold higher than in **NI**.

Different behavior patterns can be observed in fatty acids at 30 °C: the saturated fatty acids **P** and **S** showed a constant increase in concentration reaching a plateau between 4 and 3 h of imbibition, respectively; while the unsaturated acid **Ln** showed a maximum concentration after 3 h of imbibition, followed by a decrease the next hour, even lower compared with **NI**. Concentration dynamics of **O** and **L** were very similar to **Ln**; however, it is not possible to clearly distinguish the decrease in concentration at 4 h of imbibition. The maximum concentration observed in **Ln** at 30 °C occurs one hour before maximum concentrations were reached in treatments at 10 and 20 °C.

During the first 3 h of imbibition, **P** and **S** concentration at 10 °C was significantly lower ($F_{2, 42}$ = 12.11; $p < 0.001$) than concentrations at 20 and 30 °C. At 4 h of imbibition, **S** concentration at 10 and 20 °C experiences an increase, separating it from treatment at 30 °C. On the other hand, at 4 h, only the increase in the concentration of **P** was observed at 10 °C, while concentration at 20 °C was not significantly different between 3 h and 4 h of imbibition.

For **O**, it was observed that at 10 °C, the concentration remains undetectable during all imbibition time, while at 20 °C experienced an increase in the concentration of 0.5-fold after 2 h of imbibition. The highest concentrations of **O** were observed at 30 °C, remaining between 0.4–0.6 mg g^{-1} from 0–5 h of imbibition. **L** and **Ln** concentrations at 10 & 30 °C were close during the first 3 h of imbibition regarding with treatment at 20 °C; however, during the fourth hour of imbibition at the same temperature, only **Ln** reached a second maximum concentration, while **L** concentration remained close to **NI** (Figure 4).

Three **Ln** isomers were identified by their double bond position and configuration [53]: *6Z, 9Z, 12Z* (*γ*–linolenic acid); *9Z, 12E, 15Z*, and *6Z, 9Z, 11E* (Figure 5). *Trans*-fatty acid isomers were detected at 20 and 30 °C; however, only at 30 °C isomers were detected in all replicates. Maximum isomer concentration was observed after 3–4 h of imbibition, i.e., FII$_{end}$, time at which a decrease in **Ln** was observed (Figure 6). Together, the total concentration of **Ln** and its *trans*-isomers at 4 h of imbibition (≥6.8 mg g^{-1}) represents ~77% of the concentration of **Ln** observed in **NI** (8.8 mg g^{-1}).

4. Discussion

4.1. Imbibition

We observed that treatments at 10 and 20 °C reach at constant weight at 4 h of imbibition; while treatment at 30 °C reaches constant weight at 3 h, we associated this change with a shorter FII of germination and consequently, earlier germination at 30 °C. It is known that temperature affects water uptake by seeds [23]; in this context, it has been suggesting that, at suboptimal temperatures, there are changes in membranes configuration, affecting the retention of solutes, including sugars, organic acids, ions, amino acids, and proteins, affecting the efficiency of germination [26,27]; also, the rates of metabolic reactions underlying germination are affected by temperature [29].

In another imbibition assays with complete chia seeds [44,45,55,56], it has been reported that seeds reach up a constant weight between 2–4 h at temperatures ranging from 20–28 °C, similar behavior has been observed in some members of the *Plantago* genus [57,58].

The course of chia seed imbibition also has been explored by Muñoz et al. [45] as part of mucilage release characterization at 18–20 °C. The maximum weights reported by Muñoz et al. [45] were about 3 g for 100 mg of isolated mucilage and 1 g for 100 mg of demucilaged seeds (combined weight of 4 g) at 2.5 h of imbibition. We observed a maximum weight value of 5.3 g reached at 4 h of imbibition for seeds at 20 °C, while treatments at 10 and 30 °C reach a weight of 5.7 g at the same hour for 400 mg of seeds with intact mucilage. A similar effect has been observed in *Dillenia indica* (Dilleniaceae) another myxospermic angiosperm with copious mucilage, where intact seeds have higher water uptake than seeds without mucilage or seeds with excised embryos [59].

4.2. Germination

It has been observed that *Salvia hispanica* L. is tolerant to freezing in all development stages [60,61] and grows at a minimum temperature of 11 °C and a maximum of 36 °C, with an optimum range of 16–26 °C [44]. According to this evidence, we observed germination at 10, 20, and 30 °C, with final germination above 80%. Maximum total germination has been observed at 20–30 °C, accordingly with their natural environmental conditions, i.e., tropical, and subtropical environments, elevations of 400 to 2500 m. a. s. l. and mild temperatures [62]. The same was observed by Paiva et al. [48,63], where the highest germination was observed at constant 25 °C and alternating temperatures of 25–30 °C, in their assays the first count of germination was observed on the second day of sowing. In another research, chia seed germination has been tested during assays at 20–35 °C, they observed a germination time of 2 days at 22 and 32 °C [64]; in contrast, we count seeds with radicle protrusion from the first day of imbibition in all treatments.

Germination rate (GR) can change with temperature of imbibition and with the features acquired by cultivars throughout its domestication process [23]; specifically, during chia germination assays

driven at 20, 25, and 30 °C, was observed a higher GR at 25 °C (13.1 ± 0.1 seeds day^{-1}) compared with treatments at 20 and 30 °C (12.6 ± 0.1 and 9.7 ± 0.1 seeds day^{-1}, respectively) [65]. In contrast, we observed a GR 2-fold faster at 30 °C (18.5 ± 1.4 seeds day^{-1}) than the observed by Nadtochii et al. [65] (9.7 ± 0.1 seeds day^{-1}); while at 20 °C, a similar GR was observed in both studies. Likewise, final germination was quite similar between their results and ours. Other studies also support the influence of temperature as the main indicator associated with chia seed germination [48,64–68]. In comparative experiments, it was shown that the germination of chia seeds at low temperature (below 20 °C) and high temperature (above 30 °C) limits plant growth.

We observed a delay in germination at 10 °C, i.e., t_{50} of 5.64 ± 0.20 days; in this sense, Bita & Gerats [69] suggest that at low temperatures metabolic rates are reduced and the growth process is affected from germination to seedling stage. Another explanation arises from evidence with the myxospermous seed-mucilage *Lavandula subnuda* (Lamiaceae) and *Plantago ciliate* (Plantaginaceae), where mucilage presence increased moisture uptake and inhibited germination at lower temperatures (night/day temperatures of 15/25 °C). It has been suggested that mucilage inhibits germination under excessive moist conditions by preventing the diffusion of oxygen to the embryo [70]. Upon germination, the progressive depletion of oxygen generates conditions that almost achieve anaerobiosis, and fermentation is triggered as the main source of cellular ATP, supporting the reduction of electron transferring compounds, e.g., NAD and NADP, and inevitably leading to ROS (reactive oxygen species) accumulation [71]. The fact that chia germinates satisfactorily under all our conditions, reflects their potential resilience to adverse environmental conditions.

4.3. Fatty Acids Analysis

Chia seed oil has been extensively studied related to their quality and PUFAs high levels, in this subject it has been observed that differences in fatty acid concentrations depend on the extraction method, chia variety, and storage conditions [13,18,72–75].

Although hydrated chia seeds are the most common way it is consumed, few reports have been conducted on imbibed seeds. Zare et al., [76] observed that concentrations of oleic, linoleic, and linolenic acids of seeds soaked in water at 23 °C and after 24 h of imbibition was about ≥7, ≥10 and ≥32 mg/g of seeds, respectively; we observed similar concentrations for linoleic and linolenic acids. Although the concentrations observed are similar and agree with the 50–67% reported in the literature for ω-3 fatty acids [18,72], the concentrations that we observed are approximately 20 h earlier than those observed by Zare et al., [76]. Although fatty acid concentrations in control treatments between both works are similar, the differences in fatty acid concentrations of soaked seeds can be attributed to the experimental conditions and extraction method. Notably, it has been observed that water improves the extractability of fatty acids due to cell wall weakening, and therefore accessibility of oil bodies to the extraction solvent [76]. During our assays, it was observed that at 30 °C treatment, the maximum weight due to water absorption by seeds was reached after 3 h, and accordingly with the evidence, we found an increase in concentration in all fatty acids. At 10 °C maximum weight and **P**, **S**, **L**, and **Ln** were reached at 4 h of imbibition, while at 20 °C the maximum weight was also reached after 4 h of imbibition, at this time only **S** and **Ln** reached maximum concentration, at the same temperature, maximum concentrations of **P**, **O** and **L** were reached after 1–2 h of imbibition.

At 20 °C, we observed a decrease in the concentration of all fatty acids after 3 h of imbibition, after that, at 4 h, only **S** and **Ln** experience an increase in their concentration, part of the increase in **S** and **Ln** concentration could be explained by their use as energy reserves and nutrient mobilization in metabolically active seeds during FII, while the subsequent increase in **S** and **Ln** during the FII$_{end}$ is due to the synthesis of new nutrients and solutes that underlies this germination phase. A negative correlation between α-linolenic acid contents and the 18-C more saturated fatty acids, oleic and linoleic it has been observed in almond [77], chestnuts [78], soybeans [79], flaxseed [80], and chia [81].

The inverse association is supported by the biosynthesis of α-linolenic fatty acid through the process of desaturation of stearic [82,83] and oleic fatty acid [83,84], via linoleic fatty acid by the specific activity of desaturase enzymes, part of the increase observed in Ln concentration could be explained by this metabolic process.

At 30 °C, the temperature at which we observed a higher GR and a lower t_{50}, the increase in the concentration of **P**, **S**, and **O** from 0–3 h of imbibition and a constant concentration in **L** and **Ln** along the 5 h of imbibition, could be related with a higher germination efficiency [26,85] and with the optimum temperature range for chia seed germination (16–26 °C) reported by Ayerza & Coates [44].

During FI cellular process as genetic material damage reparation, mRNA degradation and synthesis, mitochondrial reparation, and the increase of cellular respiration are favored [23,28]. At 20 °C, the observed increase at the end of FI in concentrations of all fatty acids, except **O**, are supported by the evidence that fatty acid synthesis occurs during early germination in *Pisum sativum* seeds, where after a short lag phase, the incorporation of marked lipids proceeded linearly, being palmitic and stearic acid the first to be synthesized followed by long-chain saturated fatty acid the synthesis [86]. This evidence suggests that enzymes for fatty acid synthesis are already present in dry seeds and participate in the synthesis of fatty acids once a critical water content of the seeds is achieved. Therefore, at 20 °C, the humidity threshold is reached during the first hour of imbibition. While at the same temperature, during FII$_{end}$, the increase in concentrations of all fatty acids, except for **O**, is supported by the fact that seeds with higher proportions of saturated and unsaturated oils would be favored because they would have more energy available and an enhanced membrane fluidity without delaying or slowing germination [87]. Fatty acid synthesis at FI also would be favored by their conversion to sucrose [88,89] and their utilization for energy via the TCA cycle during the subsequent FII, our results agree with this evidence.

Lipids are the main reserve energy compounds for the embryo in oil crops, lipid fluidity mainly depends on the fatty acid unsaturation profile, since saturated fatty acids are solid at low temperatures (**P**, **S**, and **O**) than unsaturated ones (**L** and **Ln**) and increasing the number of unsaturations increases the fluidity [90]. Cell membrane fluidity is essential for organisms to maintain the function of important metabolic systems such as the electron transport chain [91,92], the set of mechanisms developed to change their cell membrane composition to maintain cell membrane fluidity and functionality in response to shifting environmental conditions, is known as homeoviscous adaptation [93]. Although fatty acid synthesis during germination is associated to cell membranes functionality and ultimately the seed germination, the possible effects of the fatty acid composition of the reserve lipids on seed germination at different temperatures remain almost completely unexplored. The possible mechanisms involved in these responses include variations in membrane functionality and reserve lipids' breakdown during germination [90].

The increase in the concentration of saturated **P**, **S**, and **O** is related to more energy for growth, also saturated fatty acids in membrane lipids increase the lipid melting temperature and prevent a heat-induced increase in the membrane fluidity, modulating their metabolism in response to increasing temperatures [94]. Therefore, to maintain membrane fluidity, plants increase the content of saturated and monounsaturated fatty acids. On the other hand, the constant concentration of unsaturated **L** and **Ln** during all the five hours of imbibition, suggests a balance between its breakdown and synthesis, this balance could be related to its continuous use for the maintenance of the permeability and the activity of membrane-associated enzymes [87,95].

The increase of *trans*-isomers of fatty acids observed at 30 °C is mainly associated with cell defense against oxidative stress [30,31]. Higher plants exposed to excess heat, at least 5 °C above their optimal growing conditions exhibit a characteristic set of cellular and metabolic responses required for the plants to survive under the high-temperature conditions [96], including membrane functions [97]. The detrimental effects of warmer temperatures on chlorophyll and the photosynthetic apparatus are also associated with the production of injurious reactive oxygen species (ROS) and lipid peroxidation [98,99], related evidence has been observed in the legume *Medicago truncatula* [35].

However, the seeds used in our study seem to have an optimal germination range close to 30 °C; thus, isomers formation that occurs at temperatures favorable for germination, are mainly associated with changes in physicochemical properties of membranes, affecting configuration and fluidity. In this context, it is known that *trans* geometric isomer of fatty acids has a much higher melting point and remains solid at room temperature. Our results also suggest that this response is delayed as a function of temperature. Another explanation is the role of these isomers during signal events [96], however, this hypothesis needs to be explored extensively.

On the other hand, it has been observed that many tropical plants suffer frost damage when they are exposed to temperatures slightly below 0 °C and cold damage has been sometimes been reported at temperatures close to 5 °C [32], this evidence can be related to the observed increase in the concentration of all fatty acids, except **O**, after 4 h of imbibition at 10 °C, which are associated with the response for imbibitional damage caused by low temperature and humidity [23]. At low temperatures, a high proportion of polyunsaturated fatty acids helps maintain membrane fluidity. Another evidence supports the notion that increasing the level of polyunsaturated fatty acid can improve seed performance at low temperatures [100].

5. Conclusions

In this work, we explore the effect of temperature on seed imbibition, germination, and early events of fatty acid seed metabolism in the oilseed crop *S. hispanica*. The main conclusions are the following:

1. In *S. hispanica* a shorter FII imbibition phase is associated with earlier germination.

2. The increase in concentration in fatty acids after 3 h and a negative correlation between linoleic and linolenic acid observed at 20 °C were related to a higher germination efficiency.

3. At 30 °C, it was observed the formation of three *trans* linolenic acid isomers.

The results presented in this paper have the potential to establish the basis of future research in seed lipid and fatty acids metabolism of a species of agronomic importance and the potential to establish itself, as an experimental model for the study of fatty acid during seed germination.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bueno, M.; Di Sapio, O.; Barolo, M.; Busilacchi, H.; Quiroga, M.; Severin, C. Análisis de la calidad de los frutos de *Salvia hispanica* L. (*Lamiaceae*) comercializados en la ciudad de Rosario (Santa Fe, Argentina). *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **2010**, 9, 221–227.

2. Chicco, A.; D'Alessandro, M.; Hein, G.; Oliva, M.; Lombardo, Y. Dietary chia seed (*Salvia hispanica* L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. *The British Journal of Nutrition* **2009**, 101, 41–50.

3. Capitani, M.; Corzo-Rios, L.; Chel-Guerrero, L.; Betancur-Ancona, D.; Nolasco, S.; Tomás, M. Rheological properties of aqueous dispersions of chia (*Salvia hispanica* L.) mucilage. *Journal of Food Engineering* **2015**, 149, 70–77.

4. Imran, M.; Nadeem, M.; Manzoor, M. F.; Javed, A.; Ali, Z.; Akhtar, M. N.; Hussain, Y. Fatty acids characterization, oxidative perspectives and consumer acceptability of oil extracted from pre-treated chia (*Salvia hispanica* L.) seeds. *BioMed Central* **2016**, 15, 1–13.

5. Busilacchi, H.; Bueno, M.; Severin, C.; Di Sapio, O.; Quiroga, M.; Flores, V. Evaluation of Salvia hispanica L. cultivated in the south of Santa Fe (Argentina). *Cultivos Tropicales* **2013**, 34, 55–59.

6. Beltrán-Orozco, M.; Romero, M. Chía, alimento milenario. *Revista Industria Alimentaria* **2003**, 22–25.

7. Jamboonsri, W.; Phillips, T.; Geneve, R.; Cahill, J.; Hildebrand, D. Extending the range of an ancient crop, *Salvia hispanica* L. - a new ω3 source. *Genetic Resources and Crop Evolution* **2012**, 59, 171–178.

8. Ayerza, R. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. *Journal of the American Oil Chemists Society* **1995**, 72 (9), 1079–1081.

9. Ayerza, R.; Coates, W. Composition of chia (*Salvia hispanica*) grown in six tropical and subtropical ecosystems of South America. *Trop. Sci.* **2004**, 44, 131–135.

10. Coates, W.; Ayerza, R. Production potential of chia in northwestern Argentina. *Industrial Crops and Products* **1996**, 5, 229–233.

11. Heuer, B.; Yaniv, Z.; Ravina, I. Effect of late salinization of chia (*Salvia hispanica*), stock (Matthiola tricuspidata) and evening primrose (*Oenothera biennis*) on their oil content and quality. *Ind Crop Prod.* **2002**, 15, 163–167.

12. Ixtaina, V.; Nolasco, S.; Tomás, M. Physical properties of chia (*Salvia hispanica* L.) seeds. *Ind. Crops Prod.* **2008**, 28, 286–293.

13. Reyes-Caudillo, E.; Tecante, A.; Valdivia-López, M.A. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chem.* **2008**, 107, 656–663.

14. Mohd Ali, N.; Yeap, S.; Ho, W.; Beh, B.; Tan, S.; Tan, S. The promising future of chia, *Salvia hispanica* L. *J Biomed Biotechnol.* **2012**, 2012, 171956.

15. Ayerza, R. Crop year effects on seed yields, growing cycle length, and chemical composition of chia (*Salvia hispanica* L) growing in Ecuador and Bolivia. *Emir. J. Food Agric.* **2016**, 28: 196–200.

16. Knez Hrnčič, M.; Ivanovski, M.; Cör, D.; Knez, Ž. Chia Seeds (*Salvia hispanica* L.): An Overview-Phytochemical Profile, Isolation Methods, and Application. *Molecules* **2019**, 25, 11.

17. Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission. Opinion on the safety of 'Chia seed (*Salvia hispanica*) and ground whole Chia seed' as a food ingredient. *The EFSA Journal* **2009**, 996, 1–26.

18. Ayerza, R.; Coates, W. (2011). Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.). *Industrial Crops and Products* **2011**, 34, 1366–1371.

19. Lee, A.S. The effects of *Salvia hispanica* L. (Salba) on postprandial glycemia and subjective appetit. Thesis Master of Science. Nutritional Sciences University of Toronto, 2009.

20. Kulczyński, B.; Kobus-Cisowska, J.; Taczanowski, M.; Kmiecik, D.; Gramza-Michałowska A. The chemical composition and nutritional value of chia seeds-current State of Knowledge. *Nutrients* **2019**, 11, 1242.

21. Chia seed market – growth, trends and forecast (2020–2025). Available online: www.mordorintelligence.com/industry-reports/chia-seeds-market (accessed on 15 January 2021)

22. Rajjou, L.; Duval, M.; Gallardo, K.; Catusse, J.; Bally, J.; Job, C.; Job, D. (2012). Seed germination and vigor. *Annu Rev Plant Biol*. **2012**, 63, 507–33.

23. Bewley, J.; Bradford, K.; Hilhorst, H.; Nonogaki, H. Seeds: physiology of development, germination and dormancy, 3rd ed.; Springer: Gewerbestr Cham, Switzerland, 2013, pp. 85–246.

24. Nonogaki, H.; Bassel, G.; Bewley, J. Germination - Still a mystery. *Plant Science* **2010**, 179, 574–581.

25. Nonogaki, H. Seed Biology Updates - Highlights and New Discoveries in Seed Dormancy and Germination Research. *Frontiers in plant science* **2017**, 8, 524.

26. Benech-Arnold, R.; Sánchez, R.; Forcella, F.; Kruk, B.; Ghersa, C. Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* **2000**, 67, 105–122.

27. Batlla, D.; Benech-Arnold, R. A framework for the interpretation of temperature effects on dormancy and germination in seed populations showing dormancy. *Seed Science Research* **2015**, 25, 147–158.

28. Bewley, J.; Black, M. Seeds: Physiology of Development and Germination 2nd ed.; Springer: Gewerbestr Cham, Switzerland, 1994, pp. 147–197.

29. Marcos Filho, J. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola* **2015**, 72, 363–374.

30. Ferreri, C.; Chatgilialoglu, C. Encyclopedia of Radicals in Chemistry, Biology and Materials 1st ed.; Wiley: Toronto, Canada, 2012, pp. 1599–1623.

31. Jouhet, J. Importance of the hexagonal lipid phase in biological membrane organization. *Front. Plant Sci*. **2013**, 4, 494.

32. Lukatkin, A.; Brazaitytė, A. Bobinas, A.; P. Duchovskis. Chilling injury in chilling-sensitive plants: a review. *Zemdirbyste-agriculture* **2012**, 99, 111–124.

33. Zacheo, G.; Cappelloa, A.R, Perronea, L.M.; Gnoni, G.V. Analysis of factors influencing lipid oxidation of almond seeds during accelerated ageing. *LWT - Food Science and Technology* **1998**, 31, 6–9.

34. Oenel, A.; Fekete, A.; Krischke, M.; Faul, S.; Gresser, G.; Havaux, M.; Mueller, M.; Berger, S. Enzymatic and non-enzymatic mechanisms contribute to lipid oxidation during seed aging. *Plant Cell Physiol*. **2017**, 58, 925–933.

35. Doria, E.; Pagano, A.; Ferreri, C, Larocca, A.V.; Macovei, A.; Araújo, S.S.; Balestrazzi, A. How Does the Seed Pre-Germinative Metabolism Fight Against Imbibition Damage? Emerging Roles of Fatty Acid Cohort and Antioxidant Defence. *Front Plant Sci*. **2019**, 10, 1505.

36. Huang, R.; Liu, Z.; Xing, M.; Yang, Y.; Wu, X.; Liu, H.; Liang, W. Heat Stress suppresses *Brassica napus* seed oil accumulation by inhibition of photosynthesis and BnWRI1 pathway. *Plant and Cell Physiology* **2019**, 60, 1457–1470.

37. Lin, Y.-x.; Xin, X.; Yin, G.-k.; He, J.-j. Zhou, Y.-z.; Chen, J.-y.; Lu, X.-x. Membrane phospholipids remodeling upon imbibition in *Brassica napus* L. seeds. *Biochemical and Biophysical Research Communications* **2019**, 515, 289–295.

38. Sudhakaran, I. (2020). Effect of Seed Ageing in Biochemical and Molecular Changes in Oilseeds: A Review. *Agricultural Reviews* **2020**, 41, 408–412.

39. Noblet, A.; Leymarie, J.; Bailly, C. (2017). Chilling temperature remodels phospholipidome of *Zea mays* seeds during imbibition. *Sci. Rep*. **2017**, 7, 8886.

40. Iqbal, M.; Iqbal Raja, N.; Yasmeen, F.; Hussain, M.; Ejaz, M.; Shah, M. A. Impacts of heat stress on wheat: a critical review. *Adv. Crop Sci. Tech*. **2017**, 5, 1.

41. Fahad, S.; Bajwa, A. A.; Nazir, U.; Anjum, S. A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S.; Ihsan, M.Z.; Alharby, H.; Wu, C.; Wang, D.; Huang, J. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Frontiers in Plant Science* **2017**, 8, 1147.

42. Mene-Saffrane, L.; Dubugnon, L.; Chetelat, A.; Stolz, S.; Gouhier-Darimont, C.; Farmer, E. Nonenzymatic oxidation of trienoic fatty acids contributes to reactive oxygen species management in Arabidopsis. *J. Biol. Chem*. **2009**, 284, 1702–1708.

43. Liu, W.; Lu, G. H. Cis-trans isomerization of unsaturated fatty acids in edible oils to prepare trans fat. *Grasas Y Aceites* **2018**, 69, e268.

44. Ayerza, R.; Coates, W. Influence of environment on growing period and yield, protein, oil and α-linolenic content of three chia (*Salvia hispanica* L.) selections. *Industrial Crops and Products* **2009**, 30, 321–324.

45. Muñoz, L.; Cobos, A.; Diaz, O.; Aguilera, J. Chia seeds: microstructure, mucilage extraction and hydration. *J. Food Eng*. **2012**, 108, 216–224.

46. Salgado-Cruz, M. Chanona-Pérez, J.; Farrera-Rebollo, R. Méndez-Méndez, J. Díaz-Ramírez, M. Chia (*Salvia hispanica* L.) seed mucilage release characterization. A microstructural and image analysis study. *Industrial Crops and Products* **2013**, 51, 453–462.

47. Sampayo-Maldonado, S.; Ordoñez-Salanueva, C.A.; Mattana, E.; Ulian, T.; Way, M.; Castillo-Lorenzo, E.; Dávila-Aranda, P.D.; Lira-Saade, R.; Téllez-Valdéz, O.; Rodriguez-Arevalo, N.I.; Flores-Ortíz, C.M. Thermal Time and Cardinal Temperatures for Germination of *Cedrela odorata* L. *Forest* **2019**, 10, 41.

48. Paiva, E.; Barros-Torres, S.; da Silva, F.; Nogueira, N.; Freitas, R.; Leite, M. Light regime and temperature on seed germination in Salvia hispanica L. *Acta Scientiarum. Agronomy* **2016**, *38*, 513–519.

49. International Seed Testing Association - ISTA. International Rules for Seed Testing, 2020 ed.; ISTA: Bassersdorf, Switzerland, 2020, pp. 1–4.

50. Ordoñez-Salanueva, C.; Seal, C.; Pritchard, H.; Orozco-Segovia, A.; Canales-Martínez, M.; Flores-Ortíz, C. Cardinal temperatures and thermal time in *Polaskia Beckeb* (Cactaceae) species: Effect of projected soil temperature increase and nurse interaction on germination timing. *J. Arid Environ*. **2015**, 115, 73–80.

51. Maguire, J. D. Speed of Germination—Aid In Selection And Evaluation for Seedling Emergence And Vigor. *Crop Science* **1962**, 2, 176–177.

52. Priestley, D.; Wermer, B.; Leopold, C.; McBride, M. Organic free radical levels in seeds and pollen: The effects of hydration and aging. *Physiologia Plantarum* **1985**, 64, 88–94.

53. NIST/EPA/NIH Mass spectral library. Available online: www.chemdata.nist.gov (accessed on 30 January 2021).

54. Bewley, J. Seed Germination and Dormancy. *The Plant cell* **1997**, 9, 1055–1066.

55. Nayani, S.; Rao, S. Extraction of Mucilage from Chia Seeds and its Application as Fat Replacer in Biscuits, *International Journal of Engineering Research Technology* **2020**, 9, 922–927.

56. Geneve, R.; Hildebrand, D.; Phillips, T.; Kirk, J.; Al-Amery, M. Seed germination and mucilage production in chia (*Salvia hispanica*). *Acta Hortic*. **2019**, 1249, 153–156.

57. Singh, B.; Chauhan, G.S.; Bhatt, S.S.; Kumar, K. Metal ion sorption and swelling studies of psyllium and acrylic acid based hydrogels. *Carbohydrate Polymers* **2006**, 64, 50–56.

58. Singh, B.; Chauhan, G.S.; Kumar, S.; Chauhan, N. Synthesis, characterization and swelling responses of pH sensitive psyllium and polyacrylamide based hydrogels for the use in drug delivery (I). *Carbohydrate Polymers* **2007**, 67, 190–200.

59. Thapliyal, R.C.; Phartyal, S. S.; Baskin, J. M.; Baskin, C. C. Role of mucilage in germination of *Dillenia indica* (Dilleniaceae) seeds. *Australian Journal of Botany* **2008**, 56, 583–589.

60. Lobo, R.; Alcocer, M.; Fuentes, F. Rodríguez, W. Morandini, M.; Devani, M. Desarrollo del cultivo de chía en Tucumán, República Argentina. *EEAOC-Avance Agroindustrial* **201**1, 32, 27–30.

61. Bochicchio, R.; Rossi, R.; Labella, R.; Bitella, B.; Permiola, M.; Amato, M. Effect of sowing density and nitrogen top-dress fertilization on growth and yield of Chia (*Salvia hispanica* L.) in a Mediterranean environment. *Italian Journal of Agronomy* **2015**, 10, 163–166.

62. Orozco, G.; Durán, N.; González, D.; Zarazúa, P.; Ramírez, G.; Mena, S. Proyecciones de cambio climático y potencial productivo para *Salvia hispanica* L. en las zonas agrícolas de México. *Revista Mexicana de Ciencias Agrícolas* **2014**, 10, 1831–1842.

63. Paiva, E.; Barros-Torres, S.; Alves, Costa, T.; da Silva, F.; de Sousa, L.; Dallabona, J. Germination and biochemical components of Salvia hispanica L. seeds at different salinity levels and temperatures. *Acta Scientiarum. Agronomy* **2018**, 40, e39396.

64. Gómez-Favela, M.; Gutiérrez-Dorado, R.; Cuevas-Rodríguez, E.; Canizalez-Román, V.; Del Rosario León-Sicairos, C.; Milán-Carrillo, J.; Reyes-Moreno, C. Improvement of Chia Seeds with Antioxidant Activity, GABA, Essential Amino Acids, and Dietary Fiber by Controlled Germination Bioprocess. *Plant Foods Hum Nutr*. **2017**, 72(, 345–352.

65. Nadtochii, L.; Kuznetcova, D.; Proskura, A.; Apalko, A.; Nazarova, V.; Srinivasan, M. Investigation of various factors on the germination of chia seeds sprouts (*Salvia hispanica* L.). *Agronomy Research* **2019**, 17, 1390–1400.

66. Stefanello, R.; das Neves, L.A.S.; Abbad, M.A.B.; Viana, B.B. Physiological response of chia seeds (*Salvia hispanica*-Lamiales: Lamiaceae) to saline stress. *Biotemas* **2015a**, 28, 35–39.

67. Stefanello, R.; das Neves, L.A.S.; Abbad, M.A.B.; Viana, B.B. Germination and vigor of chia seeds (*Salvia hispanica* L.-Lamiaceae) under different temperatures and light conditions. *Brazilian Journal of Medicinal Plants* **2015b**, 17, 1182–1186.

68. Possenti, J.C.; Donazzolo, J.; Gullo, K.; Voss, L.C.; Danner, M.A. Influence of temperature and substrate on chia seeds germination. *Científica* **2016**, 44, 235–238.

69. Bita, C.; Gerats, T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science* **2013**, 4, 1–18.

70. Western, T.L. The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. *Seed Sci. Res*. **2012**, 22, 1–25.

71. Ma, Z.; Marsolais, F.; Bykova, N. V.; Igamberdiev, A. U. Nitric oxide and reactive oxygen species mediate metabolic changes in barley seed embryo during germination. *Front. Plant Sci*. **2016**, 7, 138.

72. Segura-Campos, M. R.; Ciau-Solis, N.; Rosado-Rubio, G.; Chel-Guerrero, L.; Betancur-Ancona, D. Chemical and functional properties of chia seed (*Salvia hispanica* L.) gum. *International Journal of Food Science* **2014**, 2014, 1–5.

73. Silva, C.; Garcia, V.A.S.; Zanette, C.M. Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: Oil yield, fatty acids profile and technological analysis of defatted meal. *Int. Food Res. J*. **2016**, 23, 998–1004.

74. Dąbrowski, G.; Konopka, I.; Czaplicki, S.; Tańska, M. Composition and oxidative stability of oil from *Salvia hispanica* L. seeds in relation to extraction method. *Eur. J. Lipid Sci. Technol*. **2017**, 119, 1600209.

75. Bodoira, R.; Penci, M.; Ribotta, P.; Martínez, M. Chia (*Salvia hispanica* L.) oil stability: Study of the effect of natural antioxidants. *LWT* **2017**, 75, 107–113.

76. Zare, T.; Rupasinghe, T.W.T.; Boughton, B.A.; Roessner, U. The changes in the release level of polyunsaturated fatty acids (ω-3 and ω-6) and lipids in the untreated and water-soaked chia seed. *Food Res Int*. **2019**, 126, 108665.
77. Abdallah, A.; Ahumada, M.; Gradziel, T. Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. *J. Am. Soc. Hort*. **1998**, 123, 1029−1033.
78. Borges, O. P.; Soeiro Carvalho, J.; Reis Correia, P.; Paula Silva, A. Lipid and fatty acid profiles of Castanea sativa Mill. Chestnuts of 17 native Portuguese cultivars. *Journal of Food Composition and Analysi*s **2007**, 20, 80–89.
79. Thomas, J.M.G.; Boote, K.J, Allen, L.H.Jr.; Gallo- Meagher, M.; Davis, J.M. Elevated temperature and carbon dioxide effects on soybean seed composition and transcript abundance. *Crop Sci*. **2003**, 43, 1548−1557.
80. Wakjira, A.; Labuschagne, M.T.; Hugo, A. Variability in oil content and fatty acid composition of Ethiopian cultivars of linseed. *J. Sci. Food Agric*. **2004**, 84, 601−607.
81. Ayerza, R. The seed's protein and oil content, fatty acid composition, and growing cycle length of a single genotype of chia (*Salvia hispanica* L.) as affected by environmental factors. *J Oleo Sci*. **2009**, 58, 347−54.
82. Nakamura, M.; Nara, T. Structure, function, and dietary regulation of $\Delta 6$, $\Delta 5$, and $\Delta 9$ desaturases. *Annu. Rev. Nutr.* **2004**, 24, 345–376.
83. Lee, J.; Lee, H.; Kang, S.; Park, W. Fatty Acid Desaturases, Polyunsaturated Fatty Acid Regulation, and Biotechnological Advances. *Nutrients*, **2016**, 8, 23.
84. Dybing, C.; Zimmerman, D. Fatty acid accumulation in maturing flaxseeds as influenced by environment. *Plant Physiol*. **1966**, 41, 1465−1470.
85. Batlla, D.; Benech-Arnold, R. L. Predicting changes in dormancy level in weed seed soil banks: Implications for weed management. *Crop Protection* **2007**, 26, 189–197.
86. Harwood, J. L.; Stumpf, P. K. Fat Metabolism in Higher Plants: XL. Synthesis of Fatty Acids in the Initial Stage of Seed Germination. *Plant physiology* **1970**, 46, 500–508.
87. Linder, C. Adaptive Evolution of Seed Oils in Plants: Accounting for the Biogeographic Distribution of Saturated and Unsaturated Fatty Acids in Seed Oils. *Am Nat*. **2000**, 156, 442–458.
88. Pritchard, S.; Charlton, W.; Baker, A.; Graham, I. Germination and storage reserve mobilization are regulated independently in Arabidopsis. *Plant Journal* **2002**, 31, 639−647.
89. Rosental, L.; Nonogaki, H.; Fait, A. Activation and regulation of primary metabolism during seed germination. *Seed Science Research* **2014**, 24, 1−15.
90. Izquierdo, N.; Benech-Arnold, R.; Batlla, D.; Belo, R.; Tognetti, J. Seed composition in oil crops. In Oilseed Crops, 1st ed.; Ahmad, P. Ed.; Wiley: Toronto, Canada, 2017, pp. 34–51.
91. Hazel, J. R. Thermal Adaptation in Biological Membranes: Is Homeoviscous Adaptation the Explanation? *Annual Review of Physiology*. **1995**, 57, 19–42.
92. Denich, T. J.; Beaudette, L. A.; Lee, H.; Trevors, J. T. Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. *J. Microbiol. Methods*. **2003**, 52, 149–182.
93. Sinensky, M. Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A*. **1974**, 71, 522–525.
94. Zhang, M.; Barg, R.; Yin, M.; Gueta-Dahan, Y.; Leikin-Frenkel, A.; Salts, Y.; Shabtai, S.; Ben-Hayyim G. Modulated fatty acid desaturation via overexpression of two distinct omega-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. *Plant J*. **2005**, 44, 361−71.
95. Larkindale, J.; Huang, B. Thermotolerance and antioxidant systems in *Agrostis stolonifera*: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. *J. Plant Physiol*. **2004**, 161, 405–413.

96. Guy, C. Molecular responses of plants to cold shock and cold acclimation. *J. Mol. Microbiol. Biotechnol*. **1999**, 1, 231–242.

97. Weis, E.; Berry, J. A. Plants and high temperature stress. *Symp. Soc. Exp. Biol*. **1988**, 42, 329−346.

98. Camejo, D.; Jiménez, A.; Alarcón, J. J.; Torres, W.; Gómez, J.; Sevilla, F. Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants. *Funct. Plant Biol*. **2006**, 33, 177–187.

99. Guo, T.; Zhang, G.; Zhang, Y. Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. *Colloids Surf B: Biointerfaces* **2007**, 57, 182–188.

100. Oteng, A.; Kersten, S. Mechanisms of Action of *trans* Fatty Acids. *Adv Nutr*. **2020**, 11, 697-708.

Tables and Figures
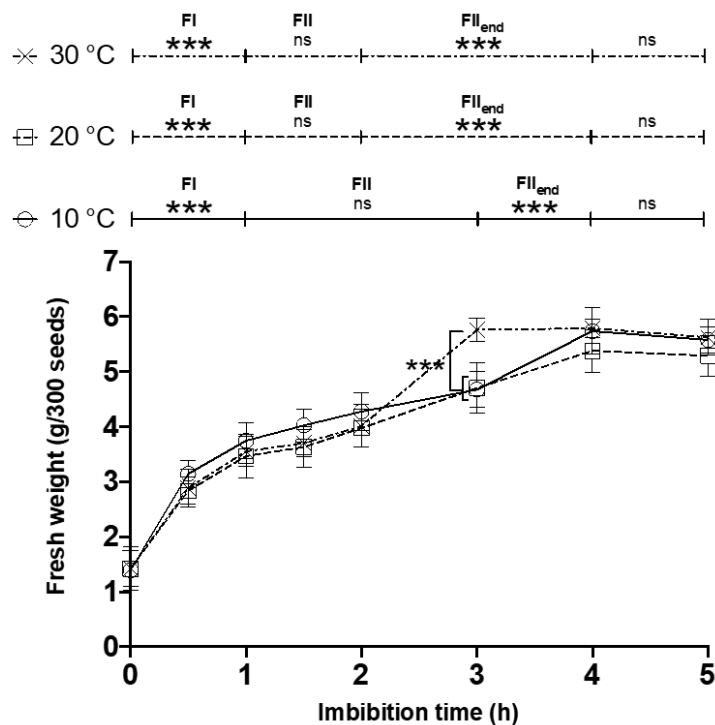
Figure 1. Imbibition curves of *S. hispanica* seeds at 10, 20 and 30 °C. Values are expressed as mean ± SD of five independent replicates. In the top is indicated the temporality of the imbibition phases FI, FII and FII$_{end}$ in each of the temperature treatments. Statistical analysis was performed using two-way ANOVA followed by a Tukey multiple comparison test. Asterisks correspond to data with statistical differences in time intervals and between temperature treatments ($p < 0.001$).
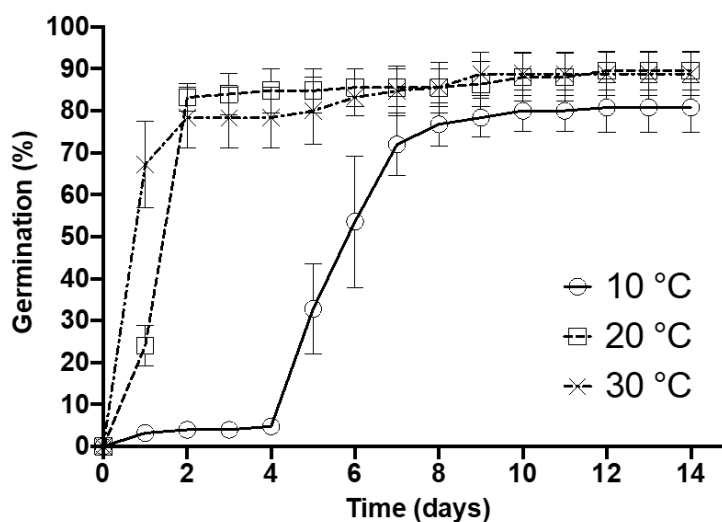
Figure 2. Cumulative germination of *S. hispanica* at 10, 20 and 30 °C. Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis followed by a Dunn's multiple comparison test.

Table 1. Final germination percent and median germination time (t_{50}) of seeds during imbibition at 10, 20, and 30 °C. Final germination is the percentage of seeds in which the germination process reaches the end; while median germination time (t_{50}) is the time to reach 50% of final germination.

Temperature	Final Germination (%)	Median Germination Time t_{50} (Days)
10 °C	80.8 ± 5.93	5.64 ± 0.20 ***
20 °C	89.6 ± 4.56	1.27 ± 0.01 ***
30 °C	88.8 ± 5.21	0.58 ± 0.09 ***

Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis ($p < 0.001$) followed by a Dunn's test multiple comparison test. Asterisks indicate significant differences between treatments (*** $p < 0.001$).
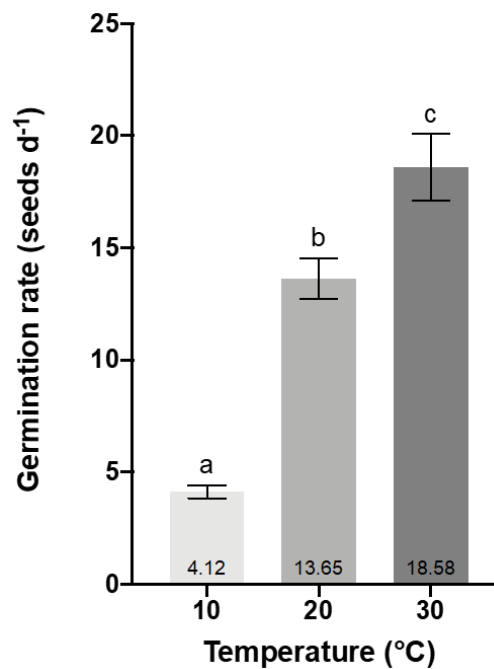
Figure 3. Germination rate (number of germinated seeds per day) at 10, 20 and 30 °C. Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis followed by a Dunn's multiple comparison test. Different letters indicate significant differences ($p < 0.001$).
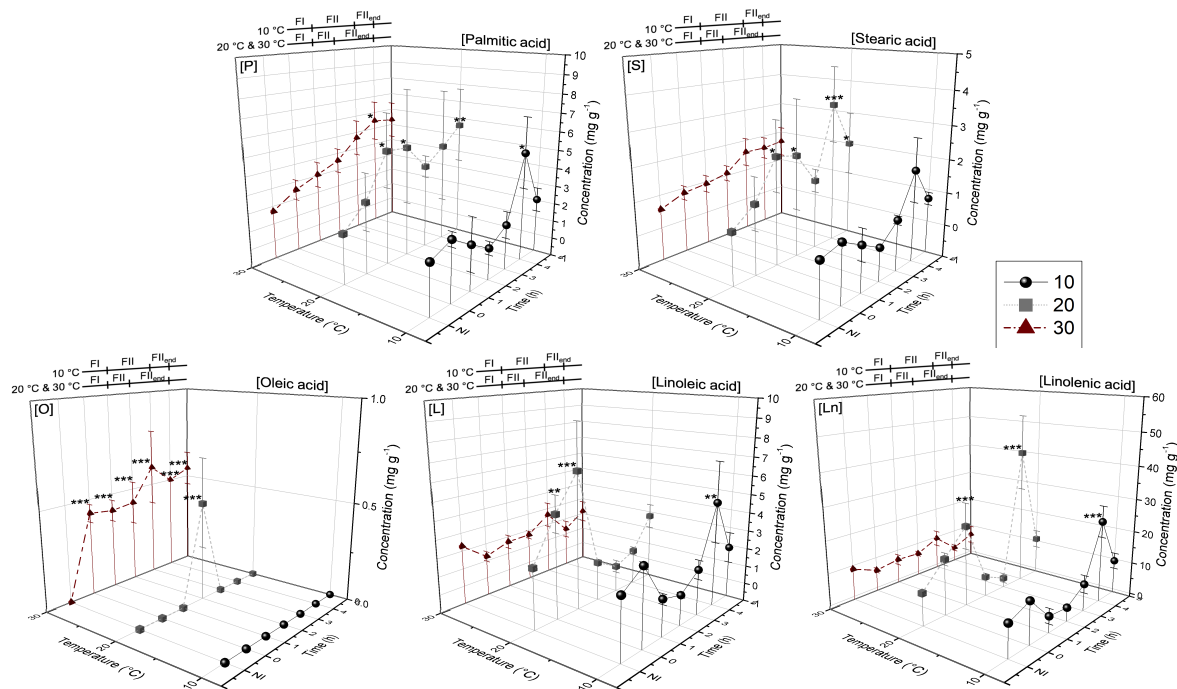
Figure 4. Fatty acid concentration changes in *S. hispanica* seeds related with imbibition stages (FI, FII and FII$_{end}$) at 10, 20 and 30 °C. Concentrations were calculated relative to the weight of non-imbibed seeds (NI) within the total weight. Values are expressed as mean ± SD of three independent replicates. Statistical analysis was performed using two-way ANOVA followed by a Dunnett multiple comparison test. Asterisks correspond to data with statistical differences regard with control concentration in NI (* $p = 0.033$; ** $p = 0.002$; *** $p < 0.001$).
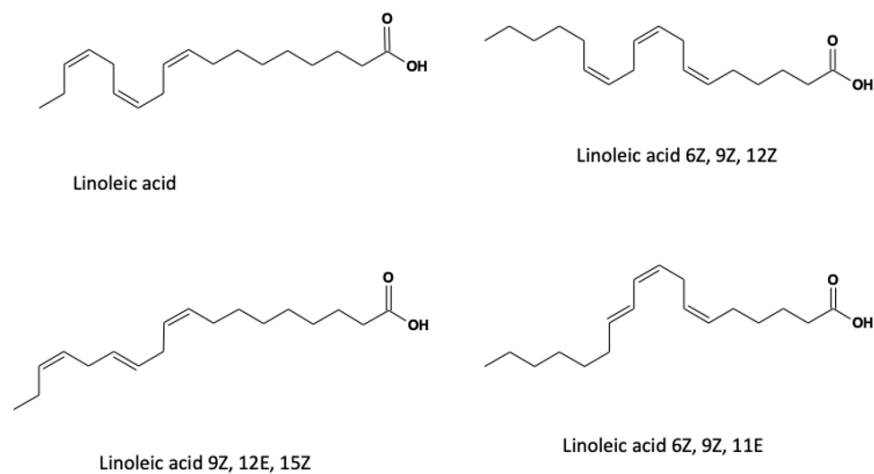
Figure 5. Linoleic acid isomers found in *S. hispanica* during seeds imbibition.
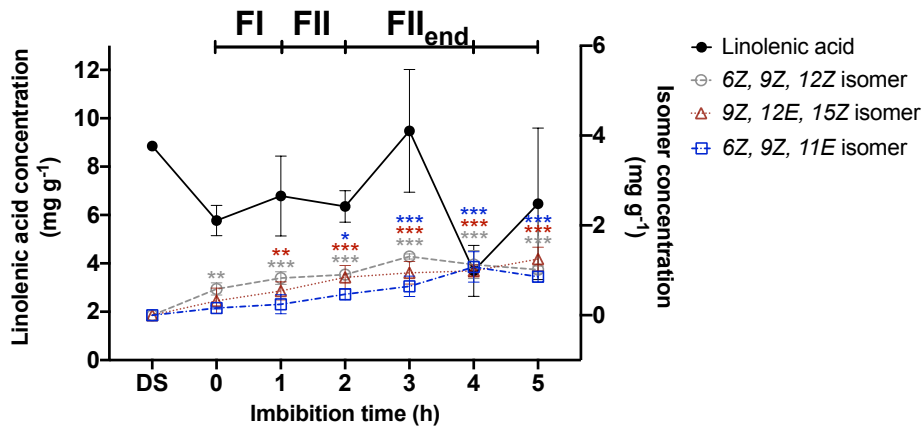
Figure 6. Fatty acid isomers in *S. hispanica* seeds. The imbibition stages (FI, FII and FII_end) are indicated throughout the imbibition trend at 30 °C. Concentrations were calculated relative to the weight of non-imbibed seeds (NI) within the total weight. Values are expressed as mean ± SD of three independent replicates. Asterisks correspond to data with statistical differences regard with control concentration in NI (* $p = 0.033$; ** $p = 0.002$; *** $p < 0.001$); the colors of the asterisks indicate significant differences to the corresponding isomer with the same symbol color. Left y-axis corresponds to the concentration of Ln; while the right y-axis corresponds to the isomers' concentration.

Quantifying cardinal temperatures of chia (*Salvia hispanica* L.) using non-linear regression models

Quantifying cardinal temperatures of chia (*Salvia hispanica* L.) using non-linear regression models

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Abstract: Temperature is the main factor that impacts germination, and therefore the success of annual crops, such as chia (*Salvia hispanica* L.), whose seeds are known for their high nutritional value related to its oil. The effect of temperature on germination is related to cardinal-temperature concepts that describe the range of temperature over which seeds of a particular species can germinate. Therefore, in this study, in addition to calculated germinative parameters such as Total Germination and Germination Rate of *S. hispanica* seeds, the effectiveness of non-linear models for estimating the cardinal temperatures of chia seeds was also determined. We observed that germination of *S. hispanica*, occurred in cold to moderate-high temperatures (10–35 °C), having an optimal range between 25–35 °C, with the highest GR and t_{50} at 30 °C. The temperatures higher than 35 °C significantly reduced germination. Output parameters of the different non-linear models showed that the response of chia germination to temperature was best explained by beta models (B). Cardinal temperatures calculated by the B1 model for chia germination were: 2.52 ± 6.82 °C for the base, 30.45 ± 0.32 °C for optimum, and 48.58 ± 2.93 °C for ceiling temperature.

Keywords: beta functions; cardinal temperatures; intersected-line models; *Salvia hispanica* L.; segmented non-linear regressions

1. Introduction

Chia (*Salvia hispanica* L.) is a summer biannual herbaceous and oleaginous plant, belonging to the family of *Lamiaceae*, rich in officinal and aromatic species with essential oils (EOs) making them valuable in many fields as cosmetics, food, medicine [1,2] and in agriculture as antimicrobial agents [3–5]. This oilseed crop is native to the region comprising mountainous areas from midwestern Mexico to northern Guatemala [6-8]. Historically, chia has been cultivated in subtropical and frost-free regions [9], specifically in the mountainous areas of the Pacific Ocean slope [10]. Currently, chia is cultivated in Australia, Bolivia, Colombia, Guatemala, Mexico, Peru, and Argentina [11].

Chia seeds vary in size from 1 to 2 mm with oval and flatted shapes; with colors from black to white, with or without gray and black spots [12–15]. Seed compositions are 15–25% protein, 30-33% fat, 26-41% carbohydrates, 18-30% fiber, 4-5% ashes, and also minerals, vitamins, and dry matter [12,16]. The seed oil represents 25-40% of the total seed weight and is composed of almost 50-57% of linolenic and 17-26% linoleic acid (ω-3 and ω-6 fatty acids, respectively), dietary fiber (over 30% of

the total weight) and proteins of high biological value (around 19% of the total weight) [17–20]. So far, there is no evidence of adverse effects or allergenicity caused by whole or ground chia seeds [21]. These chia characteristics have a positive impact on nutrition and health, and since 2009 was approved as a novel food by the European Parliament and the European Council [22].

Due to public health awareness and the demand for functional food with innumerable health benefits, chia production has experienced an increase in its production worldwide [16]. On this subject, during the year 2018, the chia seed worldwide market was valued at USD 66.5 million; by the year 2024, the market is projected to reach a value of USD 88.1 million [23]. Experimental evidence about *S. hispanica* is still in progress, especially concerning seed technology, including the role of temperature on germinative behavior [24–26]. Therefore, it is necessary to obtain enough knowledge about the factors underlying germination and plant development, following different approaches to support chia agricultural practices.

Germination and seedling establishment comprises two critical stages, on which the success of the next generation depends. Germination is affected by temperature, water availability, and gaseous environment [27]. From these environmental parameters, when water availability is not a limitation, the temperature is the main factor controlling germination, exerting influence on germination rate, latency level, and seed deterioration rate. Germination rate is mainly affected by temperature because it is related to water absorption by seeds [28,29]. Also, biochemical reaction rates underlaying the metabolic networks are affected [30]. In addition, the time at which germination occurs could be affected by temperature [31].

Thermal-germination models, which usually contain certain assumptions about within-population variability in germination-rate response to temperature, are one way to characterize germination reaction to temperature [32]. These assumptions are most often related to cardinal-temperature concepts that describe the range of temperature over which seeds of a particular species can germinate. It has been recognized three cardinal temperatures: base temperature (T_b) below which germination does not proceed; an optimal temperature (T_o) at which the rate of germination is highest; and a maximum or ceiling temperature (T_c) above which germination ceases [33–40]. The T_b for germination of any fraction of the seed population is considered to be a constant, while T_c varies among each percentile fraction in a normal distribution [35,36]. The temperature has an impact on plant growth and development, so estimating the cardinal temperatures is essential.

Because germination is one of the most important factors in the success of annual crops, playing a key role in crop production, practical research in plant science usually attempts to establish the minimum temperature required for germination or its maximum range. To improve establishment success rates and to reduce costs, it is essential to have a good understanding of seed germination requirements of species of agricultural importance. Although several models including linear and non-linear functions are available to estimate cardinal temperatures, a suitable model for the specific crop should be selected.

Therefore, in this study, in addition to calculated germinative parameters such as Total Germination and Germination Rate of *S. hispanica* seeds, the effectiveness of non-linear models (segmented and beta) for estimating the cardinal temperatures of chia seeds was also determined. Consequently, due to its economic potential based on its positive effects on human health, such knowledge may be useful for identifying the best planting dates for this oilseed crop in a range of climates and regions; and importantly, its resistance and distribution into climate change scenario.

2. Results

2.1. Germination

No significant differences were observed in final germination percentage between the treatments at 10, 15, 20, 25, 30, and 35 °C (H(7) = 28.27, $p < 0.001$ for Kruskal-Wallis Test and $p > 0.999$ for Dunn's *post hoc* test). However, there were significant differences (H(7) = 28.27, $p < 0.001$ for Kruskal-Wallis Test and $p < 0.001$ for Dunn's test) between each of these treatments regarding the

treatments at 40 °C and 45 °C, as well as between these last two treatments (H(7) = 28.27, *p* < 0.001 for Kruskal-Wallis Test and *p* < 0.001 for Dunn's test). In the group of treatments of 10-35 °C, average germination reached >88%, while the highest final germination observed was 98% at 20 °C. High temperatures, i. e. 40 °C and 45 °C, inhibited germination, reaching final germination values of 44%, and 11%, respectively, the latter being the lowest value observed of the eight treatments.

The time required to reach 50% germination (t_{50}) was significantly different between treatments (H(5) = 26.11, *p* < 0.001 for Kruskal-Wallis Test and *p* < 0.001 for Dunn's test). The highest t_{50} value was observed at 30 °C (0.30 ± 0.10 days) and the lowest at 10 °C (5.50 ± 0.44 days); while the treatments at 40 °C and 45 °C, did not reach 50% germination. t_{50} at 30 °C was 18.3-fold, 6.6-fold, 4.4-fold, 1.8-fold, and 1.2-fold faster than 10, 15, 20, 25, and 35 °C, respectively (Table 1). To reach t_{50}, 1–5 days elapsed at temperatures below 20 °C, whereas, in the temperature range of 25–35 °C, less than one day was required.

The GR was significantly different between the treatments (H(7) = 36.58, *p* < 0.001 for Kruskal-Wallis Test, and *p* < 0.001 for Dunn's test). It is possible to distinguish three different groups: 10–20 °C, 25–35 °C, and 40–45 °C. In the range of 10–20 °C, a gradual increase in GR was observed, reaching a plateau within the range of 25–35 °C as significant differences (H(7) = 36.58, *p* < 0.001 for Kruskal-Wallis Test and *p* > 0.999 for Dunn's test) were observed in this range; subsequently, a decrease was observed in the range of 40–45 °C. The highest GR was observed at 30 °C with 22 seeds per day and the lowest at 45 °C with about 2 seeds per day (Figure 2).

2.2. Cardinal temperatures determination by linear and non-linear regression models

Table 2 summarizes the six non-linear models used for cardinal temperature determination fitted to the reciprocal of the germination time versus temperature data for each of the 10-80% percentiles for each of the treatments (10–45 °C).

For the S1 model (Figure 3, S1) the estimated average base, optimum, and ceiling temperatures were: 6.90 ± 1.86 °C; 33.45 ± 2.76 °C and 42.83 ± 3.88 °C, respectively (Table 3). For the S2 model calculated Tb, To and Tc were: 6.65 ± 2.55 °C, 36.97 ± 5.70 °C, and 44.96 ± 1.45 °C, respectively (Figure 3, S2). In both models, it was only possible to calculate the cardinal temperatures from 10–40% percentiles, due to the few available points of 50–80% percentiles to perform the respective non-linear regressions. On the S3 model (Figure 3, S3) calculated Tb, To and Tc were: 6.52 ± 2.55 °C, 32.60 ± 1.20 °C, and 41.34 ± 3.74°C, respectively. On B1 model Tb, To and Tc for 10–80% were: 2.52 ± 6.82 °C, 30.45 ± 0.32 °C, and 48.58 ± 2.93 °C, respectively. On B2 model Tb, To and Tc were: 9.74 ± 2.23 °C, 31.24 ± 0.21 °C, and 44.10 ± 1.48 °C, respectively. Finally, on B3 model were: 4.97 ± 4.06 °C, 28.44 ± 2.28 °C, and 44.26 ± 2.83 °C, for Tb, To and Tc, respectively. The optimum temperature for all six models was very close to 30 °C, the temperature at which the higher GR and t_{50} were observed (Table 1, Figure 2).

For segmented models, the Tb, To and Tc values varied among percentiles in the three models (Figure 3; Table 3, 4, and 5). For the S1 model, the Tb variation difference from the lowest to the highest percentile temperature estimation was 5.94 °C, To was 1.5 °C and the Tc was 6.91 °C; the variation range was wider for To than Tb and Tc. In the S2 model, Tb varies in a wider range of 7.03 °C, To varies about 18.15 °C and Tc varies in a range of 2.45 °C. For the S3 model, Tb varies in a range of 9.1 °C, To varies 4.05 °C, and Tc in a range of 11.62 °C. For the B1 model, Tb variation difference was 17.38 °C (for 50-80% estimated Tb was negative), To difference was 0.87 °C and Tc difference was 6.47 °C; for B2 model Tb variation difference was 9.48 °C, To was 5.42 °C and Tc difference was 7.38 °C, and finally for B3 model Tb variation difference was 12.85 °C, To was about 1.13 °C, finally Tc difference was 16.49 °C.

In the S1 model, Tb values tend to decrease until population percentage reaches 40%, where Tb was 9.02 °C; from this point on, the temperature tends to drop again until reaches 80% (4.15 °C); To tends to increase until reaches 40% and then decrease from this point until reaches 60%, and then rises from 60% to 80% (35.88 °C). Finally, for the same model, Tc tends to decrease from 10% to 30%, from this point on it was not possible to perform the respective linear regressions due to the lack of

empirical data of 40–80%. For S2 Tb only tends to decrease from 9.62 to 2.59 °C; To only tends to increase and Tb only tends to decrease, again the lack of empirical data from 40–80% was a limitation to perform the corresponding regressions. For S3, Tb follows a tendency to decrease, To only increase, from 10% to 20% and subsequently remains constant near to 30 °C; while Tc decrease from 10% to 30%, then increases at 40% and 50%, subsequently dropping from this point on. For all beta models, Tb tends to decrease, To remains constant in the three models, while Tc tends to increase in B1 and B3 models, following an expected normal distribution, while in the B2 model Tc tends to decrease.

RMSE, R^2, and adjusted R^2 were calculated for all regression lines in the sub-optimal range in the intersected S1 and S2 model, while output parameters for 50–80% in the supra-optimal range of those models were unable to be calculated due to the lack of experimental data, where at least seven experimental points are needed to perform the corresponding regressions. For the remaining models, output parameters were calculated for a single regression, that spanned both ranges; all models met the assumptions of linearity, homoscedasticity, normality, and independence. In all models the RMSE, R^2, and adjusted R^2 were prone to decrease as the population percentage increases; except for B3, where RMSE increases from 10% to 30% and from this point on tends to decrease. On the other hand, R^2 and adjusted R^2 increase from 10% to 20%, then decrease from 20% to 30%, afterward increase from 30% to 50%, and finally decrease from this point on.
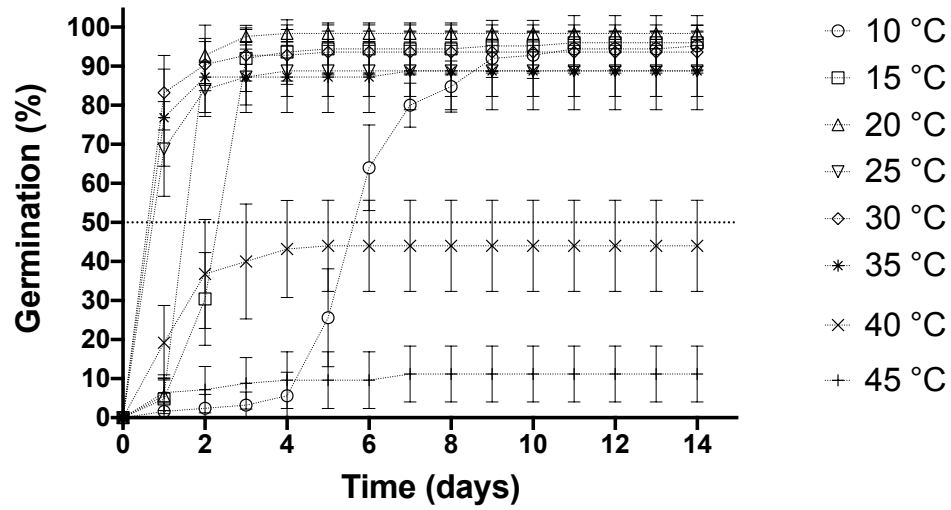
Figure 1. Cumulative germination of S. hispanica at 10, 15, 20, 25, 30, 35, 40, and 45 °C. Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis followed by a Dunn's multiple comparison test.

Table 1. Final germination percent and median germination time (t50) of seeds during imbibition at 10, 15, 20, 25, 30, 35, 40, and 45 °C. Final germination is the percentage of seeds in which the germination process reaches the end; while median germination time (t50) is the time to reach 50% of final germination.

Temperature	Final Germination (%)	Median Germination Time t_{50} (Days)
10 °C	95.20 ± 5.21[a]	5.50 ± 0.44[a]
15 °C	96.00 ± 6.92[a]	2.00 ± 0.15[b]
20 °C	98.40 ± 2.19[a]	1.32 ± 0.07[c]
25 °C	88.80 ± 6.57[a]	0.56 ± 0.19[d]
30 °C	93.60 ± 5.36[a]	0.30 ± 0.10[d]
35 °C	88.80 ± 9.96[a]	0.38 ± 0.22[d]
40 °C	44.00 ± 11.66[b]	ND
45 °C	11.20 ± 7.15[c]	ND

Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis (p < 0.001) followed by a Dunn's test multiple comparison test. The values that share the same letters did not present statistically significant differences (p < 0.001).
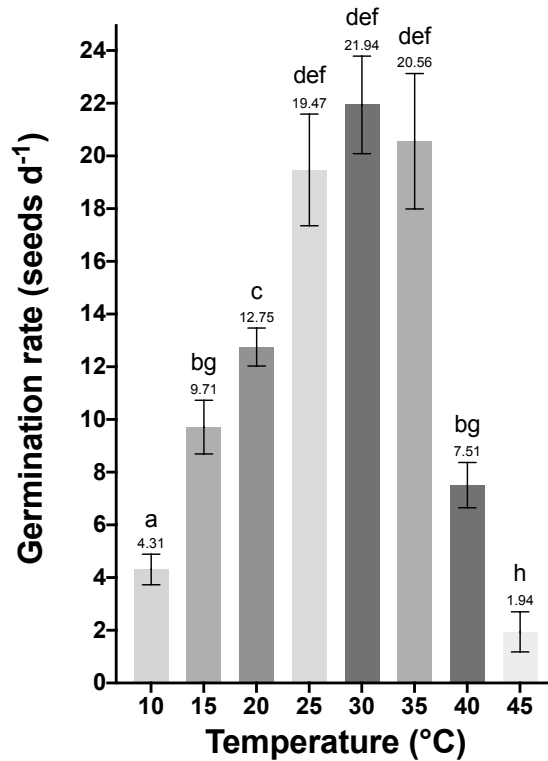
Figure 2: Germination rate per day of seeds at 10-45 °C. Values are shown as means ± SD of 5 replicates with 25 seeds each. Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis followed by a Dunn's multiple comparison test. Different letters indicate significant differences ($p < 0.001$).

Table 2. Non-linear regression models fitted to reciprocal of GR versus temperature data for 10-80% percentiles to determine cardinal temperatures of chia seeds.

Model; reference	Formula		Conditions
Segmented 1 (intersected model: two-segment no-linear regression and simple linear regression) [76]; **S1**	$f(T) = \dfrac{T - Tb}{To - Tb}$	If Tb<T≤To, for 10-30 °C	Sub- and supra-optimal range: two-segment non-linear regression for 10-80%. No constricted
	$f(T) = \dfrac{Tc - T}{Tc - To}$	If To<T<Tc, for 30-45 °C	
	$f(T) = 0$	If T≤Tb or T≥Tc	
Segmented 2 (three-segment non-linear regression) [76]; **S2**	$f(T) = \dfrac{T - Tb}{To - Tb}$	If Tb<T≤To, for 10-20 °C & 20-30 °C	Sub- and supra-optimal range: two-segment non-linear regression for 10-80%. Sub-optimal range constricted to pass through 20 °C as inflection point; supra-optimal range constricted to pass through 40 °C as the inflection point
	$f(T) = \dfrac{Tc - T}{Tc - To}$	If To<T<Tc, for 30-45 °C	
	$f(T) = 0$	If T≤Tb or T≥Tc	
Segmented 3 (three-segment non-linear regression) [76]; **S3**	$f(T) = \dfrac{T - Tb}{To - Tb}$	If Tb<T≤To, for 10-20 °C & 20-30 °C	Three-segment non-linear regression for sub- and supra-optimal range; no-constrained.
	$f(T) = \dfrac{Tc - T}{Tc - To}$	If To<T<Tc, for 30-45 °C	
	$f(T) = 0$	If T≤Tb or T≥Tc	
Beta 1 (four-parameters) [81]; **B1**	$f(T) = \left(\dfrac{T - Tb}{To - Tb}\right)^{\alpha} \times \left(\dfrac{Tc - T}{Tc - To}\right)^{\beta}$	$\alpha = 5$; $\beta = 4$	One non-linear regression; no-constrained.
Beta 2 (five-parameters) [81]; **B2**	$f(T) = \left(\left(\dfrac{T - Tb}{To - Tb}\right)^{\frac{Tc-To}{To-Tb}}\right)^{\alpha} \times \left(\left(\dfrac{Tc - T}{Tc - To}\right)^{\frac{Tc-To}{To-Tb}}\right)^{\beta}$	$\alpha = 8$; $\beta = 6$	One non-linear regression; no-constrained.
Beta 3 [89]; **B3**	$f(T) = (A0) \times \left(e^{(-A1 \times (X/A2 - 1)^{2 + 1/(X - A3)})}\right)$		One non-linear regression; no-constrained.
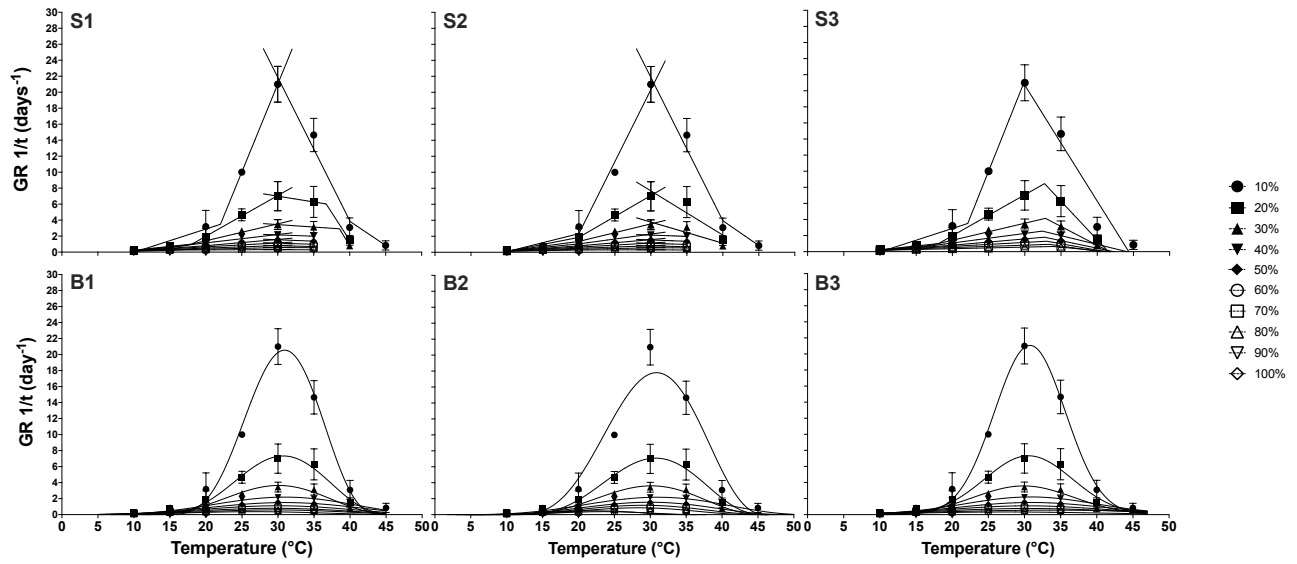
Figure 3: Relationship between the reciprocal of the GR and the germination temperature of the percentiles in the cardinal temperature range. Symbols represent experimental data; while the solid lines correspond to the predicted values by segmented (S1, S2, and S3) and beta functions (B1, B2, and B3).

Table 3. Estimated parameters for segmented model (S1) of *Salvia hispanica* seeds. Root mean square of deviations (RMSE) and coefficient of determination (R^2) for the relationship between emergence rates.

Parameter	S1															
	10%		**20%**		**30%**		**40%**		**50%**		**60%**		**70%**		**80%**	
Tb (°C)	10.09		7.45		7.29		9.02		6.86		6.56		4.93		4.15	
Mean Tb (°C)							**6.90 ± 1.86**									
To (°C)	31.09		31.43		32.54		37.49		36.28		29.93		32.98		35.88	
Mean To (°C)							**33.45 ± 2.76**									
Tc (°C)	47.31		40.77		40.40		ND		ND		ND		ND		ND	
Mean Tc (°C)							**42.83 ± 3.88**									
Range	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**
RMSE	1.28	1.88	0.91	1.46	0.44	0.55	0.32	ND	0.22	ND	0.17	ND	0.13	ND	0.11	ND
R²	0.97	0.95	0.88	0.75	0.88	0.83	0.84	ND	0.83	ND	0.80	ND	0.74	ND	0.58	ND
Adjusted R²	0.97	0.94	0.87	0.68	0.87	0.78	0.82	ND	0.81	ND	0.77	ND	0.71	ND	0.52	ND

Table 4. Estimated parameters for segmented model (S2) of *Salvia hispanica* seeds. Root mean square of deviations (RMSE) and coefficient of determination (R²) for the relationship between emergence rates

	S2															
Parameter	**10%**		**20%**		**30%**		**40%**		**50%**		**60%**		**70%**		**80%**	
Tb (°C)	9.62		9.41		8.74		8.05		6.96		6.10		4.67		2.59	
Mean Tb (°C)	**6.65 ± 2.55**															
To (°C)	31.07		32.28		33.29		36.05		36.70		37.71		39.51		49.22	
Mean To (°C)	**36.97 ± 5.70**															
Tc (°C)	46.64		44.06		44.19		ND		ND		ND		ND		ND	
Mean Tc (°C)	**44.96 ± 1.45**															
Range	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**	**Sub**	**Supra**
RMSE	1.46	1.88	0.93	1.76	0.45	0.74	0.31	ND	0.22	ND	0.17	ND	0.13	ND	0.11	ND
R²	0.96	0.95	0.88	0.63	0.88	0.69	0.85	ND	0.83	ND	0.80	ND	0.74	ND	0.58	ND
Adjusted R²	0.96	0.94	0.87	0.57	0.87	0.64	0.83	ND	0.82	ND	0.78	ND	0.72	ND	0.54	ND

Table 5. Estimated parameters for segmented models (S3) of *Salvia hispanica* seeds. Root mean square of deviations (RMSE) and coefficient of determination (R^2) for the relationship between emergence rates.

Parameter	S3							
	10%	20%	30%	40%	50%	60%	70%	80%
Tb (°C)	10.09	7.45	7.29	7.09	6.86	6.50	5.91	0.99
Mean Tb (°C)				6.52 ± 2.55				
To (°C)	29.84	32.73	32.85	32.43	32.72	33.04	33.37	33.89
Mean To (°C)				32.60 ± 1.20				
Tc (°C)	44.46	41.05	39.85	45.71	44.73	36.59	37.00	32.84
Mean Tc (°C)				41.34 ± 3.74				
RMSE	1.88	1.04	0.47	0.32	0.22	0.18	0.14	0.11
R^2	0.94	0.86	0.87	0.85	0.83	0.79	0.70	0.64
Adjusted R^2	0.93	0.84	0.85	0.81	0.80	0.74	0.64	0.56

Table 6. Estimated parameters for beta models (B1, B2, and B3) of *Salvia hispanica* seeds. Root mean square of deviations (RMSE) and coefficient of determination (R^2) for the relationship between emergence rates.

Parameter	B1 (four-parameters)								B2 (five-parameters)								B3							
	10%	20%	30%	40%	50%	60%	70%	80%	10%	20%	30%	40%	50%	60%	70%	80%	10%	20%	30%	40%	50%	60%	70%	80%
Tb (°C)	12.88	9.38	6.85	4.42	-1.22	-2.06	-5.57	-4.50	9.97	9.98	8.14	6.19	0.71	0.49	2.18	2.16	20.10	17.11	15.37	12.91	10.33	10.05	8.80	7.25
Mean Tb (°C)	2.52 ± 6.82								4.97 ± 4.06								12.74 ± 4.45							
To (°C)	31.03	30.86	30.24	30.47	30.24	30.22	30.39	30.16	30.97	30.92	30.19	29.81	27.31	27.13	25.68	25.55	30.19	32.78	30.15	30.30	30.79	29.94	29.54	29.06
Mean To (°C)	30.45 ± 0.32								28.44 ± 2.28								30.34 ± 1.11							
Tc (°C)	44.28	46.31	46.44	46.93	51.32	50.28	52.33	50.75	46.48	46.06	46.26	47.04	44.45	43.5	41.26	39.1	40.22	41.34	44.65	49.92	55.85	50.06	53.14	56.71
Mean Tc (°C)	48.58 ± 2.93								44.26 ± 2.83								48.99 ± 6.32							
RMSE	1.53	1.10	0.53	0.36	0.23	0.19	0.15	0.13	2.26	1.10	0.52	0.36	0.36	0.28	0.25	0.22	1.66	1.72	2.30	0.31	0.22	0.18	0.15	0.03
R²	0.96	0.85	0.84	0.81	0.82	0.77	0.68	0.51	0.91	0.85	0.84	0.81	0.55	0.47	0.11	-0.37	0.99	0.96	0.95	0.99	0.99	0.98	0.98	0.96
Adjusted R²	0.96	0.84	0.83	0.80	0.81	0.75	0.65	0.47	0.90	0.84	0.83	0.80	0.52	0.43	0.04	-0.48	0.98	0.93	0.90	0.97	0.98	0.96	0.95	0.92

3. **Discussion**

3.1. Germination

It has been reported that chia grows at a minimum temperature of 11 °C and a maximum of 36 °C, with an optimal between 16–26 °C [41]. However, until now, a wide germination temperature gradient had not been assayed. In this context, we observed that chia can germinate at lower temperatures, reaching a final germination percentage above 95% at 10 °C; at this temperature, germination is delayed because more time is needed to accumulate enough day degrees to complete germination, as has been observed other short-day related species [39,42,43] and other phenological events of chia as the flowering [44]; however, until now, a thermal time coefficient for chia germination is still lacking. The fact that germination is not completely inhibited at 10 °C indicates that the base temperature is below this value and lower concerning the previously reported temperature [41].

Our observed optimal condition, i. e. the temperature(s) at which the germination percentage is high, and germination occurs the fastest, in this case, 25–35 °C is almost ten degrees above the reported range [41], this evidence agrees with our previous results [24] and with those observed by Paiva et al. [25,26]. The differences in the germination of chia varieties generated by domestication are mainly associated with different capabilities to germinate and grow under different climatic conditions [27], like those varieties produced to grow during the long days of the northern hemisphere [45,46]. Another explanation is related to storage conditions, where it's already known that humidity and high temperatures reduce seeds viability and germination [27]. These deterioration processes are related to the chemical composition of the seeds [47] and are mainly associated with factors such as water content, environmental conditions, microorganisms, package and storage conditions, among others [48]. Because of the disruption of the membrane system caused by free radical attacks on the chemical components of the membrane, the most visible physiological symptoms of seed degeneration arise during germination and seedling initial development [49].

In other surveys carried out in some members of the *Salvia* L. genus, it has been observed that golden chia (*Salvia columbariae* Benth.) reached the highest final germination at 25 °C, compared with 4 °C and 10 °C [50]; while Noroozak (*Salvia leriifolia* Benth.) has an optimal range between 15–25 °C, with a calculated Tb, To and Tc of 1.00 °C, 19.0 °C, and 36.5 °C, respectively [51]. On the other hand, in an analysis conducted on 11 different medicinal plants [52], including two belonging to the genus *Salvia*, it was observed that *Salvia sclarea* L. and *Salvia nemorosa* L., reached the highest final germination and GR in the treatments at 20 °C for *S. sclarea* L. (Final germination: 71.9%; GR: 65 seeds d^{-1}; Tb: 0.0 °C; To: 21.0 °C and Tc: 40.3 °C) and 15 °C for *S. nemorosa* L. (Final germination: 58.9%; GR: 54.3 seeds d^{-1}; Tb: 0.0 °C; To: 17.0 °C and Tc: 41.0 °C).

The GR observed in our analysis was 1.4-fold and 2.2-fold higher than the previously reported by Nadtochii et al. [53] for 25 °C, and 30 °C (13.1 ± 0.1 and 9.7 ± 0.1); while at 20 °C, GR was similar between works (12.6 ± 0.1 and 12.75 ± 0.72, respectively). In this subject, Adam *et al*. [54] showed that GR differed among species and seed lots within species. We observe that in chia, temperatures higher than 35 °C led to reduced final germination and germination rate, in this context it has been shown that temperature increased up to optimum followed by increased GR, but declined afterward [40,55–57]. Hardegree [58] reported that there was a large error in predictions of seedling emergence in early spring due to seed degradation and lowering GR at high temperatures.

Our results suggest that *S. hispanica,* due to their oil quality and quantity, has an improved seed performance at low and higher temperatures, where a high proportion of polyunsaturated fatty acids helps to maintain cellular membrane fluidity, this agrees with our observation that chia, after a lag phase at 10 °C (four days) reaches final germination above 95%, in contrast with *S. sclarea* L. and *S. nemorosa* L., which reached less than 50% at temperatures below 10 °C and that at a temperature above 40 °C, germination is significantly reduced, but not completely inhibited nonetheless [52], either by thermodormancy, thermoinhibition or directly by the death of the embryo [27,31,59]. This

is supported by the fact that in *S. sclarea* L. and *S. nemorosa* L. seeds have been observed a lower concentration of linoleic (both species with 15–20% less compared with *S. hispanica*) and α-linolenic acid (10% less in *S. sclarea* L. and almost 40% less in *S. nemorosa* L. compared with *S. hispanica*) [60,61].

Germination behavior is intrinsically connected to ecological conditions of species' natural habitats and biogeographical origin [62]. Most of the species naturally found in arid and temperate climates have the potential to germinate well at temperatures ranging from 15 to 30 °C, indicating a preference for moderate and moderate-high temperatures for germination. In this subject, there is evidence that shows that temperatures ranging from 15 °C to 25 °C led to maximum germination in species characteristic of arid environments like *Lavandula dentata* L., *Teucrium gnaphalodes* L'Hér., *Thymbra capitata* (L.) Cav., and *Thymus hyemalis* L. while higher temperatures limit germination and growth [63]. Therefore, it is not surprising that temperatures <10 °C and >35 °C affect the growth and yield of chia, either delaying or inhibiting germination, considering that this species is adapted to Central and South America annual mean temperatures that fluctuate between 11 and 36 °C [17–19,41], reflecting their distribution and climatic conditions for optimal germination.

3.2. *Cardinal temperatures determination by linear and non-linear regression models*

The temperature variation range for all models was quite different; while with the S1 and S2 models it was not possible to determine Tc for 4 percentiles (40–80%), due to restrictions related to the number of experimental points to perform the regression analysis. In all segmented models, the Tb values were less variable than To and Tc values. Finally, Tc variations were narrower for S1 and S2 models, considering that only 3 percentiles were able to estimate with these models. On the other hand, for beta models Tb variation was wider between percentiles than To and Tc variation; being To variation the narrowest from all beta models; B3 model estimated the wider for Tb and Tc variation range.

Our observed Tb downward trend in S1, S2, and S3 were according to those tendencies reported for Tb determination with two and three-piece segmented models observed in *Phalaris minor* Retz. [64] and *Silybum marianum* L. [65]. The observed trends in this species include a linear increase in germination, followed by a plateau segment, and finally a decrease in germination until reaching zero as the temperature increases; the plateau segment was not observed in our data of chia germination. Our data tendency involves higher slope values for lower population percentages, forcing regression lines from higher to lower values of x-intercept (Tb values) as the percentage of the population increases. The same phenomenon seems to occur with the Tb estimation with B2 and B3 models, whose observed trends are opposite to the observed Tb values for beta functions with five-parameters [64,65]; while our observed tendency for B1 (beta four-parameters or modified) is according with the observed in *S. marianum* L. [65]. The Tb tendencies for B2 and B3 models are related to chia 1/VG data distribution. Although the physiological meaning of this behavior remains to be explored, it has been suggested that the amount of energy reserve of the different population percentages could be related to a higher germination efficiency, related in turn with faster germination, where more energy is needed to satisfy the metabolic demands of this population, especially for processes such as the of starch and proteins hydrolysis [66]. So far, S1 and S2 were a combination of two-segments linear regressions combined in an intersected model, this is the first report of cardinal temperatures determination using this type of approximation.

Our findings established that the optimal temperature range of chia seed germination was from 28.4 to 36.97 °C, with a maximum GR observed at 33.45 ± 2.76 °C in S1, 36.97 ± 5.70 °C in S2, 32. 60 ± 1.20 °C in S3; 30.45 ± 0.32 °C in B1, 28.44 ± 2.28 °C in B2, and 30.34 ± 1.11 °C in B3; these values did not support previous findings, where it has been observed an optimal range between 16–26 °C [17–19,41]. Until now, there are few reports where the cardinal temperatures of some[6 species of the genus *Salvia* have been determined. In this context, it has been reported that seeds of *Salvia pomifera* L. and *Salvia fruticosa* Mill. have an optimal temperature range of 10–20 °C [67]. Also, it has been

found that seeds of *Salvia officinalis* L. germinated within the range of 10–25 °C and that *Salvia sclarea* L. had a broader range of optimal temperatures from 10 to 30 °C [68]. While in another survey, it has been reported that the optimum temperature for seed germination of *S. officinalis* L. was 25 °C [69]. For the majority of plant species, optimum and ceiling temperatures have been reported at 15–30°C and 30–40°C, respectively [69]; however, the optimum temperature of germination depends on genetic and environmental conditions that the plant evolved [70].

Finally, it has been reported that cardinal temperatures for *Plantago ovata* Forssk. were Tb: 4.4 °C, To: 19.0 °C and Tc: 25.5 °C; while for *Plantago psyllium* L. was Tb: 9.4 °C, To: 28.8 °C and Tc: 35.0 °C [56]; it is important to emphasize that these two species are myxospermic angiosperms with copious mucilage, as chia; owning both to the Lamiales order. Mucilage is a polymer secreted by a variety of plants and their parts, including *Aloe vera* L., *Salvia hispanica* L. seeds, *Cordia dichotoma* G.Forst., *Basella alba* L., *Plantago psyllium* L., *Cyamopsis tetragonoloba* (L.) Taub., *Cactaceae*, *Abelmoschus esculentus* (L.) Moench, *Trigonella foenum-graecum* L., *Moringa oleifera* Lam., and *Linum usitatissimum* L. [71]. The structure, components, ecological roles, and production mechanism of mucilage have been well studied in the model plant *Arabidopsis thaliana* (L.) Heynh. [72–74]. Studies involving *A. thaliana* (L.) Heynh. and other species have concluded that seed coat mucilages may have multiple roles, including the inhibition of germination under excessive moist conditions (i.e., seed dormancy) by preventing the embryo from oxygen diffusion [74]. Evidence with the seed-mucilage *Lavandula subnuda* Benth. (Lamiaceae) and *Plantago ciliata* Desf. (Plantaginaceae), have established that mucilage presence increased moisture uptake and inhibited germination at lower temperatures [74]. Upon germination, the progressive depletion of oxygen generates conditions that almost achieve anaerobiosis, and fermentation is triggered as the main source of cellular ATP, supporting the reduction of electron transferring compounds, e.g., NAD and NADP, and inevitably leading to ROS (reactive oxygen species) accumulation [75]. In the case of *S. hispanica*, we observed that in a temperature range from 10 °C to 20 °C, germination undergoes a lag phase; above that temperature, germination increases exponentially as it approaches the optimal temperature, this evidence suggests that mucilage could have a temperature were its moisture-holding capacity change, allowing germination to proceed more quickly, this agrees with our previous observations [24].

Parameters from model fitting to the reciprocal of GR versus temperature data are shown in Table 3, 4, and 5 for segmented models and Table 6 for beta models. For the sub- and supra-optimal range in S1, the root means square of deviations (RMSE) was highest for 10% and tends to decrease as the percentage of the population increases in the sub-optimal range (RMSE from 1.28 to 0.11); the same tendency was observed from 10% to 30% in the supra-optimal range (RMSE from 1.88 to 0.55 for the supra-optimal range). The same tendency was observed for S2 and S3 models with values from 1.46 to 0.11 and 1.88 to 0.11, respectively. R^2 and adjusted R^2 were >0.7 for 10–70 percentiles of all segmented models; except for the lowest values of the adjusted R^2 and adjusted R^2 of 80% (<0.6) in S1, S2, and S3 models. RMSE was lower for beta models than segmented ones, except for the B2 model ranging from 1.53 to 0.13 for the B1 model; from 2.26 to 0.22 for B2, and from 1.66 to 0.03 for the B3 model, these lower RMSE values of B1 and B3 indicate a higher fit of the beta models to our empirical data, having globally B3 the best fitting output parameters; however, this model tends to overestimate Tb values (12.74 ± 4.45 °C).

While all six models showed a good predicting ability, beta models had a better estimate for cardinal temperatures. For all segmented models a better fit can be observed as the population percentage increases; this type of function has been used for the description of data distribution with little variation in the germination rate between percentiles in an optimal range, forming a plateau, as the observed in chickpea [76], littleseed canarygrass [64], and milk thistle [65], this behavior is in contrast to the type of performance that we observed in chia, where three abrupt changes in the germination rate were observed at 20 °C, 30 °C and 40 °C, respectively. Also, it has been observed that segmented models tend to overestimate base or maximum temperature when only two segments are used making a bilinear function [77]; in the case of chia, however, we observed a realistic value

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for all models including segmented ones, where the only exception was observed in B3 model (Tb of 12.74 ± 4.45 °C); while To and Tc were quite similar for all models. This evidence agrees well with other findings where it has been observed that segmented functions adequately described the response of germination, leaf appearance, and development rate to temperature in different crops [78–80].

On the other hand, the observed lower values of RMSE and higher R^2 and adjusted R^2 for beta models, are expected because beta functions are more flexible than segmented ones, due to the curvilinear nature of beta models that provide a gradual transition between phases producing a smooth realistic curve. Beta models did not require the determination of cardinal temperatures for each subpopulation and therefore the models can be easily parameterized since they can be linearized if values of Tb and T, are predetermined from the data or external sources, or data transformation to probit units are not required [58,81]. Also, it has been observed that curvilinear models accurately predict ceiling temperatures by extrapolation when empirical data are not available [82]. Beta functions show some limitations, i. e. assume a symmetric response about optimum temperature and does not allow for any concave curvature near the base temperature [81]; however, exponential functions, like the used for B3, are flexible enough to handle nonsymmetric responses, these characteristics could explain the overestimation of the base temperature by this model, making it accurate and suitable for chia To and Tc but not for Tb.

The results of this work indicate that all assayed models fit well empirical data of chia germination in response to temperature; however, beta models have a better fit than segmented ones and that the B1 model sharply defines the cardinal temperatures of *S. hispanica*.

4. Materials and Methods

4.1. Seed acquisition and store

Medicinal variety of *S. hispanica* seeds were obtained without previous treatment, with 90% of germination and 99% of purity accordingly with the supplier (Okko super foods©; Jalisco, México; Lot/Batch: 130320/19). Seeds were kept in their shipping bags in a cold, dry seed storeroom at 10 ± 5 °C and 20 ± 5% relative humidity (RH) until imbibition assays were conducted. No previous disinfection treatment was applied in any of the experiments due to chia seeds' response at mucilage secretion level [83,84].

4.2. Germination tests

Five replicates of 25 seeds were sown randomly on agar medium (10 g L^{-1}) in Petri dishes (5.5 × 1.5 cm). Seeds were incubated at constant temperatures in germination chambers at 10 °C to 45 ± 2 °C (with intervals of 5 °C) with a 12 h photoperiod same as Cabrera-Santos et al. [24] and Sampayo-Maldonado et al. [85]. Seeds were considered germinated when radicle emerged ≥2 mm [86], after that, seedlings were removed from the Petri dish. Germination was recorded daily for 14 days, a time at which no more germination was observed.

4.3 Variables evaluated

4.3.1. Total Germination

The daily number of germinated seeds in each Petri dish was recorded. G(%) was reported as the average cumulative percentage of germinated seeds in each treatment, calculated according to:

$$G(\%) = \frac{n}{N} \times 100$$

where n is the number of seeds germinated and N is the total number of seeds.

4.3.2. Germination Rate (GR)

Germination rate or the number of germinated seeds by day was obtained with the equation proposed by Maguire [87]:

$$GR = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \cdots + \frac{G_i}{N_i} + \frac{G_n}{N_n} = \sum_{i=1}^{n} \frac{G_i}{N_i}$$

where G_i is the number of germinated seeds and N_i es the number of days after the beginning of the experiment.

The reciprocal of time for germination for each fraction of the seed population (10–100%) was calculated and plotted as a function of the temperature to observe the tendency of the data, locate the point of inflection and determinate the sub-optimal and supra-optimal temperature ranges.

4.4. Determination of cardinal temperatures by non-linear and linear regression models

To formulate and validate mathematical functions that have been used to quantify the effect of temperature on the biological time required for germination and cardinal temperatures determination, a combination of two-segment linear regressions in two intersected models, segmented 1 and 2 (S1 and S2, respectively) and four non-linear regression models: segmented 3 (S3); beta 1, 2 and 3 (B1, B2, and B3, respectively), were fitted to the reciprocal of germination rate versus temperature data. Intersected models encompass two linear regressions that fit the germination rate data as a function of temperature in the sub-optimal and supra-optimal temperature ranges. Base temperature and ceiling temperature are calculated from the x-intersection of both regression lines, while the optimum temperature is calculated from the intersection point of the two linear regression lines [27]. For S1 and S2, the first linear regression was adjusted when X<X0 and the second regression when X>X0 was in the suboptimal range; while X>X0 and X<X0 were in the sub-optimal range, respectively, while in S3 the criteria were X<X0 and X<X1. In S2 X0 was constricted at 20 °C and 40 °C, respectively. X0 and X1 were chosen when the model did not follow a gradual change in the slope of regression lines, i. e. after an abrupt breakpoint (statistically different). On the other hand, beta models are based on beta probability density distribution, often used for fitting curvilinear relationships.

For the S1 model, two-segment non-linear regressions in sub- and supra-optimal ranges were performed letting all the segmented linear regressions vary without any restriction. On the other hand, changes in slope were observed in both ranges with inflection points at 20 and 40 °C respectively; therefore, the S2 model was restricted with inflection points at those temperatures. The first segment of the regression in the sub-optimal range was used to estimate the x-intercept of each regression line; while for the supra-optimal range the second segment was used to estimate the x-intercept and therefore, ceiling temperature. An average of the x-intercept among fractions in the sub-optimal and supra-optimal temperature range was calculated to establish the Tb and Tc for each population percentile [88]. Non-linear regressions for sub- and supra-optimal were then recalculated for each fraction but constrained to pass through Tb for the sub-optimal temperature data and Tc for the supra-optimal temperature data [58]. Optimum temperature was calculated for each fraction as the intercept of sub and supra-optimal temperature-response functions [58]. Parameters for 10–40% only were obtained with these models, due to the few available points of 50-80% percentiles to perform the respective segmented regressions (at least five experimental data points). For the S3 model, three-segment non-linear regressions with no restrictions were performed, letting vary the inflection points.

Beta models (B1 and B2) were performed varying α and β parameters (α=5, β=4 for B1 and α= 8, β=6 for B3, respectively) without any other restriction. B3 model were performed with the function developed by Reyes-Ortega [89].

To determine the best estimates of the parameters (lower deviations of the intercept from 0 and of the slope from 1 correspond to increased reliability (RMSE; Eq. (3)), the coefficient of determination (R^2; Eq. (4)), and the intercept and slope of the regression of predicted vs. observed germination rate were used.

$$RMSE = \sqrt{\left(\frac{1}{n}\right)\sum\left(Y_{obs} - Y_{pred}\right)^2}$$

where Y_{obs} denotes observed value, Y_{pred} predicted value, and n the number of samples [90], and

$$R^2 = SSR/SST$$

where SSR denotes the sum of squares (SS) for regression $\left(\sum_{i=1}^{n}(\hat{Y} - \bar{Y})\right)$ and SST the total SS $\left(\sum_{i=1}^{n}(Y_i - \bar{Y})\right)$. Yi is the observed value and Y is the correspondent estimated value. Low RMSE and R^2 near 1 correspond to better model estimation.

Segmented and beta models were fitted according to Soltani et al., [76], Yin et al., [81] and Reyes-Ortega [89], respectively (Table 1). TableCurve® 2D (version 5.01 for windows, Systat Software Inc., San Jose, CA, USA, www.sigmaplot.co.uk/products/tablecurve2d) and GraphPad Prism® software (version 8.4.0 for macOS, GraphPad Software, San Diego, CA, USA, www.graphpad.com) was used to calibrate the models via the iterative optimization method.

2.5. Statistical Analysis

Germination data did not fulfill the assumption of normality, therefore significant differences in Total Germination, Median Germination Time (t_{50}), and Germination Rate (GR) were determined by Kruskal-Wallis followed by Dunn's *post hoc* test for multiple comparisons. Statistical analyses were carried out using the GraphPad Prism® software, version 8.4.0 for macOS, GraphPad Software, San Diego, CA, USA, www.graphpad.com (accessed on 10 October 2021).

5. Conclusions

In this work, we explore the effect of temperature on seed germination in the oilseed crop *S. hispanica*. The main conclusions are the following:

1. Germination of *S. hispanica* L., occurs in cold to moderate-high temperatures (10–30 °C), having an optimal range between 25–35 °C, with the highest GR and t_{50} observed at 30 °C. The temperatures higher than 35°C strongly inhibited the germination characteristics.

2. The results of this study showed that the response of chia germination to temperature was best explained by beta models, having a better fit than segmented models.

3. Cardinal temperatures for chia germination calculated by the B1 model were: 2.52 ± 6.82 °C for the base, 30.45 ± 0.32 °C for optimum, and 48.58 ± 2.93 °C for ceiling temperature.

This is the first report of cardinal temperatures determination of *Salvia hispanica* L., our data for the base, optimum, and ceiling temperatures for chia seed germination provides basic temperature requirements that can be used in further research and crop of this species; further assays must be oriented to determine thermal requirements of the different chia genotypes and varieties. As a perspective, it is necessary to carry out approaches in -omics fashion (genomic, proteomic, and metabolomic), in order to have a more complete physiological overview; meanwhile, every effort

must be oriented towards its application in the field, which by virtue of the economic and ecological situations that our societies are going through, attend to an activity of primary importance: food.

References

1. Carović-Stanko, K.; Petek, M.; Grdisa, M.; Pintar, J.; Bedekovic, D.; Herak Custic, M.; Satovic, Z. Medicinal plants of the family of Lamiaceae as functional foods - A Review. *Czech J. Food Sci.* **2016**, 34, 377–390. https://doi.org/10.17221/504/2015-CJFS

2. Perrino, E.V.; Valerio, F.; Jallali, S.; Trani, A.; Mezzapesa, G.N. Ecological and Biological Properties of *Satureja cuneifolia* Ten. and *Thymus spinulosus* Ten.: Two Wild Officinal Species of Conservation Concern in Apulia (Italy). A Preliminary Survey. *Plants* **2021**, 10, 1952. https://doi.org/10.3390/plants10091952

3. Císarová, M.; Hleba, L.; Tančinová, D.; Florková, M.; Foltinová, D.; Charousová, I.; Vrbová, K.; Božik, M.; Klouček, P. Inhibitory effect of essential oils from some Lamiaceae species on growth of *Eurotium* spp. isolated from bread. *J. Microbiol. Biotechnol. Food Sci.* **2018**, 8, 857–862. https://doi.org/10.15414/jmbfs.2018.8.2.857-862

4. Skendi, A.; Katsantonis, D.N.; Chatzopoulou, P.; Irakli, M.; Papageorgiou, M. Antifungal Activity of Aromatic Plants of the Lamiaceae Family in Bread. *Foods* **2020**, 9, 1642. https://doi.org/10.3390/foods9111642

5. Valerio, F.; Mezzapesa, G.N.; Ghannouchi, A.; Mondelli, D.; Logrieco, A.F.; Perrino, E.V. Characterization and antimicrobial properties of essential oils from four wild taxa of Lamiaceae family growing in Apulia. *Agronomy* **2021**, 11, 1431. https://doi.org/10.3390/agronomy11071431

6. Bueno, M.; Di Sapio, O.; Barolo, M.; Busilacchi, H.; Quiroga, M.; Severin, C. Análisis de la calidad de los frutos de *Salvia hispanica* L. (Lamiaceae) comercializados en la ciudad de Rosario (Santa Fe, Argentina). *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **2010**, 9, 221–227.

7. Chicco, A.; D'Alessandro, M.; Hein, G.; Oliva, M.; Lombardo, Y. Dietary chia seed (*Salvia hispanica* L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. *The British Journal of Nutrition* **2009**, 101, 41–50. https://doi.org/10.1017/S000711450899053X

8. Imran, M.; Nadeem, M.; Manzoor, M. F.; Javed, A.; Ali, Z.; Akhtar, M. N.; Hussain, Y. Fatty acids characterization, oxidative perspectives and consumer acceptability of oil extracted from pre-treated chia (*Salvia hispanica* L.) seeds. *BioMed Central* **2016**, 15, 1–13. https://doi.org/10.1186/s12944-016-0329-x

9. Capitani, M.; Corzo-Rios, L.; Chel-Guerrero, L.; Betancur-Ancona, D.; Nolasco, S.; Tomás, M. Rheological properties of aqueous dispersions of chia (*Salvia hispanica* L.) mucilage. *Journal of Food Engineering* **2015**, 149, 70–77. https://doi.org/10.1016/j.jfoodeng.2014.09.043

10. Beltrán-Orozco, M.; Romero, M. Chía, alimento milenario. *Revista Industria Alimentaria* **2003**, 22–25.

11. Busilacchi, H.; Bueno, M.; Severin, C.; Di Sapio, O.; Quiroga, M.; Flores, V. Evaluation of *Salvia hispanica* L. cultivated in the south of Santa Fe (Argentina). *Cultivos Tropicales* **2013**, 34, 55–59.

12. Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission. Opinion on the safety of 'Chia seed (*Salvia hispanica*) and ground whole Chia seed' as a food ingredient. *The EFSA Journal* **2009**, 996, 1–26.

13. Peiretti, P.G.; Meineri, G. Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (*Salvia hispanica* L.) seed supplements. *Meat Sci*. **2008**, 80(4):1116-21. https://doi.org/10.1016/j.meatsci.2008.05.003

14. Reyes-Caudillo, E.; Tecante, A.; Valdivia-López, M.A. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chem*. **2008**, 107, 656–663. https://doi.org/10.1016/j.foodchem.2007.08.062

15. Cahill, J.P.; Provance, M.C. Genetics of qualitative traits in domesticated chia (*Salvia hispanica* L.). *J Hered*. **2002**, 93(1):52-5. https://doi.org/10.1093/jhered/93.1.52

16. Mohd Ali, N.; Yeap, S.; Ho, W.; Beh, B.; Tan, S.; Tan, S. The promising future of chia, *Salvia hispanica* L. *J Biomed Biotechnol*. **2012**, 171956. https://doi.org/10.1155/2012/171956

17. Ayerza, R. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. *Journal of the American Oil Chemists Society* **1995**, 72 (9), 1079–1081. https://doi.org/10.1007/BF02660727

18. Ayerza, R.; Coates, W. Composition of chia (*Salvia hispanica*) grown in six tropical and subtropical ecosystems of South America. *Trop. Sci*. **2004**, 44, 131–135. https://doi.org/10.1002/ts.154

19. Coates, W.; Ayerza, R. Production potential of chia in northwestern Argentina. *Industrial Crops and Products* **1996**, 5, 229–233. https://doi.org/10.1016/0926-6690(96)89454-4

20. Heuer, B.; Yaniv, Z.; Ravina, I. Effect of late salinization of chia (*Salvia hispanica*), stock (*Matthiola tricuspidata*) and evening primrose (*Oenothera biennis*) on their oil content and quality. *Ind Crop Prod*. **2002**, 15, 163–167. https://doi.org/10.1016/S0926-6690(01)00107-8

21. EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). Scientific Opinion on the safety of chia seeds (*Salvia hispanica* L.) as a novel food for extended uses pursuant to Regulation (EU) 2015/2283. *EFSA Journal* **2019**, 17(4):5657, 17 pp.

22. Kulczyński, B.; Kobus-Cisowska, J.; Taczanowski, M.; Kmiecik, D.; Gramza-Michałowska A. The chemical composition and nutritional value of chia seeds-current State of Knowledge. *Nutrients* **2019**, 11, 1242. https://doi.org/10.3390/nu11061242

23. Chia seed market – growth, trends and forecast (2020–2025). Available online: www.mordorintelligence.com/industry-reports/chia-seeds-market (accessed on 15 January 2021)

24. Cabrera-Santos, D.; Ordoñez-Salanueva, C.A.; Sampayo-Maldonado, S.; Campos, J.E.; Orozco-Segovia, A.; Flores-Ortiz, C.M. Chia (*Salvia hispanica* L.) Seed Soaking, Germination, and Fatty Acid Behavior at Different Temperatures. *Agriculture* **2021**, 11, 498. https://doi.org/10.3390/agriculture11060498

25. Paiva, E.; Barros-Torres, S.; da Silva, F.; Nogueira, N.; Freitas, R.; Leite, M. Light regime and temperature on seed germination in *Salvia hispanica* L. *Acta Scientiarum. Agronomy* **2016**, 38, 513–519. https://doi.org/10.4025/actasciagron.v38i4.30544

26. Paiva, E.; Barros-Torres, S.; Alves, Costa, T.; da Silva, F.; de Sousa, L.; Dallabona, J. Germination and biochemical components of *Salvia hispanica* L. seeds at different salinity levels and temperatures. *Acta Scientiarum. Agronomy* **2018**, 40, e39396. https://doi.org/10.4025/actasciagron.v40i1.39396

27. Bewley, J.; Bradford, K.; Hilhorst, H.; Nonogaki, H. Seeds: physiology of development, germination and dormancy, 3rd ed.; Springer: Gewerbestr Cham, Switzerland, 2013, pp. 85–246.

28. Benech-Arnold, R.; Sánchez, R.; Forcella, F.; Kruk, B.; Ghersa, C. Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* **2000**, 67, 105–122. https://doi.org/10.1016/S0378-4290(00)00087-3

29. Batlla, D.; Benech-Arnold, R. A framework for the interpretation of temperature effects on dormancy and germination in seed populations showing dormancy. *Seed Science Research* **2015**, 25, 147–158. https://doi.org/10.1017/S0960258514000452

30. Marcos Filho, J. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola* **2015**, 72, 363–374. https://doi.org/10.1590/0103-9016-2015-0007

31. Bewley, J.; Black, M. Seeds: Physiology of Development and Germination 2nd ed.; Springer: Gewerbestr Cham, Switzerland, 1994, pp. 147–197.

32. Hardegree, S.; Van Vactor, S. Predicting germination response of four cool-season range grasses to field-variable temperature regimes. *Environmental and Experimental Botany* **1999**, 41(3), 209–217.

33. Garcia-Huidobro, J.; Monteith, J. L.; Squire, G. R. Time, Temperature and Germination of Pearl Millet (*Pennisetum typhoides* S. & H.): I. Constant temperature, *J. Exp. Bot.*, **1982a**, 33: 288–296. https://doi.org/10.1093/jxb/33.2.288

34. Garcia-Huidobro, J.; Monteith, J.L.; Squire, G.R. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.). II. Alternating temperature. *J. Exp. Bot.*, **1982b**, 33: 297–302. https://doi.org/10.1093/jxb/33.2.297

35. Covell, S.; Ellis, R. H.; Roberts, E. H.; Summerfield, R. J. The Influence of Temperature on Seed Germination Rate in Grain Legumes: I. A comparison of chickpea, lentil, soyabean and cowpea at constant temperatures, *Journal of Experimental Botany*, **1986**, 37(5): 705–715. https://doi.org/10.1093/jxb/37.5.705

36. Ellis, R.H.; Butcher, P.D. The effects of priming and natural differences in quality amongst onion seed lots on the response of the rate of germination to temperature and the identification of the characteristics under genotypic control, *Journal of Experimental Botany*, **1988**, 39: 935–950. https://doi.org/10.1093/jxb/39.7.935

37. Roberts, E.H. Temperature and seed germination. In: Long SP, Woodward FI, eds. Plants and temperature. Cambridge: Society of Experimental Biology, Company of Biologists, 1988, 109–132.

38. Benech-Arnold, R.L.; Ghersa, C.M.; Sanchez, R.A.; Insausti, P. Temperature effects on dormancy release and germination rate in *Sorghum halepense* (L.) Pers. seeds: a quantitative analysis. *Weed Research*, **1990**, 30: 81–89. https://doi.org/10.1111/j.1365-3180.1990.tb01690.x

39. Probert, R.J. The role of temperature in germination ecophysiology. In: Fenner M, ed. Seeds: the ecology of regeneration in plant communities. Wallingford, Oxon: CAB International, 1992, 285–325.

40. Alvarado, V.; Bradford, K.J. A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant, Cell and Environment*, **2002**, 25: 1061–1069. https://doi.org/10.1046/j.1365-3040.2002.00894.x

41. Ayerza, R.; Coates, W. Influence of environment on growing period and yield, protein, oil and α-linolenic content of three chia (*Salvia hispanica* L.) selections. *Industrial Crops and Products* **2009**, 30, 321–324. https://doi.org/10.1016/j.indcrop.2009.03.009

42. Lobo, R.; Alcocer, M.; Fuentes, F.; Rodríguez, W.; Morandini, M.; Devani, M. Desarrollo del cultivo de chía en Tucumán, República Argentina. *EEAOC-Avance Agroindustrial* **2011**, 32:27-30.

43. Jensen, E.; Robson, P.; Norris, J.; Cookson, A.; Farrar, K.; Donnison, I.; Clifton-Brown, J. Flowering induction in the bioenergy grass *Miscanthus sacchariflorus* is a quantitative short-day response, whilst delayed flowering under long days increases biomass accumulation. *Journal of Experimental Botany* **2013**, 64:541-552. https://doi.org/10.1093/jxb/ers346

44. Baginsky, C.; Arenas, J.; Escobar, H.; Garrido, M.; Valero, N.; Tello, D., Pizarro, L.; Valenzuela, A.; Morales, L.; Silva, H. Growth and yield of chia (*Salvia hispanica* L.) in the Mediterranean and desert climates of Chile. *Chilean Journal of Agricultural Research* **2016**, 76(3): 255–264. http://dx.doi.org/10.4067/S0718-58392016000300001

45. Jamboonsri, W.; Phillips, T.; Geneve, R.; Cahill, J.; Hildebrand, D. Extending the range of an ancient crop, *Salvia hispanica* L. - a new ω3 source. *Genetic Resources and Crop Evolution* **2012**, 59, 171–178. https://doi.org/10.1007/s10722-011-9673-x

46. Grimes, S.J.; Phillips, T.D.; Hahn, V.; Capezzone, F.; Graeff-Hönninger, S. Growth, Yield Performance and Quality Parameters of Three Early Flowering Chia (*Salvia hispanica* L.) Genotypes Cultivated in Southwestern Germany. *Agriculture* **2018**, 8, 154. https://doi.org/10.3390/agriculture8100154

47. Pereira, M.D.; Dias, D.C.; Borges, E.E.; Martins Filho, S.; Dias, L.A.; Soriano, P.E. Physiological Quality of Physic Nut (*Jatropha curcas* L.) seeds during storage. *Journal of Seed Science* **2013**, 35: 21-27. https://doi.org/10.1590/S2317-15372013000100003

48. Marcos-Filho; J. Seed Physiology of Cultivated Plants. 2nd Edition, ABRATES, Londrina, 2015, 660 p.

49. José, S.C., Salomão, A.N.; Costa, T.S.; Silva, J.T.; Curi, C.C. Armazenamento de sementes de girassol em temperaturas subzero: Aspectos fisiológicos e bioquímicos. *Revista Brasileira de Sementes* **2010**, 32, 29-38. https://doi.org/10.1590/S0101-31222010000400004

50. Hashemi, A.; Estilai, A. Seed germination response of golden chia (*Salvia columbariae* Benth.) to low temperature and gibberellin. *Industrial Crops and Products* **1994**, 2(2): 107–109. https://doi.org/10.1016/0926-6690(94)90091-4

51. Dashti, M.; Kafi, M.; Tavakkoli, H.; Mirza, M. Cardinal temperatures for germination of *Salvia leriifolia* Benth. *Herba Polonica.*, **2015**, 61: 5-18. https://doi.org/10.1515/hepo-2015-0006

52. Nadjafi, F.; Tabrizi, L.; Shabahang, J.; Mahdavi Damghani, A. M. Cardinal germination temperatures of some medicinal plant species. *Seed Technology* **2009**, 31(2): 156-63.

53. Nadtochii, L.; Kuznetcova, D.; Proskura, A.; Apalko, A.; Nazarova, V.; Srinivasan, M. Investigation of various factors on the germination of chia seeds sprouts (*Salvia hispanica* L.). *Agronomy Research* **2019**, 17, 1390–1400. https://doi.org/10.15159/ar.19.128

54. Adam, N.R.; Dierig, D.A.; Coffelt, T.A.; Wintermeyer, M.J.; Mackeya, B.E.; Wall, G.W. Cardinal temperatures for germination and early growth of two Lesquerella species. *Ind Crop Prod* **2007**, 25: 24-33. https://doi.org/10.1016/j.indcrop.2006.06.001

55. Iannucci, A.; Di Fonzo, N.; Martiniello, P. Temperature requirements for seed germination in four annual clovers grown under two irrigation treatments. *Seed Sci Technol* **2000**, 28: 59-66.

56. Tabrizi, L.; Nassiri Mahallati, M.; Koocheki, A. Investigations on the cardinal temperatures for germination of *Plantago ovata* and *Plantago psyllium*. *J Iran Field Crop Res* **2004**, 2(2): 143-50. https://doi.org/10.22067/GSC.V2I2.1248

57. Bannayan, M.; Nadjafi, F.; Rastgoo, M.; Tabrizi, L. Germination properties of some wild medicinal plants from Iran. *Seed Technology* **2006**, 28(1): 80-86.

58. Hardegree, S.P. Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Ann Bot*. **2006**, 97(6): 1115-1125. https://doi.org/10.1093/aob/mcl071

59. Hills, P.N.; Staden, J. Thermoinhibition of seed germination. South African Journal of Botany. **2003**, 69. 455-461. https://doi.org/10.1016/S0254-6299(15)30281-7

60. Tulukcu, E.; Yalcin, H.; Ozturk, I.; Sagdic, O. Changes in the fatty acid compositions and bioactivities of clary sage seeds depending on harvest year. *Industrial Crops and Products* **2012**, 39: 69–73. https://doi.org/10.1016/j.indcrop.2012.02.012

61. Habibvash, F.N.; Rajamand, M.A.; Heidari, R.; Sarghein, S.H.; Ricani, M.H. Chemical analysis of some Salvia species native to West Azarbaijan (Iran). *Pak J Biol Sci*. **2007**, 10(20): 3516-3524. https://doi.org/10.3923/pjbs.2007.3516.3524

62. Baskin, C.; Baskin, J.M. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego CA, USA, **2014**; pp 150–162.

63. Estrelles, E.; Albert, F.; Navarro, A.; Prieto, J.; Ibars, A.M. Germination behavior of Labiatae SW distributed in the Iberian Peninsula. In: Proceedings of the 4to European conference on the conservation of wild plants. Planta Europa. Valencia, 1999.

64. Derakhshan, A.; Gherekhloo, J.; Vidal, R.; De Prado, R. Quantitative description of the germination of Littleseed Canarygrass (*Phalaris minor*) in response to temperature. *Weed Science* **2014**, 62(2): 250-257. https://doi.org/10.1614/WS-D-13-00055.1

65. Parmoon, G.; Moosavi, S. A.; Akbari, H.; Ebadi, A. Quantifying cardinal temperatures and thermal time required for germination of *Silybum marianum* seed. *The Crop Journal* **2015**, 3(2): 145–151. https://doi.org/10.1016/j.cj.2014.11.003

66. Zhao, M.; Zhang, H.; Yan, H.; Qiu, L.; Baskin, C. C. Mobilization and Role of Starch, Protein, and Fat Reserves during Seed Germination of Six Wild Grassland Species. *Frontiers in Plant Science* **2018**, 9. https://doi.org/10.3389/fpls.2018.00234

67. Thanos, C.A.; Doussi, M.A. Ecophysiology of seed germination in endemic Labiates of Crete. *Isr J Plant Sci* **1995**; 43: 227-37. https://doi.org/10.1080/07929978.1995.10676607

68. Côme D. Germination of seeds of some aromatic plants. In: Panetsos K, Nikolaidis A. Lyrintzis G., Identification, preservation, adaptation, and cultivation of selected aromatic and medicinal plants suitable for marginal lands of the mediterranean region. Mediterranean Agronomic Institute of Chania, 1993: 111-26.

69. Hornok, L. Cultivation and processing of medicinal plants. John Wiley and Sons Publication, 1992.

70. Copeland, L.O.; McDonald, M.B. Principles of seed science and technology. Chapman and Hall Publication, 1995.

71. Tosif, M. M.; Najda, A.; Bains, A.; Kaushik, R.; Dhull, S. B.; Chawla, P.; Walasek-Janusz, M. A Comprehensive Review on Plant-Derived Mucilage: Characterization, Functional Properties, Applications, and Its Utilization for Nanocarrier Fabrication. *Polymers* **2021**, 13(7): 1066. https://doi.org/10.3390/polym13071066

72. Francoz, E.; Ranocha, P.; Burlat, V.; Dunand, C. *Arabidopsis* seed mucilage secretory cells: regulation and dynamics. *Trends Plant Sci* **2015**, 20: 515–24. https://doi.org/10.1016/j.tplants.2015.04.008

73. Haughn, G.W.; Western, T.L. *Arabidopsis* Seed Coat Mucilage is a specialized cell wall that can be used as a model for genetic analysis of plant cell wall structure and function. *Front Plant Sci* **2012**, 3:64. https://doi.org/10.3389/fpls.2012.00064

74. Western, T.L. The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. *Seed Sci Res* **2012**, 22: 1–25. https://doi.org/10.1017/S0960258511000249

75. Ma, Z.; Marsolais, F.; Bykova, N. V.; Igamberdiev, A. U. Nitric oxide and reactive oxygen species mediate metabolic changes in barley seed embryo during germination. *Front. Plant Sci.* **2016**, 7, 138. https://doi.org/10.3389/fpls.2016.00138

76. Soltani, A.; Robertson, M. J.; Torabi, B.; Yousefi-Daz, M.; Sarparast, R. Modelling seedling emergence in chickpea as influenced by temperature and sowing depth. *Agricultural and Forest Meteorology* **2006**, 138: 156–167. https://doi.org/10.1016/j.agrformet.2006.04.004

77. Craufurd, P.Q.; Qi, A.; Ellis, R.H.; Summerfield, R.J.; Roberts, E.H.; Mahalakshmi, V. Effect of temperature on time to panicle initiation and leaf appearance in sorghum. Crop Sci. **1998**, 38: 942–947. https://doi.org/10.2135/cropsci1998.0011183X003800040011x

78. Olsen, J.K.; McMahan, C.R.; Hammer, G.L. Prediction of sweet corn phenology in subtropical environments. *Agron. J.* **1993**, 85: 410–415. https://doi.org/10.2134/agronj1993.00021962008500020044x

79. Mwale, S.S.; Azam-Ali, S.N.; Clark, J.A.; Bradley, R.G.; Chatha, M.R. Effect of temperature on germination of sunflower. *Seed Sci. Technol*. **1994**, 22: 565–571.

80. Robertson, M.J.; Carberry, P.S.; Huth, N.I.; Turpin, J.E.; Probert, M.E.; Poulton, P.L.; Bell, M.; Wright, G.C.; Yeates, S.J.; Brinsmead, R.B. Simulation of growth and development of diverse legume species in APSIM. *Aust. J. Agric. Res*. **2002**, 53: 429–446. https://doi.org/10.1071/AR01106

81. Yin, X.; Kropff, M.J.; McLaren, G.; Visperas, R.M. A non- linear model for crop development as a function of temperature. *Agric. For. Meteorol*. **1995**, 77: 1–16. https://doi.org/10.1016/0168-1923(95)02236-Q

82. Kamkar, B.; Ahmadi, M.; Soltani, A.; Zeinali E. Evaluating Non-Linear regression models to describe response of wheat emergence rate to temperature. *Seed Science and Biotechnology* **2008**, 2:53–57.

83. Muñoz, L.; Cobos, A.; Diaz, O.; Aguilera, J. Chia seeds: microstructure, mucilage extraction and hydration. *J. Food Eng*. **2012**, 108, 216–224. https://doi.org/10.1016/j.jfoodeng.2011.06.037

84. Salgado-Cruz, M. Chanona-Pérez, J.; Farrera-Rebollo, R. Méndez-Méndez, J. Díaz-Ramírez, M. Chia (*Salvia hispanica* L.) seed mucilage release characterization. A microstructural and image analysis study. *Industrial Crops and Products* **2013**, 51, 453–462. https://doi.org/10.1016/j.indcrop.2013.09.036

85. Sampayo-Maldonado, S.; Ordoñez-Salanueva, C.A.; Mattana, E.; Ulian, T.; Way, M.; Castillo-Lorenzo, E.; Dávila-Aranda, P.D.; Lira-Saade, R.; Téllez-Valdéz, O.; Rodriguez-Arevalo, N.I.; Flores-Ortíz, C.M. Thermal Time and Cardinal Temperatures for Germination of *Cedrela odorata* L. *Forest* **2019**, 10, 41. https://doi.org/10.3390/f10100841

86. International Seed Testing Association - ISTA. International Rules for Seed Testing, 2020 ed.; ISTA: Bassersdorf, Switzerland, 2020, pp. 1–4.

87. Maguire, J. D. Speed of Germination—Aid In Selection And Evaluation for Seedling Emergence And Vigor. *Crop Science* **1962**, 2, 176–177. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

88. Ellis, R. H.; Covell, S.; Roberts, E. H.; Summerfield R. J. The Influence of Temperature on Seed Germination Rate in Grain Legumes: II. INTRASPECIFIC VARIATION IN CHICKPEA (*Cicer Arietinum* L.) AT CONSTANT TEMPERATURES. *Journal of Experimental Botany* **1986**, 183: 1503-515. https://doi.org/10.1093/jxb/37.10.1503

89. Reyes-Ortega. Modelo de la respuesta germinativa de diferentes poblaciones de Wigandia urens (Ruiz et Pav.) Kunth en un gradiente de temperaturas constantes. Master's degree thesis, Facultad de Ciencias, Universidad Nacional Autónoma de Mexico, Mexico, 2001.

90. Timmermans, B.G.; Vos, J.; Van Nieuwburg, J.; Stomph, T.J.; Van der Putten, P.E. Germination rates of *Solanum sisymbriifolium*: temperature response models, effects of temperature fluctuations and soil water potential, *Seed Sci. Res*. **2007**, 17: 221. https://doi.org/10.1017/S0960258507785628

Discusión y conclusiones

Salvia (del latin "salvare", es decir, "curar"), es el género más grande de la familia *Lamiaceae* con alrededor de 800 especies en todo el mundo (Hao *et al*., 2015). La mayoría de las plantas de este género son bien conocidas por sus componentes biológicamente activos (Hao *et al*., 2015; Kulczyński *et al*., 2019). Los miembros de este género, colectivamente nombrados chía, desempeñan un papel vital en la medicina tradicional. En este tema se conoce que algunas especies del género poseen actividad farmacológica significativa en el tratamiento de distintas enfermedades (Vuksan *et al*., 2007; Fernandez *et al*., 2008; Ho *et al*., 2013; Rossi *et al*., 2013; Marineli *et al*., 2015; Sierra *et al*., 2015; Silva *et al*., 2016; Chicco *et al*., 2019; Fonte-Faria *et al*., 2019; Kulczyński *et al*., 2019). En este mismo contexto es importante mencionar que México cuenta con alrededor de 328 especies pertenecientes al género *Salvia*, siendo el género con el mayor número de especies vasculares del país (Villaseñor, 2016).

Este trabajo gira en torno a *Salvia hispanica* L., especie nativa del centro de México hasta el norte de Guatemala, empleada desde la época del Cem Anáhuac (verdadero nombre de la extensión territorial que abarcaba el continente antes de la conquista y posteriormente nombrado América) hacia el 3500 a. C. por sus características nutritivas (altos niveles de proteína, antioxidantes, fibra dietética, vitaminas y minerales), pero sobre todo a su alto contenido de ácidos grasos omega 3 (ácido α-linolénico) (Guiotto *et al*., 2013). Aztecas, mayas e incas usaban las semillas para la preparación de diversas medicinas, alimentos y pinturas. Al ser una especie oleaginosa, las semillas de chía se postulan como un modelo atractivo para el estudio del metabolismo de los ácidos grasos durante la germinación; además, al tratarse de una especie mixospérmica, es decir, que produce mucílago, el

comportamiento germinativo de la chía es de particular interés debido al papel ecológico que dicha matriz polimérica ejerce sobre la germinación (retención de humedad, cimentación al sustrato y dispersión, entre otras funciones).

Existen trabajos que por separado han abordado la respuesta de la temperatura sobre la imbibición, germinación y el metabolismo de los ácidos grasos en otras especies de importancia agrícola (Abdallah *et al*., 1998; Borges *et al*., 2007; Thomas *et al*., 2003; Wakjira *et al*., 2004); sin embargo, hasta ahora estos temas no habían sido tratados en conjunto, estableciendo una relación entre la temperatura, imbibición, germinación y el metabolismo de ácidos grasos, concomitantemente con la determinación de las temperaturas cardinales de una especie oleaginosa como *S. hispanica* L.

Imbibición

El curso de la imbibición se puede dividir en tres distintas fases con respecto a la toma de agua: FI, FII y FIII; donde FI se caracteriza por una toma rápida de agua, FII por ser una fase sin cambios significativos en el peso y finalmente FIII, donde se observa una nueva toma de agua que solo experimentan aquellas semillas que ya han completado la germinación. Durante nuestros ensayos de imbibición, el último aumento de peso correspondiente a FIII se observó después de 3–4 horas, posteriormente se observó la pérdida de peso relacionada con la pérdida de mucílago, esta disminución de peso enmascaró el inicio de FIII. Debido a que FIII no se distinguió claramente, el lapso que comprendió el último aumento de peso y el punto donde inició su pérdida se consideró como una extensión de FII y se denominó FII_{end}.

Durante la imbibición de semillas de chía a 10, 20 y 30 °C, FI ocurrió dentro de la primera hora en todos los tratamientos; mientras que FII y FII$_{end}$ transcurrieron con una hora de diferencia a 20 °C y 30 °C. Esta diferencia en el tiempo que toma alcanzar el umbral máximo de peso/humedad se puede asociar con un FII más corta y consecuentemente, con una germinación más temprana a 30 °C. En este contexto, se ha sugerido que, a temperaturas subóptimas, se producen cambios en la configuración de las membranas, afectando la retención de solutos, incluidos azúcares, ácidos orgánicos, iones, aminoácidos y proteínas, afectando la eficiencia de la germinación (Benech-Arnold *et al*., 2000; Batlla *et al*., 2015); además, las tasas de las reacciones metabólicas subyacentes a la germinación se ven afectadas por la temperatura (Marcos-Filho, 2015). En otros ensayos de imbibición con semillas de chía (Ayerza & Coates, 2009; Muñoz *et al*., 2012; Geneve *et al*., 2019; Nayani & Rao, 2020), se observó que éstas alcanzan un peso constante entre 2–4 horas a temperaturas que oscilan entre 20–28 °C, un comportamiento similar se observó en algunos miembros del género *Plantago*, cuyas semillas se usan comercialmente para la producción de mucílago (Singh *et al*., 2006; 2007).

Germinación

Durante nuestros ensayos a 10, 20 y 30 °C observamos una germinación final superior al 80%; mientras que en los ensayos con el gradiente que fue de los 10 °C a los 45 °C, la germinación final del rango entre los 10–35 °C fue superior al 88%; mientras que de 40–45 °C fue menor al 50%. En otro trabajo también se observó una mayor germinación final a una temperatura constante de 25 °C y a temperaturas alternas de 25–30 °C, con respecto a tratamientos conducidos a 20 °C (Paiva *et al*., 2016; 2018). Anteriormente se ha reportado que la chía crece a una temperatura mínima de 11 °C y una máxima de 36 °C, con una

temperatura óptima entre 16–26 °C (Ayerza & Coates, 2009); sin embargo, en nuestros ensayos observamos que la chía puede germinar a temperaturas más bajas, alcanzando un porcentaje de germinación final superior al 95% a 10 °C.

Durante nuestros ensayos a 10, 20 y 30 °C, la velocidad de germinación (GR) más alta se observó a 30 °C con 18.5 semillas d^{-1} y la más baja a 10 °C con 4 semillas d^{-1}; mientras que en los ensayos conducidos de 10–45 °C fue posible distinguir tres grupos diferentes de acuerdo con su GR: 10–20 ° C, 25–35 °C y 40–45 °C. En el rango de 10–20 °C se observó un aumento gradual de la GR, llegando a una meseta dentro de los 25–35 °C, donde no se observaron diferencias significativas entre tratamientos. Posteriormente, se observó una disminución en el rango de 40–45 °C. La GR más alta se observó a 30 °C con 22 semillas por día y la más baja a 45 °C con aproximadamente 2 semillas por día.

En conjunto, la GR observada en nuestro análisis fue 1.4 y 2.2 veces mayor que la reportada previamente por Nadtochii *et al*. (2019) para 25 °C y 30 °C; mientras que, a 20 °C, la GR fue similar entre ambos trabajos. En este sentido se ha demostrado que la GR difiere entre especies, así como entre lotes de semillas de la misma especie (Adam *et al*., 2007). Varios reportes han demostrado que el aumento de temperatura hacia el intervalo óptimo conlleva un aumento en la GR, y posteriormente disminuye a medida que aumenta la temperatura (Alvarado & Bradford, 2002; Iannucci *et al*., 2000; Tabrizi *et al*., 2004; Bannayan *et al*., 2006). De manera importante, Hardegree (2006) demostró que existían errores en la predicción de la emergencia de las plántulas a principios de la primavera debido a la degradación de las semillas y la reducción de la GR a altas temperaturas. *S. hispanica* L., por su calidad y cantidad de aceite, tiene un mejor desempeño en su germinación a bajas y altas temperaturas, donde una alta proporción de ácidos grasos polinsaturados ayuda a mantener

la fluidez de las membranas celulares, esto concuerda con nuestra observación de que la chía, luego de una fase de retraso a 10 °C (cuatro días) alcanza una germinación final por arriba del 95%, en contraste con *S. sclarea* y *S. nemorosa*, que alcanzaban menos del 50% a temperaturas inferiores a 10 °C, y que a temperaturas superiores a 40 ° C la germinación se reduce significativamente, sin llegar a inhibirse por completo (Nadjafi *et al*., 2009). En esto contexto, de acuerdo con Neffati (1994), la variación en la temperatura óptima depende de las especies consideradas en los estudios mencionados, aunque para la mayoría de las especies, su germinación acontece en un rango amplio de temperaturas, aunque alrededor de los 20 °C parece optimizar su germinación. Esta variación en la temperatura óptima y la tasa de germinación entre especies constituye algunas estrategias de adaptación a diferentes condiciones ambientales. Por ejemplo, se ha demostrado que la temperatura por encima del óptimo provoca una inhibición de la germinación y un daño irreversible (Gorai *et al*., 2006; Gorai y Neffati, 2007); este fenómeno se ha observado en otro estudio con semillas de *S. officinalis*, las cuales no germinaron a 40 °C, valor que se consideró como el límite superior de temperatura a la que dicha especie germina (Oberczian y Bernath, 1988).

En conjunto, nuestros resultados indican un rango óptimo de temperatura que va de los 25– 35 ° C, con una GR y t_{50} máximos observados a 30 °C, este rango óptimo es casi diez grados por arriba del rango reportado (Ayerza & Coates, 2009), esta evidencia concuerda con lo observado por Paiva *et al*. (Paiva *et al*., 2016; 2018). Otros estudios apoyan la influencia de la temperatura como el principal indicador asociado con la germinación de la semilla de chía (Paiva *et al*., 2016; 2018; Gómez-Favela *et al*., 2017; Nadtochii *et al*., 2019; Stefanello *et al*., 2015a; 2015b; Possenti *et al*., 2016); por ejemplo, se ha observado que la germinación de

semillas de chía a baja temperatura (por debajo de 20 °C) alta temperatura (por encima de 30 °C) limita el crecimiento y desarrollo de las plantas.

El comportamiento germinativo está intrínsecamente relacionado con las condiciones ecológicas de los hábitats naturales y el origen biogeográfico de las especies. La mayoría de las especies que se encuentran naturalmente en climas áridos y templados tienen el potencial de germinar bien a temperaturas que oscilan entre 15 y 30 °C, lo que indica una preferencia por las temperaturas moderadas-altas para la germinación. En este tema, existe evidencia que muestra que temperaturas que oscilan entre 15 °C y 25 °C conducen a una germinación máxima a especies características de ambientes áridos como *Lavandula dentata* L., *Teucrium gnaphalodes* L'Hér., *Thymbra capitata* (L.) Cav., y *Thymus hyemalis* L.; mientras que las temperaturas más altas limitan su germinación y crecimiento (Estrelles *et al*., 1999). Por lo tanto, no es de extrañar que temperaturas que oscilan entre 10–20 °C y 40–45 °C afecten el crecimiento y rendimiento de la chía, considerando que esta especie está adaptada a temperaturas que fluctúan entre 11 y 36 °C (Ayerza & Coates, 2009).

Aunque algunas diferencias en los patrones de germinación de la chía pueden explicarse por las características adquiridas por los cultivares a lo largo de su proceso de domesticación (Bewley *et al*., 2013), las diferencias en la germinación de las variedades generadas por domesticación se asocian principalmente con diferentes capacidades para germinar y crecer en diferentes condiciones climáticas, como aquellas variedades producidas para crecer durante los días largos del hemisferio norte (Jamboonsri *et al*., 2012; Grimes *et al*., 2018). Otra explicación está relacionada con las condiciones de almacenamiento, donde ya se sabe que la humedad y las altas temperaturas reducen la viabilidad y germinación de las semillas (Bewley *et al*., 2013). Este proceso de deterioro está relacionado con la composición química

de las semillas (Pereira *et al*., 2013) y está principalmente asociado a factores como el contenido de agua, condiciones ambientales, empaquetamiento y microorganismos, entre otros (Marcos-Filho, 2015). Los síntomas fisiológicos más evidentes del deterioro de las semillas aparecen durante la germinación y el desarrollo inicial de las plántulas debido a la alteración del sistema de membranas como consecuencia del ataque de los radicales libres sobre los compuestos químicos de la membrana (José *et al*., 2010).

En nuestros ensayos observamos un retraso en la germinación a 10 °C, este retrasó sugiere que es necesario más tiempo para acumular suficientes grados día (°Cd) para completar la germinación, como se ha observado para la floración de la chía y otras especies de día corto emparentadas (Lobo *et al*., 2011; Jensen *et al*., 2013; Baginsky *et al*., 2016). En este tema Bita & Gerats (2013) sugieren que a bajas temperaturas las tasas metabólicas se reducen y el proceso de crecimiento se ve afectado desde la germinación hasta la etapa de plántula. Otra explicación surge de la evidencia con las especies mixospérmicas *Lavandula subnuda* (*Lamiaceae*) y *Plantago ciliate* (*Plantaginaceae*), donde la presencia de mucílago aumentó la absorción de humedad e inhibió la germinación a temperaturas bajas (temperaturas noche/día de 15/25 °C). En este contexto se ha sugerido que el mucílago inhibe la germinación en condiciones de humedad excesiva al disminuir la difusión de oxígeno al embrión (Western, 2012). Durante la germinación, el agotamiento progresivo del oxígeno genera condiciones de anaerobiosis parcial, favoreciendo la activación de rutas respiro-fermentativas como principal fuente de ATP celular, conduciendo a la reducción de compuestos acarreadores de electrones como NAD y NADP, lo que a su vez conduce a la formación y acumulación de especies reactivas de oxígeno (ROS) (Ma *et al*., 2016). El hecho de que la chía germine satisfactoriamente en todos nuestros tratamientos refleja su potencial

resistencia a condiciones ambientales adversas y, por lo tanto, que el retraso en la germinación a temperaturas bajas se relacione también con la reducción en los flujos metabólicos a temperaturas subóptimas.

Análisis de ácidos grasos

El aceite de la semilla de chía ha sido ampliamente estudiado en relación con su calidad y altos niveles de ácidos grasos polinsaturados (PUFAs). Aunque la forma más común de consumir las semillas chía es de forma hidratada, a la fecha se han realizado pocos trabajos con semillas durante su imbibición; por ejemplo, Zare *et al*. (2019) observaron que las concentraciones de ácidos oleico, linoleico y linolénico de semillas imbibidas en agua durante 24 horas a 23 °C, fueron de $\geq$7, $\geq$10 y $\geq$32 mg/g de semillas, respectivamente; durante nuestros ensayos, observamos concentraciones similares para los ácidos linoleico y linolénico. Aunque las concentraciones observadas son similares y concuerdan con el 50-67% reportado en la literatura para los ácidos grasos ω-3 (Ayerza & Coates, 2011; Segura-Campos *et al*., 2014), las concentraciones en nuestros análisis se observaron aproximadamente 20 horas antes que las observadas por Zare *et al*., (2019). Estas diferencias en las concentraciones se pueden atribuir al método de extracción, variedad de chía y condiciones de almacenamiento (Reyes-Caudillo et al., 2008; Ayerza & Coates, 2011; Segura-Campos *et al*., 2014; Silva *et al.*, 2016; Dąbrowski *et al*., 2017; Bodoira *et al*., 2017). Por ejemplo, se ha observado que el agua mejora la extractabilidad de los ácidos grasos debido al debilitamiento de la pared celular, y por lo tanto, la accesibilidad de los cuerpos oleosos al solvente de extracción (Zare *et al*., 2019).

De acuerdo con lo anterior, durante nuestros ensayos a 30 °C, el peso máximo debido a la absorción de agua se alcanzó a las 3 horas de imbibición, al mismo tiempo que se observó un aumento en la concentración en todos los ácidos grasos. A 10 °C el peso y la concentración máximos de ácido palmítico (P), esteárico (S), linoleico (L) y linolénico (Ln) se alcanzaron a las 4 horas de imbibición. De igual manera a 20 °C, el peso máximo se alcanzó a las 4 horas, sin embargo, únicamente S y Ln alcanzaron una concentración máxima; mientras que las concentraciones máximas de P, O y L se alcanzaron durante las dos primeras horas de imbibición. También se ha sugerido que diferencias en el rendimiento del aceite podrían estar relacionadas con diferencias en las condiciones climáticas, prácticas agronómicas, regímenes de fertilización y prácticas de riego, como se ha demostrado para otros cultivos oleaginosos tradicionales (Hocking *et al.*, 1997; Vegan & Chapman 2001).

En todos los ácidos grasos, se observaron dos máximos de concentración, el primero ocurrió durante FI y el segundo ocurrió desde la mitad de FII_{end} hasta su final. A 10 °C las concentraciones máximas de P, S, L y Ln se observaron a las 0 horas y posteriormente a las 4 horas de imbibición. A 20 °C las concentraciones máximas de P, S y Ln se observaron después de 1 hora y a las 4 horas de imbibición. Finalmente, a 30 °C se observó una concentración máxima de P después de 4 horas de imbibición y de S, O, L y Ln después de 3 horas. De los tres tratamientos (10, 20 y 30 °C) las concentraciones más altas de P, S, L y Ln se observaron a 20 °C.

A 20 °C, el aumento observado al final de FI en las concentraciones de todos los ácidos grasos, excepto O, podría explicarse por la síntesis *de novo* de ácidos grasos durante la germinación temprana, ya que existe evidencia de que en semillas de *Pisum sativum*, después de una breve fase de retraso, la incorporación de los lípidos marcados isotópicamente

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procedió linealmente, siendo el ácido palmítico y el ácido esteárico los primeros en ser sintetizados, seguido por la síntesis de ácidos grasos saturados de cadena larga (Harwood & Stumpf, 1970). Esta evidencia sugiere que las enzimas para la síntesis de ácidos grasos ya están presentes en semillas secas y participan en la síntesis de ácidos grasos una vez que se alcanza un contenido crítico de agua; por lo tanto, a 20 °C, el umbral de humedad se alcanza durante la primera hora de imbibición. La síntesis de ácidos grasos durante FI también se vería favorecida por su conversión en sacarosa (Pritchard *et al*., 2002; Rosental *et al*., 2014) y su uso para la obtención de energía a través del ciclo de los TCA durante la siguiente fase. Los lípidos son los principales compuestos energéticos de reserva para el embrión en los cultivos oleaginosos; por lo tanto, nuestros resultados concuerdan con estas evidencias.

También a 20 °C observamos una disminución en la concentración de todos los ácidos grasos después de 3 horas de imbibición y posteriormente, a las 4 horas, solo S y Ln experimentaron un aumento en su concentración. Parte del aumento en la concentración de estos ácidos grasos se puede explicar por su uso como reserva de energía y movilización de nutrientes en semillas metabólicamente activas durante FII, mientras que su posterior aumento, durante el FII_{end}, se debe a la síntesis de nuevos nutrientes y solutos que subyacen en esta fase de germinación. De manera importante, se ha observado una correlación negativa entre el contenido de ácido α-linolénico y los ácidos grasos 18-C más saturados, oleico y linoleico, en almendras (Abdallah *et al*., 1998), castañas (Borges *et al*., 2007), soja (Thomas *et al*., 2003), linaza (Wakjira *et al*., 2004) y chía (Ayerza, 2009). Esta relación inversa está respaldada por la biosíntesis del ácido α-linolénico a través del proceso de desaturación del ácido esteárico (Nakamura & Nara 2004; Lee *et al*., 2016) y oleico (Dybing & Zimmerman, 1966; Lee *et al*., 2016), a través del ácido linoleico mediante la actividad específica de las enzimas

desaturasas, parte del aumento observado en la concentración de Ln podría explicarse por este proceso metabólico.

A 30 °C, temperatura en que se observó una GR mayor y un t_{50} menor, también se observaron patrones diferentes para los ácidos grasos saturados e insaturados. El aumento en la concentración de P, S y O entre las 0–3 horas de imbibición y una concentración constante en L y Ln a lo largo de las siguientes 5 horas de imbibición, podría estar relacionado con una mayor eficiencia de germinación (Benech-Arnold et al., 2000; Batlla & Benech-Arnold, 2007). Esta concentración constante de L y Ln a lo largo de cinco horas de imbibición también sugiere un equilibrio entre su descomposición y síntesis. Este equilibrio podría estar relacionado con su uso continuo para el mantenimiento de la permeabilidad y la actividad de las enzimas asociadas a la membrana (Linder, 2000; Larkindale & Huang, 2004), como aquellas que participan en sistemas metabólicos importantes, como en la cadena transportadora de electrones (Hazel, 1995; Denich *et al*., 2003).

El conjunto de mecanismos desarrollados para cambiar la composición de la membrana celular durante el mantenimiento de la fluidez y la funcionalidad de la membrana celular en respuesta a los cambios en las condiciones ambientales se conoce como adaptación homeoviscosa (Sinensky, 1974). La fluidez de las membranas es dependiente del perfil de ácidos grasos de los lípidos que la componen; en este sentido se conoce que los ácidos grasos saturados como P, S y O se encuentran en estado sólido a temperaturas bajas con respecto a los ácidos grasos insaturados, como es el caso de L y Ln; por lo tanto, aumentar el número de insaturaciones, incrementa la fluidez membranal (Izquierdo *et al*., 2017). También existe evidencia que apoya la idea de que el aumento en el nivel de ácidos grasos polinsaturados puede mejorar el rendimiento de las semillas a bajas temperaturas (Oteng & Kersten, 2020).

Por otra parte, a temperaturas altas, los ácidos grasos saturados aumentan la temperatura de fusión de los lípidos y evitan el aumento en la fluidez de la membrana inducido por la temperatura, modulando su metabolismo en respuesta a su incremento (Zhang *et al*., 2005).

Aunque la síntesis de ácidos grasos durante la germinación está asociada a la funcionalidad de las membranas celulares y en última instancia, a la germinación, los posibles efectos de los ácidos grasos que componen los lípidos de reserva sobre la germinación a diferentes temperaturas permanecen casi completamente inexplorados. Los posibles mecanismos implicados en estas respuestas incluyen variaciones en la funcionalidad de la membrana y la degradación de los lípidos de reserva durante la germinación (Izquierdo *et al*., 2017).

Adicionalmente, se identificaron tres isómeros de Ln por su posición y configuración de doble enlace: *6Z, 9Z, 12Z* (ácido *γ*-linolénico); *9Z, 12E, 15Z* y *6Z, 9Z, 11E*. Estos isómeros *trans* se detectaron a 20 y 30 °C; sin embargo, solo a 30 ° C se detectaron isómeros en todas las réplicas. La concentración máxima de isómeros se observó después de 3–4 horas de imbibición, es decir, durante FII$_{end}$, momento en el que se observó una disminución de Ln.

El aumento de isómeros *trans* del ácido linolénico observados a 30 °C se puede asociar principalmente con la defensa celular contra el estrés oxidativo (Ferreri & Chatgilialoglu 2012; Jouhet, 2013). Las plantas superiores expuestas a un exceso de calor, al menos 5 °C por arriba de sus condiciones óptimas de crecimiento, exhiben un conjunto característico de respuestas celulares y metabólicas necesarias para que las plantas sobrevivan en condiciones de alta temperatura (Guy, 1999), incluidas las funciones de la membrana (Weis & Berry, 1988). Los efectos perjudiciales de temperaturas altas sobre la clorofila y el aparato fotosintético también están asociados con la producción de ROS y la peroxidación lipídica

relacionada (Camejo *et al*., 2006; Guo *et al*., 2007); evidencia relacionada se ha observado en la leguminosa *Medicago truncatula* (Doria *et al*., 2019). Sin embargo, las semillas utilizadas en nuestro estudio parecen tener un rango de germinación óptimo cercano a los 30°C; así, la formación de isómeros que ocurre a temperaturas favorables para la germinación se asocia principalmente con cambios en las propiedades fisicoquímicas de las membranas, afectando su configuración y fluidez. En este contexto, se sabe que el isómero *trans* de los ácidos grasos tiene un punto de fusión mucho más alto y permanece sólido a temperatura ambiente. Nuestros resultados también sugieren que esta respuesta es modulada en función de la temperatura. Otra explicación es el papel de estos isómeros durante eventos de señalización (Guy, 1999); sin embargo, esta hipótesis debe explorarse a profundidad.

Temperaturas cardinales

Se determinó la efectividad de seis modelos no lineales para calcular las temperaturas cardinales, ajustándolos al recíproco de los datos de velocidad de germinación en función de la temperatura para cada uno de los percentiles del 10-80% de los tratamientos de 10 a 45 °C. Los modelos se ajustaron a los datos de 1/VG en los rangos de temperatura sub-óptima que comprendió de los 10–30 °C y el rango supra-óptima que comprendió de los 30–45 °C.

El rango de variación de temperatura para todos los modelos fue diferente; mientras que con los modelos S1 y S2 no fue posible determinar la Tc para 4 percentiles (40-80%), debido a restricciones relacionadas con el número de puntos experimentales para realizar el análisis de regresión. En todos los modelos segmentados, los valores de Tb fueron menos variables que los valores de To y Tc. Las variaciones en la Tc fueron menores para los modelos S1 y S2, considerando que solo fue posible estimar tres percentiles con estos modelos. Por otro

lado, para los modelos beta la variación en la Tb fue más amplia entre percentiles que la variación en la To y Tc; observándose una menor variación en la To en todos los modelos beta.

La tendencia descendente en la Tb que se observó con S1, S2 y S3 concuerda con las tendencias reportadas para la determinación de la Tb con modelos segmentados de dos y tres segmentos observados en *Phalaris minor* (Derakhshan *et al*., 2014) y *Silybum marianum* (Parmoon *et al*., 2015). En estas especies se observó un aumento lineal en la germinación, seguido de una fase de meseta, y finalmente continúan con una disminución hasta llegar a cero a medida que aumenta la temperatura; el segmento de meseta no fue observó en nuestros datos de germinación de chía. La tendencia de nuestros datos comprendió valores en la pendiente mas altos para porcentajes de población más bajos, forzando las líneas de regresión de valores altos a bajos de intersección con el eje de las x (valores Tb) a medida que aumentó el porcentaje poblacional. El mismo fenómeno parece ocurrir con la estimación en la Tb con los modelos B2 y B3, cuyas tendencias observadas son opuestas a los valores de Tb observados para funciones de tipo beta con cinco parámetros (Derakhshan *et al*., 2014; Parmoon *et al*., 2015); mientras que nuestra tendencia observada para B1 (beta de cuatro parámetros o modificada) concuerda con la observada en *S. marianum* (Parmoon *et al*., 2015). Las tendencias de Tb para los modelos B2 y B3 están relacionadas con la distribución de los datos de 1/VG observados en chia. Aunque queda por explorar el significado fisiológico de este comportamiento, se ha sugerido que la cantidad en las reservas de energía de los diferentes porcentajes poblacionales podría estar relacionada con una mayor eficiencia de germinación, lo que se relacionada a su vez con una germinación más rápida, donde es

necesaria más energía para satisfacer las demandas metabólicas de esta población, especialmente para procesos como la hidrólisis de almidón y proteínas (Zhao *et al.*, 2018).

Nuestros hallazgos establecen que el rango óptimo de temperatura de germinación de la semilla de chía es de 28.4 a 36.97 °C, con un GR máximo observado a 33.45 ± 2.76 °C en S1, 36.97 ± 5.70 °C en S2, 32. 60 ± 1.20 ° C en S3; 30.45 ± 0.32 °C en B1, 28.44 ± 2.28 °C en B2 y 30.34 ± 1.11 °C en B3; estos valores no respaldan hallazgos previos, donde se ha observado un rango óptimo entre 16 y 26 °C (Ayerza & Coates, 2009). Hasta el momento, existen pocos reportes donde se hayan determinado las temperaturas cardinales de algunas especies *del género Salvia.* En este contexto, se ha informado que las semillas *de S. pomifera* y *S. fruticosa* tienen un rango de temperatura óptimo de los 10 a los 20 °C (Thanos, 1995). Además, se ha encontrado que semillas de *S. officinalis* germinan dentro del rango de los 10 a los 25 °C y que *S. sclarea* tiene un rango más amplio en su temperatura óptima que va de los 10 a los 30 °C (Côme, 1993). Mientras que, en otro trabajo, se reportó que la temperatura óptima para la germinación de semillas de *S. officinalis* fue de 25 °C (Hornok, 1992). Para la mayoría de las especies reportadas pertenecientes al género, se han observado temperaturas óptimas y máximas de 15 a 30 °C y de 30 a 40 °C, respectivamente (Copeland & McDonald, 1995); sin embargo, la temperatura óptima de germinación depende de las condiciones genéticas y ambientales en los que las especies ha evolucionado (Iannucci *et al.*, 2000).

Finalmente, se ha reportado que las temperaturas cardinales para *Plantago ovata* fueron Tb: 4.4 °C, To: 19.0 °C y Tc: 25.5 °C; mientras que para *P. psyllium* fueron Tb: 9.4 ° C, To: 28.8 ° C y Tc: 35.0 ° C (Tabrizi *et al*., 2004). Es importante destacar que estas dos especies son angiospermas mixospérmicas con mucílago abundante, como la chía y perteneciendo ambas al orden Lamiales. La secreción de mucílago podría desempeñar un papel importante durante

la respuesta de la germinación en función de la temperatura; este polímero puede ser secretado por varias plantas o sus diferentes partes como *Aloe vera*, *Cordia dichotoma*, *Basella alba*, *P. psyllium*, *Cyamopsis tetragonoloba*, *Cactaceae*, *Abelmoschus esculentus*, *Trigonella foenum-graecum*, *Moringa oleifera* y *Linum usitatissimum*. La estructura, los componentes, las funciones ecológicas y el mecanismo de producción del mucílago se han estudiado bien en la planta modelo *Arabidopsis thaliana* (Francoz *et al*., 2015; Haughn *et al*., 2012; Western, 2012). Los estudios conducidos en *A. thaliana* y otras especies han llegado a la conclusión de que los mucílagos de la cubierta de las semillas pueden tener múltiples funciones, incluida la inhibición de la germinación en condiciones de humedad excesiva (es decir, la latencia de las semillas) al impedir que el oxígeno difunda hacia el embrión (Haughn & Western, 2012), esta hipótesis ya ha sido abordada con anterioridad en este trabajo; sin embargo, es importante añadir que en el caso de *S. hispanica*, observamos que en el rango de temperatura de 10 °C a los 20 °C, la germinación atraviesa por una fase de retraso; por arriba de esa temperatura la germinación aumenta exponencialmente a medida que se acerca a la temperatura óptima, esta evidencia sugiere que el mucílago podría tener una temperatura en la cual su capacidad de retención de humedad cambia, lo que permite que la germinación proceda más rápidamente, esto concuerda con nuestras observaciones anteriores (Cabrera-Santos *et al*., 2021).

Para el rango sub-óptimo y supra-óptimo en S1, la raíz cuadrada media de las desviaciones (RMSE) fue más alta para el 10% y disminuyo a medida que el porcentaje de la población aumentó en el rango sub-óptimo (RMSE de 1.28 a 0.11); se observó una tendencia similar del 10% al 30% en el rango supraóptimo (RMSE de 1.88 a 0.55). La misma tendencia se observó para los modelos S2 y S3 con valores de 1.46 a 0.11 y 1.88 a 0.11, respectivamente.

Los valores de la R^2 y la R^2 ajustada fueron >0.7 para el 10–70% de todos los modelos segmentados; excepto por los valores más bajos de R^2 ajustado y R^2 ajustada del 80% (<0.6) en los modelos S1, S2 y S3. El RMSE fue más bajo para los modelos beta que para los segmentados, excepto para el modelo B2. Las variaciones de este valor fueron de 1.53 a 0.13 para el modelo B1; de 2.26 a 0.22 para B2, y de 1.66 a 0.03 para el modelo B3; los valores de RMSE bajos de B1 y B3 indican un mayor ajuste de los modelos beta a nuestros datos empíricos, obteniendo globalmente B3 los parámetros de salida con mejor ajuste; sin embargo, este modelo tendió a sobreestimar los valores de Tb (12.74 ± 4.45 °C).

Aunque los seis modelos mostraron una buena capacidad de predicción, los modelos beta tuvieron una mejor estimación de las temperaturas cardinales. En todos los modelos segmentados se pudo observar un mejor ajuste a medida que aumenta el porcentaje poblacional; este tipo de función se ha utilizado para la descripción de la distribución de datos con poca variación en la tasa de germinación entre percentiles en el rango subóptimo, formando una meseta, como se observa en garbanzo (*Cicer arietinum* L.) (Soltani *et al*., 2006), alpiste (*P. minor*) (Derakhshan *et al*., 2014) y cardo mariano (*S. marianum* L.) (Parmoon *et al*., 2015), este comportamiento contrasta con el tipo de tendencia que observamos en *S. hispanica*, donde se observaron tres cambios abruptos en la tasa de germinación: 20 °C, 30 °C y 40 °C, respectivamente. Además, se ha observado que los modelos segmentados tienden a sobrestimar la temperatura base y/o tope cuando se utilizan solo dos segmentos, creando una función bilineal (Craufurd *et al*., 1998); en el caso de la chía, sin embargo, observamos valores realistas para todos los modelos, incluyendo los segmentados, donde la única excepción se observó en el modelo B3 (Tb de 12.74 ± 4.45 °C); mientras que los valores de To y Tc fueron bastante similares entre todos los modelos. Esta

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evidencia concuerda con otros hallazgos en los que funciones segmentadas describen adecuadamente el efecto de la temperatura sobre la respuesta de la germinación, la apariencia de las hojas y la tasa de desarrollo de diferentes cultivos (Olsen *et al*., 1993; Mwalw *et al*., 1994; Robertson *et al*., 2002).

Por otro lado, los valores más bajos en el valor de RMSE y más altos en el valor de R^2 y R^2 ajustada para los modelos beta fueron los esperados debido a que las funciones beta son más flexibles que las segmentadas, debido a la naturaleza curvilínea de los modelos beta que proporcionan una transición gradual entre fases, produciendo un curva suave y realista. Además, los modelos beta no requieren la determinación de temperaturas cardinales para cada subpoblación y, por lo tanto, dichos modelos se pueden parametrizar fácilmente, ya que se pueden linealizar si los valores de Tb y T están predeterminados a partir de datos propios o fuentes externas. Además, se ha observado que los modelos curvilíneos predicen con exactitud las temperaturas topes por extrapolación cuando no se dispone de datos empíricos (Kamkar *et al.*, 2008). Las funciones beta muestran algunas limitaciones, es decir, asumen una respuesta simétrica sobre la temperatura óptima y no permiten ninguna curvatura cóncava cerca de la temperatura base (Yin *et al*., 1995); sin embargo, las funciones exponenciales, como la utilizada para B3, son lo suficientemente flexibles para manejar respuestas asimétricas, estas características podrían explicar la sobreestimación de la temperatura base por parte de este modelo, haciéndolo preciso y adecuado la estimación de la To y Tc pero no para Tb de semillas de chia.

Aunque este trabajo se enfocó en el comportamiento germinativo a partir de índices *ad hoc* en función de la temperatura, existen otros factores subyacentes, de los que hay evidencia experimental, que están involucrados en la respuesta de las semillas a la temperatura. Por

ejemplo, se ha observado la activación de genes de respuesta al frio (cold-responsive o *COR*) durante la adaptación de las semillas a temperaturas bajas (temperaturas no congelantes), los cuales desencadenan la acumulación de crioprotectores, guiando a la adquisición de tolerancia a temperaturas bajas (Thomashow, 1999). Por otra parte, también se conoce que las fluctuaciones climáticas favorecen la producción de ROS (Stanisavljevic *et al*., 2011); por lo tanto, durante la adaptación de las diferentes especies a estos cambios climáticos, con la finalidad de mantener el balance de ROS, es esencial la participación de los sistemas antioxidantes, en los cuales enzimas como superóxido dismutasas, catalasas y peroxidasas aumentan su actividad manteniendo niveles permisibles de ROS (Apel & Hirt, 2004; Carneiro *et al*., 2011). Es importante mencionar que parte del efecto de ROS se manifiesta en forma de peroxidación lipídica, lo cual podría afectar la isomerización de los ácidos grasos en *trans* observados en nuestra investigación. Por otra parte, también se conoce que proteínas de la embriogénesis tardía (late embryogenesis abundant o LEA), proteínas de choque térmico y estrés general también se encuentran implicadas en la protección y adaptación de las semillas a la temperatura (Kalemba & Pukacka, 2007). Sin embargo, las intrincadas redes regulatorias de estos mecanismos, tanto rio arriba como rio abajo, requieren futuras investigaciones; además, dichos factores, los cuales trabajan de manera orquestada, quedan por estudiar de forma integrativa, con la finalidad de dar un panorama más completo sobre el efecto de la temperatura en distintas especies.

Finalmente, los resultados de nuestra investigación indican que todos los modelos ensayados se ajustan bien a los datos empíricos de la germinación de chía en respuesta a la temperatura; sin embargo, los modelos beta tienen un mejor ajuste que los segmentados, siendo el modelo B1 el que mejor define las temperaturas cardinales de *S. hispanica*.

Las conclusiones principales de este trabajo son las siguientes:

1. En *S. hispanica* L., una fase de imbibición FII más corta se asocia con una germinación más temprana.

2. El aumento en la concentración de ácidos grasos después de 3 horas y una correlación negativa entre el ácido linoleico y linolénico observada a 20 °C, se relacionaron con una mayor eficiencia de germinación.

3. A 30 ° C, se observó la formación de tres isómeros en conformación *trans* del ácido linolénico, nuestros resultados sugieren que esta respuesta es modulada en función de la temperatura.

4. La germinación de *S. hispanica* L. ocurre en temperaturas frías a moderadas-altas (10–35 °C), con un rango óptimo entre 25–35 °C, con una mayor GR y t_{50} observados a 30 °C. Las temperaturas superiores a 35 °C inhibieron fuertemente la germinación.

5. Los resultados de este estudio mostraron que la respuesta de la germinación de chía en función de la temperatura es mejor explicada por los modelos beta, teniendo un mejor ajuste que los modelos segmentados.

6. Las temperaturas cardinales para la germinación de chía calculadas por el modelo B1 fueron: 2.52 ± 6.82 °C para la base, 30.45 ± 0.32 °C para la óptima y 48.58 ± 2.93 °C para la temperatura tope.

Los resultados presentados en este trabajo contribuyen al conocimiento sobre el metabolismo de los lípidos y ácidos grasos en semillas de una especie de importancia agronómica, y con el potencial de establecerse como modelo experimental para el estudio de ácidos grasos durante la germinación. Nuestros datos para las temperaturas base, óptima y máxima para la

germinación de las semillas de chía, proporcionan los requisitos básicos de temperatura que

pueden ser utilizados en futuras investigaciones.

Referencias bibliográficas

Abdallah, A., Ahumada, M., Gradziel, T. (1998). Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. *J. Am. Soc. Hort.*, 123: 1029−1033.

Adam, N.R., Dierig, D.A., Coffelt, T.A., Wintermeyer, M.J., Mackeya, B.E. & Wall, G.W. (2007). Cardinal temperatures for germination and early growth of two Lesquerella species. *Ind Crop Prod.*, 25: 24–33.

Aiazzi, M. T., Argüello, J. A., Pérez, A., Di Rienzo, J. & Guzman, C. A. (1996). Deterioration in *Atriplex cordobensis* (Gandoger & Stuckert) seeds: Natural and accelerated ageing. *Seed Science and Technology*, 25: 147-155.

Alvarado, V. & Bradford, K.J. (2002). A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant, Cell & Environment*, 25: 1061-1069.

Alvarado, V., & Bradford, K. (2005). Hydrothermal time analysis of seed dormancy in true (botanical) potato seeds. *Seed Science Research*, 15(2), 77-88.

Apel, K., & Hirt, H. (2004). Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. *Annual Review of Plant Biology*, 55: 373–399.

Arc, E., Chibani, K., Grappin, P., Jullien, M., Godin, B., Cueff, G., Valot, B., Balliau, T., Job, D., & Rajjou, L. (2012). Cold stratification and exogenous nitrates entail similar functional proteome adjustments during Arabidopsis seed dormancy release. *Journal of proteome research*, *11*(11), 5418–5432.

Arc, E., Galland, M., Cueff, G., Godin, B., Lounifi, I., Job, D., & Rajjou, L. (2011). Reboot the system thanks to protein post-translational modifications and proteome diversity: how quiescent seeds restart their metabolism to prepare seedling establishment. *Proteomics* 11, 1606–1618.

Ayerza, R. & Coates, W. (2009). Influence of environment on growing period and yield, protein, oil and α-linolenic content of three chia (*Salvia hispanica* L.) selections. *Industrial Crops and Products*, 30 (2): 321–324.

Ayerza, R. (1995). Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. *Journal of the American Oil Chemists Society*, 72 (9): 1079-1081.

Ayerza, R. (2009). The seed's protein and oil content, fatty acid composition, and growing cycle length of a single genotype of chia (*Salvia hispanica* L.) as affected by environmental factors. *J Oleo Sci.*, 58: 347−54.

Ayerza, R. (2010). Effects of seed color and growing locations on fatty acid content and composition of two chia (*Salvia hispanica* L.) genotypes. *Journal of the American Oil Chemists Society*, 87 (10): 1161-1165.

Ayerza, R., Coates, W. (2011). Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.). *Industrial Crops and Products*, 34: 1366–1371.

Baginsky, C., Arenas, J., Escobar, H., Garrido, M., Valero, N., Tello, D., Pizarro, L., Valenzuela, A., Morales, L., Silva, H. (2016). Growth and yield of chia (Salvia hispanica L.)

in the Mediterranean and desert climates of Chile. *Chilean Journal of Agricultural Research*, 76(3): 255–264.

Bannayan, M., Nadjafi, F., Rastgoo, M., Tabrizi, L. (2006). Germination properties of some wild medicinal plants from Iran. *Seed Technology*, 28(1): 80-86.

Bareke, T. (2018). Biology of seed development and germination physiology. *Adv Plants Agric Res.,* 8(4): 336-346.

Bates, P.D., Stymne, S. & Ohlrogge, J. (2013). Biochemical pathways in seed oil synthesis. *Curr Opin Plant Biol.*, 16:358–364.

Batlla, D. & Benech-Arnold, R. L. (2007). Predicting changes in dormancy level in weed seed soil banks: Implications for weed management. *Crop Protection*, 26: 189–197.

Batlla, D., Benech-Arnold, R. (2015). A framework for the interpretation of temperature effects on dormancy and germination in seed populations showing dormancy. *Seed Science Research*, 25: 147–158.

Baud, S. & Lepiniec, L. (2010) Prog Lipid Res 49:235–249 (Regulation of seed oil production)

Benech-Arnold, R.L., Sánchez, R.A., Forcella, F., Kruk, B.C. & Ghersa, C.M. (2000). Environmental control of dormancy in weed seed banks in soil. *Field Crops Research,* 67: 105-122.

Bewley, J. D. (1997). Seed Germination and Dormancy. *The Plant cell*, 9(7): 1055-1066.

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). Seeds: physiology of development, germination and dormancy, 3rd edition (3rd ed.). Springer.

Bewley, J.D. & Black, M. Seeds: Physiology of Development and Germination. New York: *Plenum Press*, 1994.

Bita, C., Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science*, 4: 1−18.

Black, M., Bewley, J.D. (eds). (2000). Seed technology and its biological basis. Sheffield Academic Press, Sheffield (Properties, synthesis and genetic engineering of the major seed reserves)

Bodoira, R., Penci, M., Ribotta, P., Martínez, M. (2017). Chia (*Salvia hispanica* L.) oil stability: Study of the effect of natural antioxidants. *LWT*, 75: 107−113.

Borges, O. P., Soeiro Carvalho, J., Reis Correia, P., Paula Silva, A. (2007). Lipid and fatty acid profiles of *Castanea sativa* Mill. Chestnuts of 17 native Portuguese cultivars. *Journal of Food Composition and Analysi*, 20: 80–89.

Bradford, K.J. (2002). Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science*, 50: 248-260.

Bradford, K.J. (2005). Threshold models applied to seed germination ecology. *New Phytologist* 165(2): 338-341.

Bueno, M., Di Sapio, O., Barolo, M., Busilacchi, H., Quiroga, M. & Severin, C. (2010) Análisis de la calidad de los frutos de *Salvia hispanica* L. (*Lamiaceae*) comercializados en la ciudad de Rosario (Santa Fe, Argentina). *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 9(3):221–227.

Burdge, G. C. (2006). Metabolism of alpha-linolenic acid in humans. *Prostaglandins, leukotrienes, and essential fatty acids*, *75*(3), 161–168.

Cabrera-Santos, D., Ordoñez-Salanueva, C.A., Sampayo-Maldonado, S., Campos, J.E., Orozco- Segovia, A., Flores-Ortiz, C.M. (2021). Chia (*Salvia hispanica* L.) Seed Soaking, Germination, and Fatty Acid Behavior at Different Temperatures. *Agriculture*, 11: 498.

Cahill, J. P. & Provance, M. C. (2002). Genetics of qualitative traits in domesticated chia (Salvia hispanica L.). *Journal of Heredity*: 93 (1): 52-55.

Camejo, D., Jiménez, A., Alarcón, J. J., Torres, W., Gómez, J., Sevilla, F. (2006). Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants. *Funct. Plant Biol.*, 33: 177–187.

Capitani, M. I., Corzo-Rios, L. J., Chel-Guerrero, L. A., Betancur-Ancona, D. A., Nolasco, S. M., & Tomás, M. C. (2015). Rheological properties of aqueous dispersions of chia (*Salvia hispanica* L.) mucilage. *Journal of Food Engineering*, 149: 70-77.

Carneiro, M. M. L. C., Deuner, S., Oliveira, P. V. de, Teixeira, S. B., Sousa, C. P., Bacarin, M. A., & Moraes, D. M. de. (2011). Atividade antioxidante e viabilidade de sementes de girassol após estresse hídrico e salino. *Revista Brasileira de Sementes*, 33: 752–761.

Chicco, A.G. (2009). Dietary chia seed (*Salvia hispanica* L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. *The British Journal of Nutrition*, 101(1): 41-50.

Chicco, A.G., D'Alessandro, M.E., Hein, G.J., Oliva, M.E., Lombardo, Y.B. (2009). Dietary chia seed (*Salvia hispanica* L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. *Br. J. Nutr.*, 101: 41–50.

Côme D. Germination of seeds of some aromatic plants. (1993). En: Panetsos K, Nikolaidis A. Lyrintzis G., Identification, preservation, adaptation, and cultivation of selected aromatic and medicinal plants suitable for marginal lands of the mediterranean region. Mediterranean Agronomic Institute of Chania, pp: 111–26.

Copeland, L.O., McDonald, M.B. (1995). *Principles of seed science and technology*. Chapman and Hall Publication.

Covell, S., Ellis, R.H., Roberts, E.H. & Summerfield, R.J. (1986). The influence of temperature on seed germination rate in grain legumes. 1: A comparison of chickpea, lentil, soybean and cowpea at constant temperatures. *J. Exp. Bot.*, 37: 705-715.

Craufurd, P.Q., Qi, A., Ellis, R.H., Summerfield, R.J., Roberts, E.H. & Mahalakshmi, V. (1998). Effect of temperature on time to panicle initiation and leaf appearance in sorghum. *Crop Sci*, 38: 942–947.

Creus, A., Benmelej, A., Villafañe, N., Lombardoa, Y.B. (2017). Dietary Salba (*Salvia hispanica* L.) improves the altered metabolic fate of glucose and reduces increased collagen

deposition in the heart of insulin resistant rats. *Prostaglandins Leukot. Essent. Fat. Acids*, 121: 30–39.

Dąbrowski, G., Konopka, I., Czaplicki, S., Tańska, M. (2017). Composition and oxidative stability of oil from Salvia hispanica L. seeds in relation to extraction method. *Eur. J. Lipid Sci. Technol.*, 119: 1600209.

Denich, T. J., Beaudette, L. A., Lee, H., Trevors, J. T. (2003). Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. *J. Microbiol. Methods.*, 52, 149–182.

Derakhshan, A., Gherekhloo, J., Vidal, R., De Prado, R. (2014). Quantitative description of the germination of Littleseed Canarygrass (*Phalaris minor*) in response to temperature. *Weed Science*, 62(2): 250–257.

Donohue, K., Burhardt, L. T., Runcie, D., Bradford, K. J. & Schmitt, J. (2015). Applying developmental threshold models to evolutionary ecology. *Trends in Ecology and Evolution*, 30: 66-77.

Donohue, K., Rubio de Casas, R., Burghardt, L., Kovach, K. & Willis, C. G. (2010). Germination, postgermination adaptation and species ecological ranges. *Annual Review of Ecology, Evolution, and Systematics*, 41(1): 293-319.

Doria, E., Pagano, A., Ferreri, C, Larocca, A.V., Macovei, A., Araújo, S.S., Balestrazzi, A. (2019). How Does the Seed Pre-Germinative Metabolism Fight Against Imbibition Damage? Emerging Roles of Fatty Acid Cohort and Antioxidant Defence. *Front Plant Sci.*, 10: 1505.

Dubois, V., Breton, S., Linder, M., Fanni, J. & Parmentier, M. (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology*, 109 (7): 710-732.

Dürr, C., Dickie, J.B., Yang, X.Y. & Pritchard, H.W. (2015). Ranges of critical temperature and water potential values for the germination of seeds worldwide: Contribution to a seed trait database. *Agricultural and Forest Meteorology*, 200: 222–232.

Dybing, C., Zimmerman, D. (1966). Fatty acid accumulation in maturing flaxseeds as influenced by environment. *Plant Physiol.*, 41: 1465−1470.

Ellis, R.H., Covell, S., Roberts, E.H., Summerfield, R.J. (1986). The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *J. Exp. Bot*. 37: 1503-1515.

Estrelles, E., Albert, F., Navarro, A., Prieto, J., Ibars, A.M. (1999). Germination behavior of Labiatae SW distributed in the Iberian Peninsula. En: Proceedings of the 4to European conference on the conservation of wild plants. Planta Europa. Valencia.

Fernandez, I., Vidueiros, S.M., Ayerza, R., Coates, W., Pallaro, A. (2008) .Impact of chia (*Salvia hispanica* L.) on the immune system: Preliminary study. *Proc. Nutr. Soc*, 67, E12.

Ferreri, C., Chatgilialoglu, C. (2012). Encyclopedia of Radicals in Chemistry, Biology and Materials 1st ed., Wiley: Toronto, Canada, pp. 1599–1623.

Filho, M. (2015). Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricol*a, 72(4): 363-374.

Fonte-Faria, T., Citelli, M., Atella, G.C., Raposo, H.F., Zago, L., de Souza, T., da Silva, S.V., Barja-Fidalgo, C. (2019). Chia oil supplementation changes body composition and activates insulin signaling cascade in skeletal muscle tissue of obese animals. *Nutrition*, 58: 167–174.

Francoz, E., Ranocha, P., Burlat, V., Dunand, C. (2015). *Arabidopsis* seed mucilage secretory cells: regulation and dynamics. *Trends Plant Sci.*, 20: 515–24.

Gallardo, K., Job, C., Groot, S. P., Puype, M., Demol, H., Vandekerckhove, J., & Job, D. (2002). Proteomics of *Arabidopsis* seed germination: a comparative study of wild-type and gibberellin-deficient seeds. *Plant Physiol.,* 129:823–37.

García-Huidobro, J., Monteith, J.L. & Squire, G.R. (1982). Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.). I. Constant temperature. *Journal of Experimental Botany* 33: 288-296.

Geneve, R., Hildebrand, D., Phillips, T., Kirk, J., Al-Amery, M. (2019). Seed germination and mucilage production in chia (*Salvia hispanica*). *Acta Hortic.*, 1249: 153−156.

Gidrol, X., Serghini, H., Noubhani, A., Mocçuot, B. & Mazliak, P. (1989). Biochemical changes induced by accelerated aging in sunflower seeds. I. Lipid peroxidation and membrane damage. *Physiologia Plantarum*, 76: 591-597.

Gill, I. & Valivety, R. (1997). Polyunsaturated fatty acids, part 1: occurrence, biological activities and applications. *Trends Biotechnol.,* 15:401– 409.

Gómez-Favela, M., Gutiérrez-Dorado, R., Cuevas-Rodríguez, E., Canizalez-Román, V., Del Rosario León-Sicairos, C., Milán-Carrillo, J., Reyes-Moreno, C. (2017). Improvement of

Chia Seeds with Antioxidant Activity, GABA, Essential Amino Acids, and Dietary Fiber by Controlled Germination Bioprocess. *Plant Foods Hum Nutr*., 72: 345−352.

Gorai, M. & Neffati, M. (2007). Germination responses of *Reaumuria vermiculata* to salinity and temperature. *Ann. Appl. Biol*., 151: 53–59.

Gorai, M., Vadel, M.A. & Neffati, M. (2006). Seed germination characteristics of *Phragmites communis*: Effects of temperature and salinity. *Belg. J. Bot*., 139: 78–86.

Grimes, S.J., Phillips, T.D., Hahn, V., Capezzone, F., Graeff-Hönninger, S. (2018). Growth, Yield Performance and Quality Parameters of Three Early Flowering Chia (*Salvia hispanica* L.) Genotypes Cultivated in Southwestern Germany. *Agriculture*, 8: 154.

Grubb, P.J. (1977). The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews*, 52: 107–145.

Guiotto, E. N., Ixtaina, V. Y., Tomás, M. C. and Nolasco, S. M. (2013). Moisture-dependent engineering properties of chia (*Salvia hispanica* L.) seeds. *Food Industry Intech*., 381-397.

Guo, T., Zhang, G., Zhang, Y. (2007). Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. *Colloids Surf B: Biointerfaces*, 57: 182–188.

Guy, C. (1999). Molecular responses of plants to cold shock and cold acclimation. *J. Mol. Microbiol. Biotechnol*., 1: 231– 242.

Hadas, A. (1982) En: Khan, A.A. (ed). The physiology and biochemistry of seed development, dormancy and germination. Elsevier Biomedical Press, Amsterdam, pp 507–527 (Water movement to seeds in soils and seed water uptake).

Hao, D. (2015). 14 – Phytochemical and biological research of Salvia medicinal resources. En: Da Cheng Hao, Xiao-Jie Gu, Pei Gen Xiao (Ed.), Medicinal Plants (587-639). Woodhead Publishing.

Hardegree, S. (2006). Predicting germination response to temperature. I. Cardinal temperature models and subpopulation-specific regression. *Ann Bot*, 97: 1115–25.

Harman, G. E. & Mattick, L. R. (1976). Association of lipid oxidation with seed ageing and death. *Nature*, 260: 323-324.

Harwood, J. L., Stumpf, P. K. (1970). Fat Metabolism in Higher Plants: XL. Synthesis of fatty acids in the initial stage of seed germination. *Plant physiology*, 46: 500–508.

Haughn, G.W., Western, T.L. (2012). *Arabidopsis* Seed Coat Mucilage is a specialized cell wall that can be used as a model for genetic analysis of plant cell wall structure and function. *Front Plant Sci.*, 3: 64.

Hazel, J. R. (1995). Thermal Adaptation in Biological Membranes: Is Homeoviscous Adaptation the Explanation? *Annual Review of Physiology.*, 57: 19–42.

He, D., Han, C., Yao, J., Shen, S. & Yang, P. (2011). Constructing the metabolic and regulatory pathways in germinating rice seeds through proteomic approach. *Proteomics* 11, 2693–2713.

Ho, H., Lee, A.S., Jovanovski, E., Jenkins, A.L., Desouza, R., Vuksan, V. (2013). Effect of whole and ground Salba seeds (*Salvia hispanica* L.) on postprandial glycemia in healthy volunteers: A randomized controlled, dose-response trial. *Eur. J. Clin. Nutr.*, 67: 786–788.

Hocking, P.J., Kirkegaard, J.A., Angus, J.F., Gibson, A.H. & Koetz, E.A. (1997). Comparison of canola, Indian mustard and linola in two contrasting environments. I. Effects of nitrogen fertilizer on dry-matter production, seed yield and seed quality. *Field Crop Research*, 49: 107–125.

Hornok, L. (1992). *Cultivation and processing of medicinal plants*. John Wiley and Sons Publication.

Howell, K. A., Cheng, K., Murcha, M. W., Jenkin, L. E., Millar, A. H., & Whelan, J. (2007). Oxygen initiation of respiration and mitochondrial biogenesis in rice. *J. Biol. Chem.* 282, 15619–15631.

Howell, K. A., Millar, A. H. & Whelan, J. (2006). Ordered assembly of mitochondria during rice germination begins with pro-mitochondrial structures rich in components of the protein import apparatus. *Plant Mol. Biol.* 60, 201–223.

Howell, K. A., Narsai, R., Carroll, A., Ivanova, A., Lohse, M., Usadel, B., Millar, A. H., & Whelan, J. (2009). Mapping metabolic and transcript temporal switches during germination in rice highlights specific transcription factors and the role of RNA instability in the germination process. *Plant Physiol.* 149, 961–980.

Huang, A. (1994). Structure of plant seed oil bodies. Current Opinion in Structural Biology, 4(4): 493-498.

Iannucci, A., Di Fonzo, N., Martiniello, P. (2000). Temperature requirements for seed germination in four annual clovers grown under two irrigation treatments. *Seed Sci Technol*, 28: 59–66

Imran, M., Nadeem, M., Manzoor, M. F., Javed, A., Ali, Z., Akhtar, M. N., & Hussain, Y. (2016). Fatty acids characterization, oxidative perspectives and consumer acceptability of oil extracted from pre-treated chia (*Salvia hispanica* L.) seeds. *BioMed Central*, 15(162): 1-13.

Ixtaina, V. Martínez, M., Spotorno, V., Mateo, C., Maestri, D. Diehl, B., Nolasco, S. & Tomás, M. (2011). Characterization of chia seed oils obtained by pressing and solvent extraction. Journal of Food Composition and Analysis. *Journal of Food Composition and Analysis*, 24(2): 166-174.

Izquierdo, N., Benech-Arnold, R., Batlla, D., Belo, R.G. & Tognetti, J. (2017). Seed composition in oil crops. In Oilseed Crops, P. Ahmad (Ed.).

Jamboonsri, W. Phillips, T. D., Geneve, R. L., Cahill, J. P. & Hildebrand, D. F. (2012). Extending the range of an ancient crop, *Salvia hispanica L.*-a new $\omega 3$ source. *Genetic Resources and Crop Evolution*, 59(2): 171–178.

Jensen, E., Robson, P., Norris, J., Cookson, A., Farrar, K., Donnison, I., Clifton-Brown, J. (2013). Flowering induction in the bioenergy grass *Miscanthus sacchariflorus* is a quantitative short-day response, whilst delayed flowering under long days increases biomass accumulation. *Journal of Experimental Botany*, 64: 541–552.

Jin, F., Nieman, D. C., Sha, W., Guoxiang Xie, G., Qiu, Y., & Jia, W. (2012). Supplementation of milled chia seeds increases plasma ALA and EPA in postmenopausal women. *Plant Foods for Human Nutrition*, 67(2): 105-110.

José, S.C., Salomão, A.N., Costa, T.S., Silva, J.T., Curi, C.C. (2010). Armazenamento de sementes de girassol em temperaturas subzero: Aspectos fisiológicos e bioquímicos. *Revista Brasileira de Sementes*, 32: 29–38.

Jouhet, J. (2013). Importance of the hexagonal lipid phase in biological membrane organization. *Front. Plant Sci.*, 4: 494.

Kalemba, E. & Pukacka, S. (2007). Possible roles of LEA proteins and sHSPs in seed protection: A short review. *Biol. Lett.* 44: 3–16.

Kamkar, B. Ahmadi, M., Soltani, A. & Zeinali, E. (2008). Evaluating Non-Linear regression models to describe response of wheat emergence rate to temperature. *Seed Science and Biotechnology*, 2: 53–57.

Kim, S. T., Kang, S. Y., Wang, Y., Kim, S. G., Hwang Du, H. & Kang, K. Y. (2008). Analysis of embryonic proteome modulation by GA and ABA from germinating rice seeds. *Proteomics* 8, 3577–3587.

Kim, S. T., Wang, Y., Kang, S. Y., Kim, S. G., Rakwal, R., Kim, Y. C., et al. (2009). Developing rice embryo proteomics reveals essential role for embryonic proteins in regulation of seed germination. *J. Proteome Res.* 8, 3598–3605.

Krishnan, S. & Dayanandan, P. (2003). Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa* L.). *J. Biosci.* 28, 455–469.

Kulczyński, B., Kobus-Cisowska, J., Taczanowski, M., Kmiecik, D., & Gramza-Michałowska, A. (2019). The Chemical Composition and Nutritional Value of Chia Seeds-Current State of Knowledge. *Nutrients*, 11(6): 1242.

Larkindale, J., Huang, B. (2004). Thermotolerance and antioxidant systems in Agrostis stolonifera: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. *J. Plant Physiol.*, 161: 405–413.

Lee, J., Lee, H., Kang, S., Park, W. (2016). Fatty Acid Desaturases, Polyunsaturated Fatty Acid Regulation, and Biotechnological Advances. *Nutrients*, 8: 23.

Linder, C. (2000). Adaptive evolution of seed oils in plants: Accounting for the biogeographic distribution of saturated and unsaturated fatty acids in seed oils. *Am Nat.*, 156: 442−458.

Lobo, R., Alcocer, M., Fuentes, F., Rodríguez, W., Morandini, M., Devani, M. (2011). Desarrollo del cultivo de chía en Tucumán, República Argentina. *EEAOC-Avance Agroindustrial*, 32: 27–30.

Lopez-Molina L., Mongrand, S., McLachlin, D.T., Chait, B.T. & Chua, N.H. (2002). ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. *Plant J.*, 32:317–28.

Lopez-Molina, L., Mongrand, S. & Chua, N.H. (2001). A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, 98:4782–87

Ma, Z., Marsolais, F., Bykova, N. V., Igamberdiev, A. U. (2016). Nitric oxide and reactive oxygen species mediate metabolic changes in barley seed embryo during germination. *Front. Plant Sci.*, 7: 138.

Marcos-Filho, J. (2015). Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*,72: 363−374. 53.

Marcos-Filho, J. Seed Physiology of Cultivated Plants. 2nd Edition, ABRATES, Londrina, 2015, 660 p.

Marineli, R. Moura, C.S., Moraes, É.A., Lenquiste, S.A., Lollo, P.C., Morato, P.N., Amaya-Farfan, J., Maróstica, M.R., Jr. (2015). Chia (*Salvia hispanica* L.) enhances HSP, PGC-1α expressions and improves glucose tolerance in diet-induced obese rats. Nutrition, 31: 740–748.

Mcgill, Brian & Enquist, Brian & Weiher, Evan & Westoby, Mark. (2006). Rebuilding Community Ecology from Functional Traits. *Trends in ecology & evolution*. 21: 178-85.

Mohd Ali, N., Yeap, S. K., Ho, W. Y., Beh, B. K., Tan, S. W., & Tan, S. G. (2012). The promising future of chia, *Salvia hispanica* L. *Journal of biomedicine & biotechnology*, 171956.

Morello, J.R., Motilva, M.J., Tovar, M.J., Romero, M.P. (2004). Changes in commercial virgin olive oil (CV Arbequina) during storage with special emphasis on the phenolic fraction. *J. Food Chem*. 85: 357-364.

Morrison, W. R. & Smith, L. M. (1964). Preparation of fatty acid methyl ester and dimethylcetals from lipids with boron fluoride-methanol. *Journal of Lipid Research*, 5: 600-608.

Muñoz, L., Cobos, A., Diaz, O., Aguilera, J. (2012). Chia seeds: microstructure, mucilage extraction and hydration. *J. Food Eng*., 108: 216−224.

Mwale, S.S., Azam-Ali, S.N., Clark, J.A., Bradley, R.G., Chatha, M.R. (1994). Effect of temperature on germination of sunflower. *Seed Sci. Technol*., 22: 565–571.

Nadjafi, F., Tabrizi, L., Shabahang, J., Mahdavi Damghani, A. M. (2009). Cardinal germination temperatures of some medicinal plant species. *Seed Technology*, 31(2): 156–63.

Nadtochii, L., Kuznetcova, D., Proskura, A., Apalko, A., Nazarova, V., Srinivasan, M. (2019). Investigation of various factors on the germination of chia seeds sprouts (*Salvia hispanica* L.). *Agronomy Research*, 17: 1390–1400.

Nakamura, M., Nara, T. (2004). Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. *Annu. Rev. Nutr*., 24: 345–376.

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M. & Kamiya, Y. (2010). Abscisic acid and the control of seed dormancy and germination. *Seed Sci. Res*., 20: 55–67.

Nayani, S., Rao, S. (2020). Extraction of Mucilage from Chia Seeds and its Application as Fat Replacer in Biscuits. *International Journal of Engineering Research Technology*, 9: 922−927.

Neffati, M. (1994). Caractérisation morphologique de certaines espèces végétales nord-africaines. Implications pour l'amélioration pastorale. Tesis de doctorado. Universidad de Gent, Bélgica.

Oberczian, G. & Bernath, J. (1988). The germination of *Salvia officinalis* L. and *Salvia sclarea* L. seeds affected by temperature and light. *Herba H*ung., 27: 31–38.

Ogawa, M., Hanada, A., Yamauchi, Y., Kuwahara, A., Kamiya, Y., & Yamaguchi, S. (2003). Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell* 15:1591–604.

Okagami, N. & Terui, K. (1996). Differences in the Rates of Metabolism of Various Triacylglycerols during Seed Germination and the Subsequent Growth of Seedlings of *Dioscorea tokoro*, a Perennial Herb. *Plant and Cell Physiology*, 37(3): 273–277.

Olsen, J.K., McMahan, C.R., Hammer, G.L. (1993). Prediction of sweet corn phenology in subtropical environments. *Agron. J.*, 85: 410–415.

Ordoñez-Salanueva, C.A., Seal, C.E., Pritchard, H.W., Orozco-Segovia, A., Canales-Martínez, M., Flores-Ortíz, C.M. (2015). Cardinal temperatures and thermal time in Polaskia Beckeb (Cactaceae) species: Effect of projected soil temperature increase and nurse interaction on germination timing. *J. Arid Environ.*, 115: 73–80.

Oteng, A., Kersten, S. (2020). Mechanisms of action of trans fatty acids. *Adv Nutr.*, 11: 697–708.

Paiva, E., Barros-Torres, S., Alves, Costa, T., da Silva, F., de Sousa, L., Dallabona, J. (2018). Germination and biochemical components of *Salvia hispanica* L. seeds at different salinity levels and temperatures. *Acta Scientiarum Agronomy*, 40: e39396.

Paiva, E., Barros-Torres, S., da Silva, F., Nogueira, N., Freitas, R., Leite, M. (2016). Light regime and temperature on seed germination in *Salvia hispanica* L. *Acta Scientiarum. Agronomy*, 38: 513−519.

Parmoon, G., Moosavi, S. A., Akbari, H., Ebadi, A. (2015). Quantifying cardinal temperatures and thermal time required for germination of *Silybum marianum* seed. *The Crop Journal*, 3(2): 145–151.

Peiretti, P. G. & Gai, F. (2009). Fatty acid and nutritive quality of chia (*Salvia hispanica* L.) seeds and plant during growth. *Animal Feed Science and Technology*, 148 (2-4): 267- 275.

Peiretti, P. G. & Meineri, G. (2008). Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (*Salvia hispanica* L.) seed supplements. *Meat Science*, 80 (4): 1116-1121.

Pereira, M.D., Dias, D.C., Borges, E.E., Martins Filho, S., Dias, L.A., Soriano, P.E. (2013). Physiological Quality of Physic Nut (*Jatropha curcas* L.) seeds during storage. *Journal of Seed Science*, 35: 21–27.

Possenti, J.C., Donazzolo, J., Gullo, K., Voss, L.C., & Danner, M.A. (2016). Influence of temperature and substrate on chia seeds germination. *Científica, Jaboticabal*, 44 (2): 235- 238.

Poudyal, H., Panchal, S., Waanders, J., Ward, L., & Brown, L. (2012). Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl CoA desaturase- and induces cardiac and hepatic protection in diet induced obese rats. *The Journal of Nutritional Biochemistry*, 23(2): 153-162.

Priestley, D. A. & Leopold, A. C. (1983). Lipid changes during natural aging of soybean seeds. *Physiologia Plantarum*, 59: 467-470.

Priestley, D. A., B. G. Werner, A. C. Leopold & M. B. McBride. (1985). Organical free radical levels in seed and pollen: The effects of hydration and aging. *Physiologia Plantarum*, 64:88-94.

Pritchard, S., Charlton, W., Baker, A., Graham, I. (2002). Germination and storage reserve mobilization are regulated independently in *Arabidopsis*. *Plant Journal*, 31: 639−647.

Qu, R. & Huang A. (1990). Oleosin structure and location. *J Biol Chem.*, 6:2238–2243.

Rajjou, L., Belghazi, M., Huguet, R., Robin, C., Moreau, A., Job, C., & Job, D. (2006). Proteomic investigation of the effect of salicylic acid on *Arabidopsis* seed germination and establishment of early defense mechanisms. *Plant Physiol.*, 141:910–23.

Rajjou, L., Gallardo, K., Debeaujon, I., Vandekerckhove, J., Job, C., & Job, D. (2004). The effect of α-amanitin on the *Arabidopsis* seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. *Plant Physiol.*, 134:1598–613

Reyes-Caudillo, E., A. Tecante, & M. A. Valdivia-López. (2008). Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chemistry*, 107 (2): 656–663.

Robertson, M.J., Carberry, P.S., Huth, N.I., Turpin, J.E., Probert, M.E., Poulton, P.L., Bell, M., Wright, G.C., Yeates, S.J., Brinsmead, R.B. (2002). Simulation of growth and development of diverse legume species in APSIM. *Aust. J. Agric. Res*., 53: 429–446.

Rosental, L., Nonogaki, H., Fait, A. (2014). Activation and regulation of primary metabolism during seed germination. *Seed Science Research*, 24: 1−15.

Rossi, A.S., Oliva, M.E., Ferreira, M.R., Chicco, A., Lombardo, Y.B. (2013). Dietary chia seed induced changes in hepatic transcription factors and their target lipogenic and oxidative enzyme activities in dyslipidaemic insulin-resistant rats. *Br. J. Nutr.*, 109: 1617–1627.

Salgado-Cruz, M. & Chanona-Pérez, J. & Farrera-Rebollo, R. & Méndez-Méndez, J. & Díaz-Ramírez, M. (2013). Chia (*Salvia hispanica* L.) seed mucilage release characterization. A microstructural and image analysis study. *Industrial Crops and Products*, 51: 453-462.

Schwartz, D.M. & Wolins, N.E. (2007). A simple and rapid method to assay triacylglycerol in cells and tissues. *J. Lipid Res*., 48:(11): 2514-2520.

Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission on the safety of 'Chia seed (*Salvia hispanica*) and ground whole Chia seed' as a food ingredient. *The EFSA Journal* (2009) 996: 1–26.

Segura-Campos, M. R., Ciau-Solis, N., Rosado-Rubio, G., Chel-Guerrero, L., Betancur-Ancona, D. (2014). Chemical and functional properties of chia seed (*Salvia hispanica* L.) gum. *International Journal of Food Science*, 2014: 1−5.

Sierra, L., Roco, J., Alarcon, G., Medina, M., Nieuwenhove, C.V., Bruno, M.P., Jerez, S. (2015). Dietary intervention with *Salvia hispanica* (Chia) oil improves vascular function in rabbits under hypercholesterolaemic conditions. *J. Funct. Foods*, 14: 641–649.

Silva, B.P., Dias, D.M., de Castro Moreira, M.E., Toledo, R.C., da Matt, S.L., Lucia, C.M., Martino, H.S., Pinheiro-Sant'Ana, H.M. (2016). Chia seed shows good protein quality, hypoglycemic effect and improves the lipid profile and liver and intestinal morphology of wistar rats. Plant Foods Hum. *Nutr*., 71: 225–230.

Silva, C., Garcia, V.A.S., Zanette, C.M. (2016) .Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: Oil yield, fatty acids profile and technological analysis of defatted meal. *Int. Food Res. J.*, 23: 998–1004.

Sinensky, M. (1974). Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli. Proc. Natl. Acad. Sci. U.S.A*, 71: 522–525.

Singh, B., Chauhan, G.S., Bhatt, S.S., Kumar, K. (2006). Metal ion sorption and swelling studies of psyllium and acrylic acid based hydrogels. *Carbohydrate Polymers*, 64: 50−56.

Singh, B., Chauhan, G.S., Kumar, S., Chauhan, N. (2007). Synthesis, characterization and swelling responses of pH sensitive psyllium and polyacrylamide based hydrogels for the use in drug delivery (I). *Carbohydrate Polymers*, 67: 190−200.

Soltani, A., Robertson, M. J., Torabi, B., Yousefi-Daz, M., Sarparast, R. (2006). Modelling seedling emergence in chickpea as influenced by temperature and sowing depth. *Agricultural and Forest Meteorology*, 138: 156–167.

Stanisavljevic, N.S., Nikolic, D.B., Jovanovic, Z.S., Samardzic, J.T., Radovic, S.R. & Maksimovic, V.R. (2011). Antioxidative enzymes in the response of buckwheat (*Fagopyrum esculentu*m Moench) to complete submergence. *Archives of Biological Sciences*, 63: 399–405.

Stefanello, R., das Neves, L.A.S., Abbad, M.A.B., Viana, B.B. (2015a). Physiological response of chia seeds (*Salvia hispanica*-Lamiales: Lamiaceae) to saline stress. *Biotemas*, 28: 35–39.

Stefanello, R., das Neves, L.A.S., Abbad, M.A.B., Viana, B.B. (2015b). Germination and vigor of chia seeds (Salvia hispanica L.-Lamiaceae) under different temperatures and light conditions. *Brazilian Journal of Medicinal Plants*, 17: 1182–1186.

Steinmaus, S. J., Timothy, S., Prather, Holt, J.D. (2000). Estimation of base temperatures for nine weed species. *Journal of Experimental Botany*, 51 (343): 275–286.

Tabrizi, L., Nassiri Mahallati, M., Koocheki, A. (2004). Investigations on the cardinal temperatures for germination of *Plantago ovata* and *Plantago psyllium*. *J Iran Field Crop Res*, 2(2): 143–50.

Tammela, P., Salo-Väänänen P., Laakso I., Hopia A., Vuorela H. & Nygren M. (2005) Tocopherols, tocotrienols and fatty acids as indicators of natural ageing in *Pinus sylvestris* seeds. *Scandinavian Journal of Forest Research*, 20 (5): 378-384.

Thanos, C.A., Doussi, M.A. (1995). Ecophysiology of seed germination in endemic Labiates of Crete. *Isr J Plant Sci.*, 43: 227–37.

Thomas, J.M.G., Boote, K.J, Allen, L.H.Jr., Gallo-Meagher, M., Davis, J.M. (2003). Elevated temperature and carbon dioxide effects on soybean seed composition and transcript abundance. *Crop Sci*, 43: 1548−1557.

Thomashow, M.F. (1999). Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. *Annu Rev Plant Physiol Plant Mol Biol.,* 50: 571–599.

Trudgill, D.L., Squire, G.R. & Thimpson, K. (2000). A thermal time basis for comparing the germination requirements of some British herbaceous plants. *New Phytologist*, 145: 107-114.

Vega, A.J. & Chapman, S. (2001). Genotype by environment interaction and indirect selection for yield in sunflower: three-mod principal component analysis of oil and biomass yield across environments in Argentina. *Field Crops Research*, 72: 39–50.

Villaseñor, J. L. (2016). Checklist of the native vascular plants of Mexico. *Revista Mexicana de Biodiversidad*, 87(3), 559–902.

Vuksan, V., Whitham, D., Sievenpiper, J.L., Jenkins, A.L., Rogovik, A.L., Bazinet, R.P., Vidgen, E., Hanna, A. (2007). Supplementation of conventional therapy with the novel grain Salba (Salvia hispanica L.) improves major and emerging cardiovascular risk factors in type 2 diabetes: Results of a randomized controlled trial. *Diabetes Care*, 30: 2804–2810.

Wakjira, A., Labuschagne, M.T., Hugo, A. (2004). Variability in oil content and fatty acid composition of Ethiopian cultivars of linseed. *J. Sci. Food Agric*., 84: 601−607.

Weis, E., Berry, J. A. Plants and high temperature stress. *Symp. Soc. Exp. Biol*., 42: 329−346.

Western, T.L. (2012). The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. *Seed Sci Res*, 22: 1–25.

Wilson, D. O. & McDonald, M. B. (1986). The lipid peroxidation model of seed ageing. *Seed Science and Technology*, 14: 269-300.

Wolk, W. D., Dillon, P. F., Copeland, L. F., & Dilley, D. R. (1989). Dynamics of Imbibition in Phaseolus vulgaris L. in Relation to Initial Seed Moisture Content. *Plant physiology*, 89(3): 805–810.

Xia, Q., Ponnaiah, M., Cueff, G., Rajjou, L., Prodhomme, D., Gibon, Y., Bailly, C., Corbineau, F., Meimoun, P. & El-Maarouf-Bouteau, H. (2018). Integrating proteomics and enzymatic profiling to decipher seed metabolism affected by temperature in seed dormancy and germination. *Plant science : an international journal of experimental plant biology*, *269*, 118–125.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. & Shen, S. (2007). Proteomic analysis of rice (*Oryza sativa*) seeds during germination. *Proteomics* 7, 3358–3368.

Yin, X., Kropff, M.J., McLaren, G. & Visperas, R.M. (1995). A non- linear model for crop development as a function of temperature. *Agric For Meteorol*, 77: 1–16.

Zacheo, G. & Cappello, A. & Perrone, L. M. & Gnoni, G. (1998). Analysis of Factors Influencing Lipid Oxidation of Almond Seeds during Accelerated Ageing. *Lwt - Food Science and Technology*, 31: 6-9.

Zare, T., Rupasinghe, T.W.T., Boughton, B.A., Roessner, U. (2019). The changes in the release level of polyunsaturated fatty acids (ω-3 and ω-6) and lipids in the untreated and water-soaked chia seed. *Food Res Int.*, 126: 108665.

Zhang, M., Barg, R., Yin, M., Gueta-Dahan, Y., Leikin-Frenkel, A., Salts, Y., Shabtai, S., Ben-Hayyim G. (2005). Modulated fatty acid desaturation via overexpression of two distinct omega-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. *Plant J.*, 44: 361−71. 61.

Zhao, M., Zhang, H., Yan, H., Qiu, L., Baskin, C. C. (2018). Mobilization and Role of Starch, Protein, and Fat Reserves during Seed Germination of Six Wild Grassland Species. *Frontiers in Plant Science*, 2018: 9.