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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

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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 proper-

ties 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. $\boldsymbol{G}(\%)$ was reported as the average cumulative percentage of germinated seeds in each treatment, calculated according to:

$$\boldsymbol{G}(\%) = \frac{\boldsymbol{n}}{\boldsymbol{N}} \times \mathbf{100} \quad (1)$$

where $\boldsymbol{n}$ is the number of seeds germinated and $\boldsymbol{N}$ 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]:

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

where $\boldsymbol{G_i}$ is the number of germinated seeds and $\boldsymbol{N_i}$ 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}.

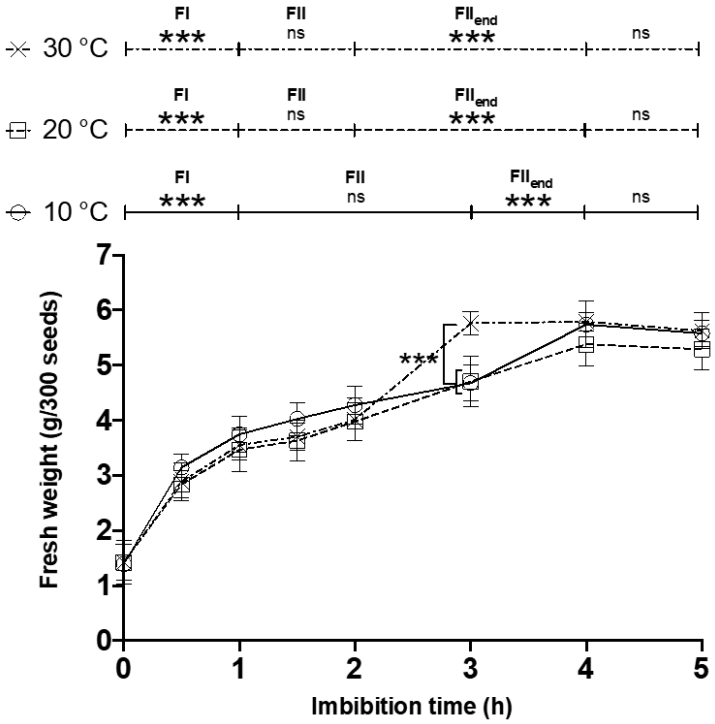

Figure 1. Imbibition curves of *S. hispanica* seeds at 10, 20 and 30 °C. Values are expressed as mean ± SD of five independent replicates. In the top is indicated the temporality of the imbibition phases FI, FII and FII_{end} in each of the temperature treatments. Statistical analysis was performed using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

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).

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 (*** $p < 0.001$).

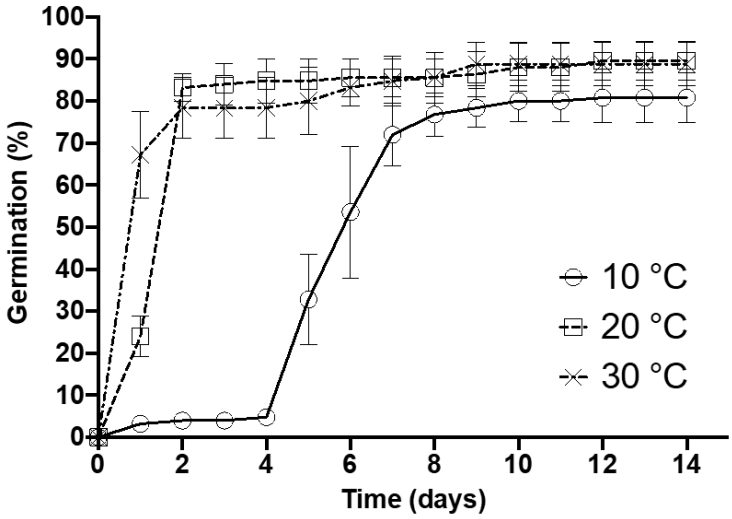

Figure 2. Cumulative germination of *S. hispanica* at 10, 20 and 30 °C. Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis followed by a Dunn's multiple comparison test.

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).

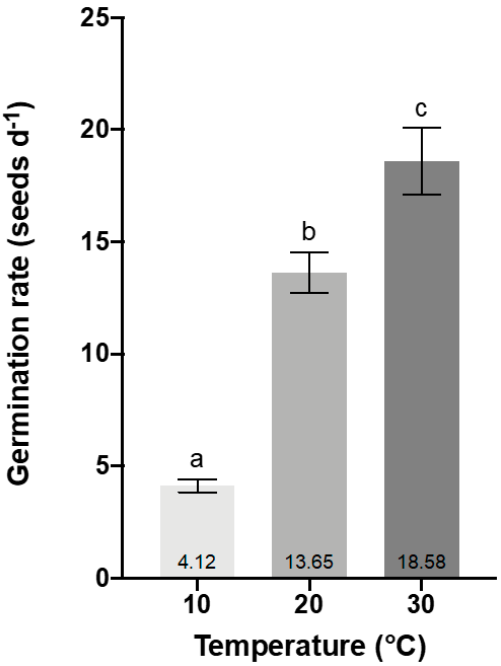

Figure 3. Germination rate (number of germinated seeds per day) at 10, 20 and 30 °C. Values are expressed as mean ± SD of five independent replicates. Statistical analysis was performed using Kruskal-Wallis followed by a Dunn's multiple comparison test. Different letters indicate significant differences ($p < 0.001$).

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.

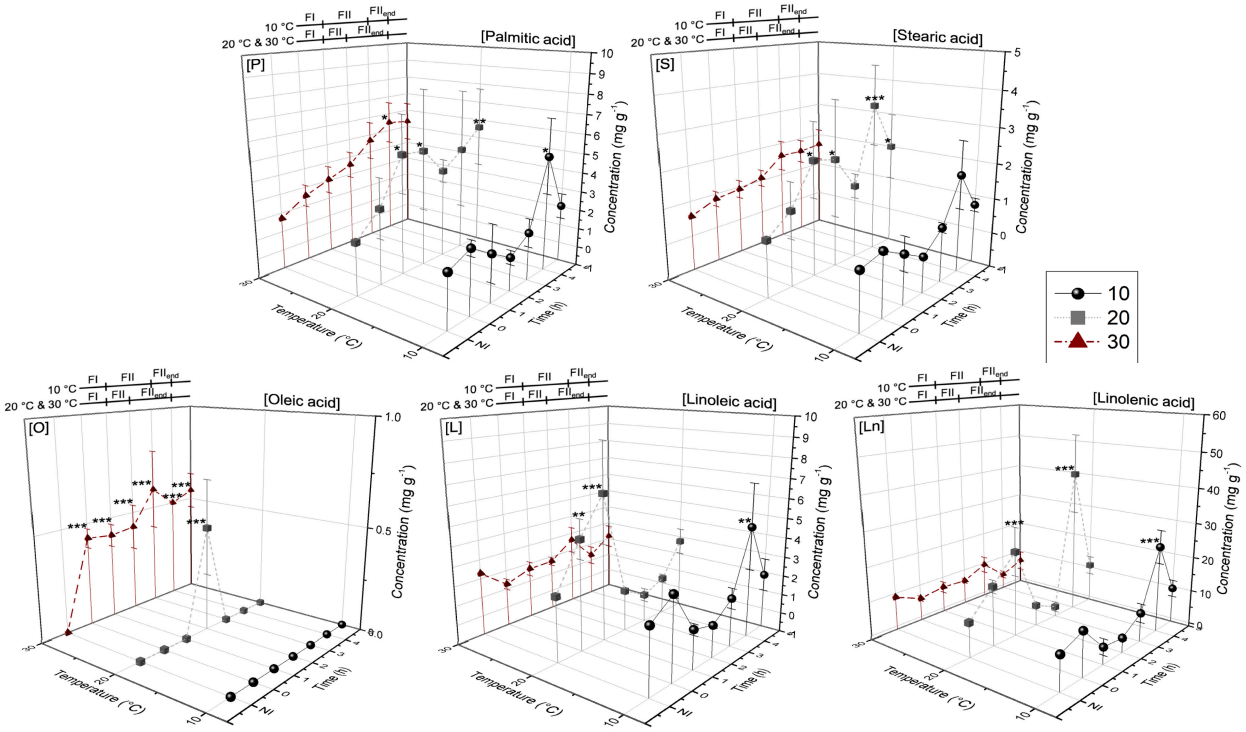

Figure 4. Fatty acid concentration changes in *S. hispanica* seeds related with imbibition stages (FI, FII and FII_{end}) at 10, 20 and 30 °C. Concentrations were calculated relative to the weight of non-imbibed seeds (NI) within the total weight. Values are expressed as mean ± SD of three independent 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 (* $p = 0.033$; ** $p = 0.002$; *** $p < 0.001$).

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}).

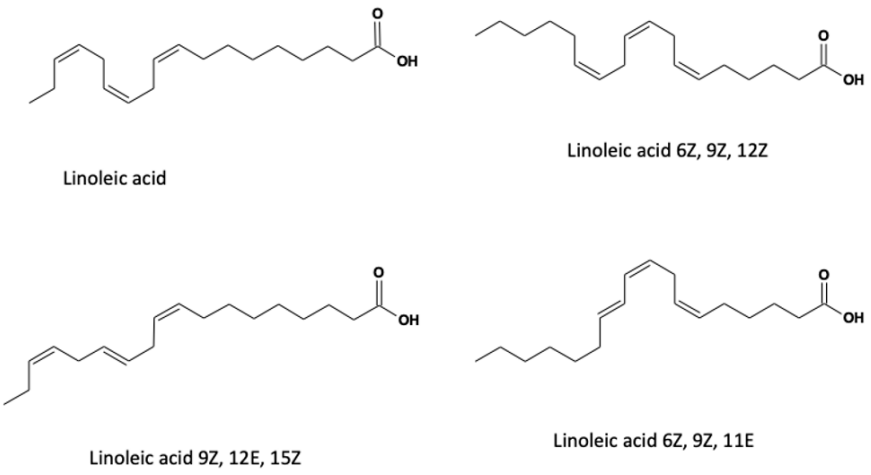

Figure 5. Linoleic acid isomers found in *S. hispanica* during seeds imbibition.

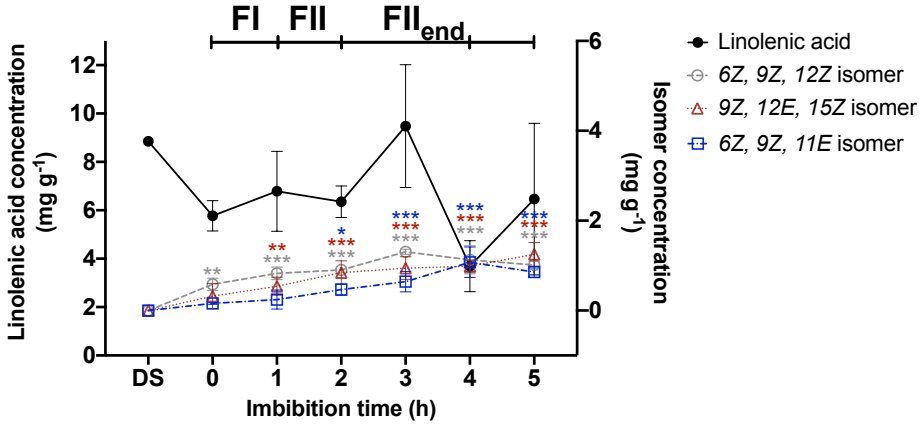

Figure 6. Fatty acid isomers in *S. hispanica* seeds. The imbibition stages (FI, FII and FII_{end}) are indicated throughout the imbibition trend at 30 °C. Concentrations were calculated relative to the weight of non-imbibed seeds (NI) within the total weight. Values are expressed as mean ± SD of three 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.

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 issue 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.

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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. *Br. J. Nutr.* **2009**, *101*, 41–50. [CrossRef] [PubMed]
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. *J. Food Eng.* **2015**, *149*, 70–77. [CrossRef]
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 Cent.* **2016**, *15*, 1–13. [CrossRef]
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). *Cultiv. Trop.* **2013**, *34*, 55–59.
6. Beltrán-Orozco, M.; Romero, M. Chía, alimento milenario. *Rev. Ind. Aliment.* **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. *Genet. Resour. Crop. Evol.* **2012**, *59*, 171–178. [CrossRef]

8. Ayerza, R. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1079–1081. [CrossRef]
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. [CrossRef]
10. Coates, W.; Ayerza, R. Production potential of chia in northwestern Argentina. *Ind. Crop. Prod.* **1996**, *5*, 229–233. [CrossRef]
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. Crops Prod.* **2002**, *15*, 163–167. [CrossRef]
12. Ixtaina, V.; Nolasco, S.; Tomás, M. Physical properties of chia (*Salvia hispanica* L.) seeds. *Ind. Crops Prod.* **2008**, *28*, 286–293. [CrossRef]
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. [CrossRef]
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. [CrossRef] [PubMed]
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. [CrossRef] [PubMed]
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. *EFSA J.* **2009**, *996*, 1–26.
18. Ayerza, R.; Coates, W. Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.). *Ind. Crops Prod.* **2011**, *34*, 1366–1371. [CrossRef]
19. Lee, A.S. The Effects of *Salvia hispanica* L. (Salba) on Postprandial Glycemia and Subjective Appetit. Master's Thesis, Nutritional Sciences University of Toronto, Toronto, ON, Canada, 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. [CrossRef]
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. Seed germination and vigor. *Annu. Rev. Plant. Biol.* **2012**, *63*, 507–533. [CrossRef]
23. Bewley, J.; Bradford, K.; Hilhorst, H.; Nonogaki, H. *Seeds: Physiology of Development, Germination and Dormancy*, 3rd ed.; Springer: Cham, Switzerland, 2013; pp. 85–246.
24. Nonogaki, H.; Bassel, G.; Bewley, J. Germination—Still a mystery. *Plant Sci.* **2010**, *179*, 574–581. [CrossRef]
25. Nonogaki, H. Seed Biology Updates—Highlights and New Discoveries in Seed Dormancy and Germination Research. *Front. Plant Sci.* **2017**, *8*, 524. [CrossRef] [PubMed]
26. Benech-Arnold, R.; Sánchez, R.; Forcella, F.; Kruk, B.; Ghersa, C. Environmental control of dormancy in weed seed banks in soil. *Field Crop. Res.* **2000**, *67*, 105–122. [CrossRef]
27. Batlla, D.; Benech-Arnold, R. A framework for the interpretation of temperature effects on dormancy and germination in seed populations showing dormancy. *Seed Sci. Res.* **2015**, *25*, 147–158. [CrossRef]
28. Bewley, J.; Black, M. *Seeds: Physiology of Development and Germination*, 2nd ed.; Springer: Cham, Switzerland, 1994; pp. 147–197.
29. Marcos Filho, J. Seed vigor testing: An overview of the past, present and future perspective. *Sci. Agric.* **2015**, *72*, 363–374. [CrossRef]
30. Ferreri, C.; Chatgilialoglu, C. *Encyclopedia of Radicals in Chemistry, Biology and Materials*, 1st ed.; Wiley: Toronto, ON, Canada, 2012; pp. 1599–1623.
31. Jouhet, J. Importance of the hexagonal lipid phase in biological membrane organization. *Front. Plant Sci.* **2013**, *4*, 494. [CrossRef]
32. Lukatkin, A.; Brazaitytė, A.; Bobinas, A.; Duchovskis, P. Chilling injury in chilling-sensitive plants: A review. *Zemdirb. Agric.* **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 Sci. Technol.* **1998**, *31*, 6–9. [CrossRef]
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. [CrossRef] [PubMed]
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. [CrossRef]
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 Cell Physiol.* **2019**, *60*, 1457–1470. [CrossRef] [PubMed]
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. *Biochem. Biophys. Res. Commun.* **2019**, *515*, 289–295. [CrossRef] [PubMed]
38. Sudhakaran, I. Effect of Seed Ageing in Biochemical and Molecular Changes in Oilseeds: A Review. *Agric. Rev.* **2020**, *41*, 408–412.

39. Noblet, A.; Leymarie, J.; Bailly, C. Chilling temperature remodels phospholipidome of *Zea mays* seeds during imbibition. *Sci. Rep.* **2017**, *7*, 8886. [CrossRef] [PubMed]
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. [CrossRef]
41. Fahad, S.; Bajwa, A.A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S.; et al. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Front. Plant Sci.* **2017**, *8*, 1147. [CrossRef]
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. [CrossRef]
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. [CrossRef]
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. *Ind. Crop. Prod.* **2009**, *30*, 321–324. [CrossRef]
45. Muñoz, L.; Cobos, A.; Diaz, O.; Aguilera, J. Chia seeds: Microstructure, mucilage extraction and hydration. *J. Food Eng.* **2012**, *108*, 216–224. [CrossRef]
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. *Ind. Crop. Prod.* **2013**, *51*, 453–462. [CrossRef]
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.; et al. Thermal Time and Cardinal Temperatures for Germination of *Cedrela odorata* L. *Forest* **2019**, *10*, 41. [CrossRef]
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. Agron.* **2016**, *38*, 513–519. [CrossRef]
49. International Seed Testing Association—ISTA. *International Rules for Seed Testing*, 2020th 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. [CrossRef]
51. Maguire, J.D. Speed of Germination—Aid in Selection and Evaluation for Seedling Emergence and Vigor. *Crop Sci.* **1962**, *2*, 176–177. [CrossRef]
52. Priestley, D.; Wermer, B.; Leopold, C.; McBride, M. Organic free radical levels in seeds and pollen: The effects of hydration and aging. *Physiol. Plant.* **1985**, *64*, 88–94. [CrossRef]
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. *Plant Cell* **1997**, *9*, 1055–1066. [CrossRef] [PubMed]
55. Nayani, S.; Rao, S. Extraction of Mucilage from Chia Seeds and its Application as Fat Replacer in Biscuits. *Int. J. Eng. Res. Technol.* **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. [CrossRef]
57. Singh, B.; Chauhan, G.S.; Bhatt, S.S.; Kumar, K. Metal ion sorption and swelling studies of psyllium and acrylic acid based hydrogels. *Carbohydr. Polym.* **2006**, *64*, 50–56. [CrossRef]
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). *Carbohydr. Polym.* **2007**, *67*, 190–200. [CrossRef]
59. Thapliyal, R.C.; Phartyal, S.S.; Baskin, J.M.; Baskin, C.C. Role of mucilage in germination of *Dillenia indica* (Dilleniaceae) seeds. *Aust. J. Bot.* **2008**, *56*, 583–589. [CrossRef]
60. Lobo, R.; Alcocer, M.; Fuentes, F.; Rodríguez, W.; Morandini, M.; Devani, M. Desarrollo del cultivo de chía en Tucumán, República Argentina. *EEAOC-Av. Agroind.* **2011**, *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. *Ital. J. Agron.* **2015**, *10*, 163–166. [CrossRef]
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. *Rev. Mex. De Cienc. Agrícolas* **2014**, *10*, 1831–1842.
63. Paiva, E.P.D.; Torres, S.B.; Alves, T.R.C.; Sá, F.V.D.S.; Leite, M.D.S.; Dombroski, J.L.D. Germination and biochemical components of *Salvia hispanica* L. seeds at different salinity levels and temperatures. *Acta Scientiarum. Agron.* **2018**, *40*, e39396. [CrossRef]
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. [CrossRef] [PubMed]
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.). *Agron. Res.* **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* **2015**, *28*, 35–39. [CrossRef]

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. *Braz. J. Med. Plants* **2015**, *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. [CrossRef]
69. Bita, C.; Gerats, T. Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci.* **2013**, *4*, 1–18. [CrossRef] [PubMed]
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. [CrossRef]
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. [CrossRef] [PubMed]
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. *Int. J. Food Sci.* **2014**, *2014*, 1–5. [CrossRef] [PubMed]
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. [CrossRef]
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. [CrossRef]
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. [CrossRef]
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. [CrossRef]
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. *J. Food Compos. Anal.* **2007**, *20*, 80–89. [CrossRef]
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. [CrossRef]
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. [CrossRef]
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*, 347–354. [CrossRef] [PubMed]
82. Nakamura, M.; Nara, T. Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. *Annu. Rev. Nutr.* **2004**, *24*, 345–376. [CrossRef] [PubMed]
83. Lee, J.; Lee, H.; Kang, S.; Park, W. Fatty Acid Desaturases, Polyunsaturated Fatty Acid Regulation, and Biotechnological Advances. *Nutrients* **2016**, *8*, 23. [CrossRef] [PubMed]
84. Dybing, C.; Zimmerman, D. Fatty acid accumulation in maturing flaxseeds as influenced by environment. *Plant Physiol.* **1966**, *41*, 1465–1470. [CrossRef]
85. Batlla, D.; Benech-Arnold, R.L. Predicting changes in dormancy level in weed seed soil banks: Implications for weed management. *Crop Prot.* **2007**, *26*, 189–197. [CrossRef]
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 Physiol.* **1970**, *46*, 500–508. [CrossRef]
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. [CrossRef] [PubMed]
88. Pritchard, S.; Charlton, W.; Baker, A.; Graham, I. Germination and storage reserve mobilization are regulated independently in Arabidopsis. *Plant J.* **2002**, *31*, 639–647. [CrossRef] [PubMed]
89. Rosental, L.; Nonogaki, H.; Fait, A. Activation and regulation of primary metabolism during seed germination. *Seed Sci. Res.* **2014**, *24*, 1–15. [CrossRef]
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, ON, Canada, 2017; pp. 34–51.
91. Hazel, J.R. Thermal Adaptation in Biological Membranes: Is Homeoviscous Adaptation the Explanation? *Annu. Rev. Physiol.* **1995**, *57*, 19–42. [CrossRef]
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. [CrossRef]
93. Sinensky, M. Homeoviscous adaptation—A homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 522–525. [CrossRef]
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*, 361–371. [CrossRef]
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. [CrossRef]

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. [PubMed]
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. [CrossRef]
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. [CrossRef] [PubMed]
100. Oteng, A.; Kersten, S. Mechanisms of Action of *trans* Fatty Acids. *Adv. Nutr.* **2020**, *11*, 697–708. [CrossRef] [PubMed]