3-15-2019

Impact of Row Spacing, Sowing Density and Nitrogen Fertilization on Yield and Quality Traits of Chia (*Salvia Hispanica* L.) Cultivated in Southwestern Germany

Samantha J. Grimes
University of Hohenheim, Germany

Timothy D. Phillips
University of Kentucky, tphillip@uky.edu

Filippo Capezzone
University of Hohenheim, Germany

Simone Graeff-Hönninger
University of Kentucky

Repository Citation

Grimes, Samantha J.; Phillips, Timothy D.; Capezzone, Filippo; and Graeff-Hönninger, Simone, "Impact of Row Spacing, Sowing Density and Nitrogen Fertilization on Yield and Quality Traits of Chia (*Salvia Hispanica* L.) Cultivated in Southwestern Germany" (2019). *Plant and Soil Sciences Faculty Publications*. 130.
https://uknowledge.uky.edu/pss_facpub/130

Impact of Row Spacing, Sowing Density and Nitrogen Fertilization on Yield and Quality Traits of Chia (*Salvia Hispanica* L.) Cultivated in Southwestern Germany

Notes/Citation Information
Published in *Agronomy*, v. 9, issue 3, 136, p. 1-21.

Digital Object Identifier (DOI)
https://doi.org/10.3390/agronomy9030136

agronomy

MDPI

Article

Impact of Row Spacing, Sowing Density and Nitrogen Fertilization on Yield and Quality Traits of chia (*Salvia Hispanica* L.) Cultivated in southwestern Germany

Samantha J. Grimes [1,*], Timothy D. Phillips [2], Filippo Capezzone [3] and Simone Graeff-Hönninger [1]

[1] Department of Agronomy, Institute of Crop Science, University of Hohenheim, 70599 Stuttgart, Germany; simone.graeff@uni-hohenheim.de
[2] Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546-0312, USA; tim.phillips@uky.edu
[3] Department of Biostatistics, Institute of Crop Science, University of Hohenheim, 70599 Stuttgart, Germany; filippo.capezzone@uni-hohenheim.de
* Correspondence: s.grimes@uni-hohenheim; Tel.: +49-711-459-23066

Received: 18 February 2019; Accepted: 6 March 2019; Published: 15 March 2019

Abstract: To obtain high chia seed yields and seed qualities, a suitable crop management system needs to be developed for the given growing conditions in southwestern Germany. Field experiments were conducted at the experimental station Ihinger Hof in two consecutive years (2016, 2017). The study aimed to evaluate yield and quality traits of chia depending on different (i) row spacing (35, 50 and 75 cm), (ii) sowing densities (1, 1.5 and 2 kg ha^{-1}) and, (iii) N-fertilization rates (0, 20 and 40 kg N ha^{-1}). It consisted of three independent, completely randomized field experiments with three replications. Results showed that chia seed yields ranged from 618.39 to 1171.33 kg ha^{-1} and that a thousand seed mass of 1.14 to 1.24 g could be obtained. Crude protein-, crude oil- and mucilage contents varied from 18.11–23.91%, 32.16–33.78% and 10.00–13.74%, respectively. Results indicated that the year of cultivation and the accompanied environmental conditions, like precipitation or temperature, influenced the determined traits more than the applied agronomic practices. As average seed yields exceeded those obtained in the countries of origin (Mexico, Guatemala) while having comparable quality characteristics, chia holds great potential as an alternative crop for farmers in southwestern Germany.

Keywords: *Salvia hispanica*; crop management; seed yield; seed quality traits

1. Introduction

The gold of the Aztecs, or what we refer to as chia (*Salvia hispanica* L.) today, is a summer annual herbaceous plant belonging to the *Lamiaceae* family. Its traditional areas of cultivation are from North Central Mexico into Guatemala [1]. In recent years, chia seeds gained popularity due to their exceptional nutritional composition as consumer food choices are increasingly focused on the positive effects that diet and nutrition can have on overall health [1–3]. In addition to the health aspect and trends, consumers increasingly value local and regional food products and buy them in high proportions [4].

Growth and development of chia, originally a short-day flowering species, is driven by day length. Vegetative plant growth takes place under long day conditions (>12 h). Subsequently, its generative growth is stimulated by short day conditions (<12 h) [1]. Its photoperiodic sensitivity is precisely the reason why chia plants are unable to reach seed maturity under the climatic conditions of southwestern Germany, as the plants are killed before flowering by frost. Hence, its photoperiodic sensitivity is the bottleneck for its expansion to the currently existing northern and southern agricultural cultivation borders [5].

Jamboonsri et al. and Hildebrand et al. bred a chia genotype that induced inflorescences under long day photoperiods of about 12–15 h enabling seed production under day length conditions of greater than 12 h [1,6]. This milestone achievement enabled Grimes et al. [7] to successfully cultivate different chia genotypes under the climatic conditions given in southwestern Germany obtaining seed yields and qualities in line with the current literature.

However, besides solving the physiological hindrances for growing chia far north, a corresponding production system for chia has yet to be developed to advise farmers and growers. To date, scientific literature on crop management techniques, physiological aspects and agronomic characterization of chia is generally quite scarce, even more so for the production of chia outside of its Mesoamerican origin [8–11]. In regard to reported sowing densities in the literature, Bochicchio et al. [12] stated that growth and yield of chia were highest at sowing densities of 125 plants m^{-2} which is contrary to Bilalis et al. [13] who stated that chia growth was not affected by sowing rate. In terms of quality traits, Bilalis et al. [13] showed that the crude protein of chia significantly increased as sowing rates increased.

Furthermore, the large degree of plasticity in chia in terms of vegetative and generative growth, representing a compensatory mechanism related to agronomic traits, makes it complicated to evaluate the direct influence of different row spacing in chia. When it comes to determining possible applicable row spacing, weed management is an inherent issue as there are currently no approved herbicides available for chia in Germany. Tested pre-emergence herbicides had a significantly adverse effect on chia growth and biomass yield [14]. Therefore, in many cases row spacing is adjusted to present hoeing techniques, allowing mechanical weed control, rather than maximizing seed yield per area.

In addition to sowing density and row spacing, nitrogen fertilization plays a critical role concerning seed yield and seed quality traits. It was shown by Bochicchio et al. [12] that N-top-dressing had a negative effect on chia seed yields whereas Bilalis et al. [13] reported that chia growth was not affected by fertilization. An assessment of optimized nitrogen fertilization is necessary in order to evaluate the direct effects on plant maturity, seed yield, and protein content, respectively.

In this context the present study aimed at developing a cropping system for chia under the climatic conditions of southwestern Germany while examining the impact of different (i) row spacing (35, 50 and 75 cm), (ii) sowing densities (1, 1.5 and 2 kg ha^{-1}), and (iii) N-fertilizer rates (0, 20 and 40 kg N ha^{-1}) on final yield traits and grain quality (protein, oil and mucilage content) during a two-year field study.

2. Materials and Methods

2.1. Site Description

Field trials were conducted in two consecutive growing seasons (2016, 2017) at the experimental station Ihinger Hof (University of Hohenheim, Upper Neckarland, Lat. N 48°44′40,70″ Lon. E 8°55′26,36″). Precipitation during the experimental period at Ihinger Hof in 2016 amounted to 306.3 mm and the mean temperature during the experimental period was 14.2 °C. In 2017, precipitation amounted to 401.7 mm while the mean temperature was 14.4 °C. Meteorological data was obtained by the weather station at Ihinger Hof (Figure 1).

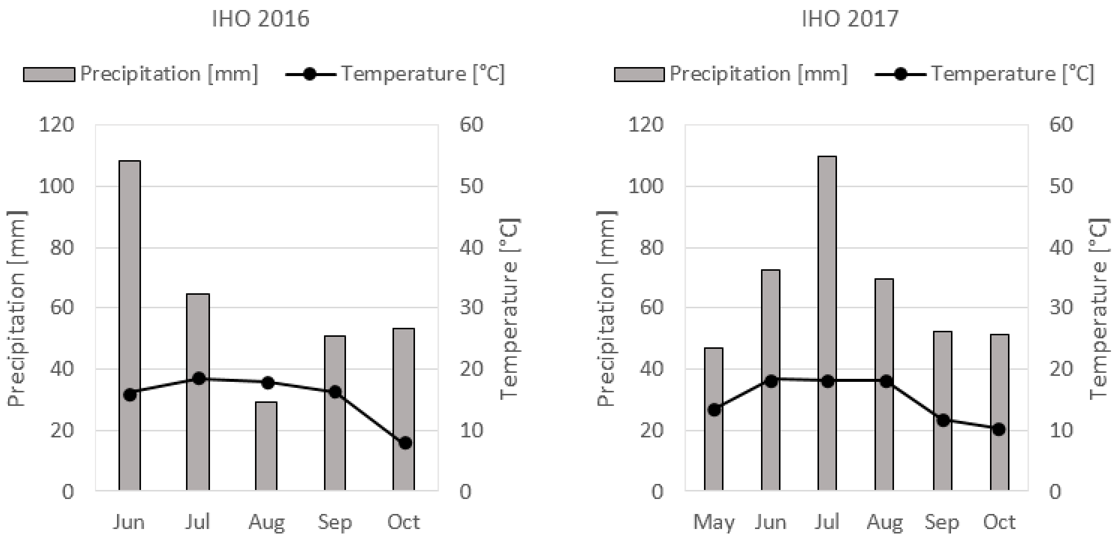

Figure 1. Precipitation [mm, bars] and mean temperature (°C; ●) during the experimental period at Ihinger Hof in 2016 and 2017 (June–October, and May–October, respectively).

Growing degree-days (GDD) for both locations were calculated using the growing degree-day Equation (1) of McMaster and Wilhelm [15], where TMAX is defined as the daily maximum air temperature and TMIN is defined as the daily minimum air temperature and TBASE as the crop base temperature. The crop base temperature of 10 °C according to Baginsky et al. [2], was subtracted from the daily average air temperature.

$$GDD = \left[\frac{(T_{MAX} + T_{MIN})}{2}\right] - T_{BASE} \tag{1}$$

If the daily maximum air temperature was less than the crop base temperature, $((T_{MAX} + T_{MIN})/2) < T_{BASE}$, $then$ $((T_{MAX} + T_{MIN})/2) = T_{BASE}$.

Experimental soils were characterized as Pelosol brown soil in 2016 and 2017 according to the IIUSS Working Group WRB [16]. In both years, soil samples were collected in X-form across the experimental site on which the three spatially separated field trials were conducted to a depth of 0–90 cm (0–30, 30–60, 60–90 cm) using a soil auger assembled to a composite sample.

The composition of the clay, sand and silt contents of the topsoil in both years at Ihinger Hof were quite similar. The sand content was about 8%, the silt content was about 67%, and clay content amounted to 25% in 2016 (Table 1). The experimental site in 2017 showed contents of sand, silt, and clay of about 3%, 70% and, 27%, respectively. The pH value was 7.45 in 2016 and 6.63 in 2017. Before sowing, soil mineral nitrogen content (N_{min}) from 0 to 90 cm amounted to 22.96 kg ha^{-1} in 2016 and 100.28 kg ha^{-1} in 2017.

Table 1. Soil characteristics of the experimental site at Ihinger Hof in 2016 and 2017.

Year	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH	N_{min} [a] (kg ha^{-1})
2016	30	7.85	66.95	25.2	7.45	6.54
	60					5.10
	90					11.32
2017	30	2.72	70.18	27.1	6.63	59.46
	60	2.41	70.44	27.15		27.58
	90	3.29	63.61	33.1		13.24

[a] N_{min} = soil mineral nitrogen content.

2.2. Trial Setup

Three spatially separated field trials were carried out on the same homogenously cultivated experimental site in order to evaluate the impact of different (i) row spacing (35, 50 and 75 cm), (ii) sowing densities (1, 1.5 and 2 kg ha^{-1}), and (iii) N-fertilization rates (0, 20 and 40 kg N ha^{-1}) on yield and quality traits of the blue flowering, charcoal-seeded genotype G8. This genotype was generated by gamma ray-mutagenesis according to Hildebrand et al. [6] and was provided by the University of Kentucky, USA. Field trials were carried out as randomized complete block designs with three replications for each treatment. Experimental fields differed between 2016 and 2017. In 2016 the size of each single field experiment amounted to 57.5 m × 20 m (plot size: 4 m × 15 m) and 42.5 m × 15 m (plot size: 3 m × 10 m) in 2017. The previous crop in 2016 was silage maize (*Zea mays* L.) whereas in 2017 it was winter wheat (*Triticum aestivum* L.). The following cultivation measures have been performed identically across the three conducted trials during the two consecutive growing seasons in 2016 and 2017.

In 2016, two passes of seedbed preparation to a depth of 8 cm were carried out prior to sowing. The first pass was conducted with the help of a Fendt 716 (AGCO GmbH, Duluth, Georgia, USA) equipped with a rotary harrow (LEMKEN GmbH and Co. KG, Alpen, Germany). The second pass was again done with a Fendt 716 as an equipment device and a semi-mounted seedbed combination System-Korund (LEMKEN GmbH and Co. KG, Alpen, Germany). In 2017, prior to sowing, one pass of seedbed preparation was conducted to a depth of 6 cm with a Fendt 414 (AGCO GmbH, Duluth, GA 30096, USA) representing the equipment device and mounted crumble rollers (Kverneland Group Deutschland GmbH, Soest, Germany), respectively.

Mechanical sowing of all field trials took place on 1 June in 2016 and on 17 May 2017, respectively, using the Fendt 207 (AGCO GmbH, Duluth, GA 30096, USA) and the 121 Deppe D82 Sower (Agrar Markt DEPPE GmbH, Rosdorf, Germany) at a speed of 2.8 km h^{-1} and a depth of 1 cm.

Fertilization in 2016 took place with a fertilizer spreader "Amazone" (AMAZONEN-WERKE H. Dreyer GmbH and Co. KG, Hasbergen, Germany) whereas in 2017 a universal fertilization spreader "UKS 230" (RAUCH Landmaschinenfabrik GmbH, Sinzheim, Germany) was attached to a Fendt 207 (AGCO GmbH, Duluth, GA 30096, USA).

Mechanical weed control was performed by the "CHOPSTAR 25–59 cm" row hoe equipped with finger weeders (Einböck GmbH and CoKG, Dorf an der Pram, Austria) attached to the Fendt 207 (AGCO GmbH, Duluth, GA, USA) in 2016. In 2017 the Fendt 207 (AGCO GmbH, Duluth, GA, USA) was equipped with a rotary hoe (Rau Landtechnik GmbH, Brigachtal, Germany) in order to perform the mechanical weed control.

In 2017, 150 g ha^{-1} Lambda-Cyhalothrin dissolved in 300 l H_2O ha^{-1} (Lambda WG, ADAMA Deutschland GmbH, Cologne, Germany) was applied 16 days after sowing with the help of a spraying system (Dammann, Buxtehude-Hedendorf, Germany) which was put on a Fendt equipment device (AGCO GmbH, Duluth, GA, USA) to eliminate flea beetle infestation.

2.2.1. Row Spacing Trial

In 2016, seedbed preparation was carried out 80 and 12 days prior to sowing, whereas in 2017 seedbed preparation was conducted one day before sowing. Three different row spacing (35, 50, 75 cm) were established in triplicates for each treatment in both consecutive growing seasons. A sowing rate of 1.5 kg ha^{-1} was realized. Nitrogen fertilization (20 kg N ha^{-1}) as calcium ammonium nitrate was applied eight (2016) and 15 (2017) days after sowing. Mechanical weed control was performed 32 and 33 days after sowing in 2016, and 27 and 29 days after sowing in 2017, respectively.

2.2.2. Sowing Density Trial

Seedbed preparation in 2016 took place 16 and 12 days before sowing and in 2017 one day before sowing. In both consecutive growing seasons, a row spacing of 50 cm was implemented, 20 kg N ha^{-1} were applied as calcium ammonium nitrate ten (2016) and 15 (2017) days after sowing. Three different sowing densities (1, 1.5, 2 kg ha^{-1}) were established in triplicates for each treatment. In 2016, mechanical weed control took place 34 days after sowing whereas in 2017 it took place 29 days after sowing, respectively.

2.2.3. Fertilizer Trial

Seedbed preparation in 2016 was performed 16, 12 and five days prior to sowing and one day before sowing in 2017. Nitrogen fertilizer (0, 20, 40 kg N ha^{-1}) was applied as calcium ammonium nitrate in triplicates for each treatment ten days after sowing in 2016 and 15 days after sowing in 2017. A row spacing of 50 cm and a sowing rate of 1.5 kg ha^{-1} was implemented in both consecutive growing seasons. Weed control was performed mechanically 34 (2016) and 29 (2017) days after sowing.

2.3. Experimental Procedure

2.3.1. Soil Mineral Nitrogen

Soil mineral nitrogen content was determined prior to sowing according to Bassler and Hoffman [17] as precisely described in Grimes et al. [7].

2.3.2. Yield Traits

Mechanical harvest took place with a plot combine Classic (Wintersteiger AG, Ried, Austria). On the basis of absolute dry matter (0% grain moisture), the plot seed yield was determined. In order to determine thousand seed mass (TSM), one thousand seeds per plot were counted using a Contador (Pfeuffer GmbH, Kitzingen, Germany) and weighed in order to calculate the mean thousand seed mass.

2.3.3. Quality Traits

Mucilage extraction took place according to Muñoz et al. [18]. Total seed nitrogen content was determined according to Dumas [19], using a vario MACRO cube CHNS (Elementar Analysensysteme GmbH, Langenselbold, Germany). The values were multiplied by 5.71 (conversion factors) in order to estimate the crude protein content [20]. Crude oil content was determined according to the European Commission's Regulation 152/III H procedure B [21], using petroleum benzene as a solvent for Soxhlet extraction. By rapid saponification and esterification, fatty acid methyl esters (FAMEs) were generated in order to identify the fatty acid profile [22–24]. Procedures mentioned above were amended according to Grimes et al. [7].

2.4. Statistical Analysis

Statistical analysis was conducted using SAS software version 9.4. The three independent field trials (i) row spacing, (ii) sowing density and (iii) fertilizer rate were conducted as randomized complete block designs and were evaluated separately. The following model was used for analysis of the assessed yield traits (yield, thousand seed mass) and quality traits (crude oil and protein content, mucilage and fatty acid contents, and the corresponding ratios):

$$y_{akj} = \mu + j_a + b_{aj} + \tau_k + (j\tau)_{ak} + e_{akj} \tag{2}$$

where y_{akj} is the observation of the k-th treatment in the j-th block of the a-th year, μ is the common intercept, j_a is the effect of the a-th year, τ_k is the effect of the k-th treatment, $(j\tau)_{ak}$ is the interaction of year and treatment, b_{aj} is the effect of the aj-th block, and e_{akj} are the errors associated with y_{akj}. e_{akj} was assumed to be normally distributed with mean zero variance.

Normality of residuals and homogeneity of variance were assessed by the inspection of plots of studentized residuals. Normal distribution was assessed on the basis of quantile-quantile plots and homogeneity of variance according to plots of residuals against predicted values. If residual plots indicated any violations, the response variable was transformed using logarithm or square root transformation. If residual plots showed different variation of the two experimental years, model (2) was extended to allow year-specific residual error variance.

The fixed effects in model (2) were tested for significance using partial Weald-type *F*-tests. Denominator degrees of freedom in *F*-tests were adjusted using the method of Roy [25]. Non-significant results at the α = 5% significance level were removed from the model. The levels of factors found significant were compared with Tukey's HSD test and results were presented as a letter display.

3. Results and Discussion

As demonstrated by Grimes et al. [7] chia cultivation, in general, is feasible under the climatic conditions in southwestern Germany as new genotypes, either adapted or insensitive to photoperiod, are available. Nevertheless, in order to successfully cultivate chia, agronomic management is a crucial factor. Establishing a chia cropping system, which is profitable in terms of yield and seed quality was the initial impetus for this study [8]. In addition to the cropping system, cultivation of chia is highly dependent on environmental factors, which enables the success of cultivation in general. Generating local information by means of studies is, therefore, critical as management recommendations are, so far, based on studies conducted in countries with different climatic conditions, especially in the areas of origin of South and Central America [9].

3.1. Plant Development

Impact of Day Length and Temperature

In all three field trials flower induction took place 65 and 68 days after sowing, respectively (Table 2) after having accumulated 463.3 growing degree days in 2016 and 529.75 in 2017. Radiation, day length and mean temperature until flower induction amounted to 10,184 Wh/m^2, 15.8 h and 17.3 °C in 2016 and 10,316 Wh/m^2, 15.9 h 18.0 °C in 2017, respectively (Table 2). In 2016 and 2017, harvest maturity was reached 150 and 156 days after sowing, having accumulated 971.75 and 948.3 growing degree days, respectively (Table 2).

The results indicated that the day length threshold to induce flower formation was obtained although the minimum of 600 growing degree days was not accumulated [26], demonstrating that the adaption of chia to day length is more relevant for the selection of suitable growing areas than the accumulation of growing degree days [7]. This is in line with Coates and Ayerza [27] who stated that photoperiod is the environmental factor that most influences floral development rate in chia. Hence, predicting days to flowering is essential, because the time between emergence and flowering determines plant size, thus affecting dry matter production and final crop yields.

Table 2. Sowing dates, accumulated growing degree-days (GDD), radiation, day length and mean temperatures at Ihinger Hof in 2016 and 2017.

Sowing Date	**Flower Induction**			**Radiation (Wh/m^2) [c]**	**Day Length (h) [d]**	**Mean Temp. (°C) [e]**	**Mean Temp. (°C) [f]**	**Harvest Maturity ***		
	DAS [a]	**Date**	**GDD [b]**					**DAS**	**Date**	**GDD**
June 1st 2016	65	August 4th 2016	463.3	10,184	15.8	17.3	14.2	150	October 28th	971.75
May 17th 2017	68	July 23rd 2017	529.75	10,316	15.9	18.0	14.4	156	October 19th	948.3

[a] DAS: Days after sowing. [b] GDD: Accumulated growing degree days with a base temperature of 10 °C [25]. [c] Average global radiation based on sunshine hours until flower induction [28] available from: http://www.kimberly.uidaho.edu/water/fao56/fao56.pdf (accessed on 15 January 2018). [d] Average day length until flower induction [29]. [e] Average mean temperature until flower induction. Obtained by the weather stations at IHO and EWE. [f] Average mean temperature from flower induction to harvest maturity obtained by the weather stations at IHO. * Harvest maturity is equivalent to vegetation period.

Phenological development was completed in all trials in both years, enabling the chia plants to reach harvest maturity.

3.2. Yield Traits

3.2.1. Row Spacing Trial

Over both years, seed yield and thousand seed mass of chia varied from 704.00 to 1171.33 kg ha^{-1} and from 1.17 to 1.24 g, respectively. For both traits, neither a significant effect of row-spacing nor an interaction of row-spacing and year was found in the *F*-tests. Instead, chia seed yield and thousand seed mass were significantly influenced by year of cultivation, decreasing from 2016 to 2017 ($p = 0.0017$ and $p < 0.0001$, respectively, Table 3). Being in line with studies presented by Ayerza and Coates [30,31] stating that environmental factors such as climate, soil conditions, sowing date, and precipitation play a crucial role in chia seed yield production and may have a high impact on final yield.

Table 3. Estimated seed yield (kg ha^{-1}) and thousand seed mass (TSM) (g) of chia (Genotype G8) based on model (2) cultivated at three different row spacing (35, 50, 75 cm) at Ihinger Hof in 2016 and 2017, along with standard error of the mean (SEM) and *F*-tests ($n = 3$, $\alpha = 0.05$).

Trait	**Year**		***F*-Test**			
Row Spacing (cm)	**2016**	**2017**	j_a	b_{aj}	τ_k	$(j\tau)_{ak}$
	Seed yield (kg ha^{-1}) ‡					
35	1171.33 A/a	819.67 B/a	0.0017	0.0004	0.3809	0.9284
50	1170.33 A/a	844.33 B/a				
75	1110.0 A/a	704.00 B/a				
	SEM = 35.12	SEM = 144.42				
	TSM [g]					
35	1.23 A/a	1.17 B/a	<0.0001	0.6242	0.8895	0.7997
50	1.23 A/a	1.17 B/a				
75	1.24 A/a	1.17 B/a				
	SEM = 0.010					

Estimates, average standard errors of the mean (SEM) and *p*-values from *F*-tests are based on model (2); j_a Annual effect; b_{aj} Block effect; τ_k Treatment effect (row spacing); $(j\tau)_{ak}$ Interaction between treatment and annual effect; Letter display: Estimates at a constant level of the treatment factor that share a capital letter do not differ significantly between years at $\alpha = 0.05$. Estimates within a year that share a lowercase letter do not differ significantly between treatment factor levels at $\alpha = 0.05$; ‡ For the marked traits model (2) was extended allowing heterogeneous error variances for each year.

Although the results of the present study indicated that row spacing did not significantly influence neither yield nor TSM, the lowest seed yield was obtained at the widest row space (75 cm) (Table 3). In this regard, Yeboah et al. [32] stated that narrow-row spacing of 0.5 m × 0.5 m consistently produced the highest chia seed yields (up to 3208 kg ha^{-1}) under Ghanaian conditions (Lat ~ 10° N). This pattern could also be observed by the present study (Table 3). A field trial conducted in India in 2016 (Lat 13° N) indicated the contrary as seed yields tended to increase with increasing row spacing; obtaining a maximum chia seed yield of 597.59 kg ha^{-1} at a row spacing of 60 cm × 45 cm [33]. For mustard (*Sinapis arvensis* L.), another oilseed crop, results of Kayaçetin et al. [34] also indicated the contrary as seed yields decreased with increasing row spacing from 20 to 60 cm under Turkish conditions. For sesame (*Sesamum indicum* L.), a negative relationship was observed between seed yield and row spacing whereas thousand seed mass significantly increased with row-spacing from 37.5 to 60 cm [35]. However, based on the results of our study on chia it can be concluded that a lower row spacing can be recommended in higher yielding environments and that the selection of the row spacing can be based more or less on the equipment, which is available to the farmer.

In the early development stages, chia is highly susceptible to weed infestation, and the associated competition for light, nutrients, and water as chia's growth rate is slow compared to common weeds [8,36]. Additionally, no herbicide has been approved in Europe for chia so far [14,37]. In this context, it is noteworthy to mention that the application of post-emergence herbicides on chia seems to be possible but, the selectivity of herbicides on chia has to be evaluated under different application rates, soil types and environmental conditions to make safe suggestions for chemical control of weeds [14]. Until canopy closure, it is possible to manually and mechanically control the weeds; as soon as the canopy closes weeds are suppressed by chia itself [36,38]. Therefore, mechanical weed control was conducted twice in the first four weeks of its cultivation. Thus, weed infestation did not impose a relevant issue regarding the growth and yield of chia in the presented (i) row spacing, (ii) sowing density and (iii) N-fertilizer rate trials in both years.

Nevertheless, Pozo Pozo [37] underlined the need to investigate weed control in relation to plant density of chia which is in line with Deligios et al. [39] who stated that a direct relationship between weed's biomass and crop yield could be found in various plant cultures. Results of thousand seed mass, representing a critical yield-determining trait, were found to be in-between the first reported biometric data (1.1–1.4 g) on chia seeds cultivated under European conditions [7,40]. As mentioned for seed yield and being in accordance with the results shown in Table 3, environmental factors seemed to be more influential on thousand seed mass than the row spacing.

3.2.2. Sowing Density Trial

Statistical analysis showed that the different sowing densities significantly influenced chia seed yield ranging from 618.33 to 881.67 kg ha^{-1} (p = 0.0114), independent of the year. The lowest sowing density (1 kg ha^{-1}) resulted in significantly lower seed yields, compared to the intermediate one (1.5 kg ha^{-1}) (Table 4).

Table 4. Estimated seed yield (kg ha^{-1}) and thousand seed mass (TSM) [g] of chia (Genotype G8) based on model (2) cultivated at three different sowing densities (1, 1.5, 2 kg ha^{-1}) at Ihinger Hof in 2016 and 2017, along with standard error of the mean (SEM) and *F*-tests (n = 3, α = 0.05).

Trait	**Year**		***F*-Test**			
Sowing Density [kg ha^{-1}]	**2016**	**2017**	j_a	b_{aj}	τ_k	$(j\tau)_{ak}$
	Seed yield [kg ha^{-1}]					
1	618.33 A/a	819.67 B/a	0.1122	0.0094	0.0114	0.4283
1.5	880.01 A/b	844.33 B/b				
2	748.00 A/ab	704.00 B/ab				
	SEM = 55.12					
	TSM [g] ‡					
1	1.22 A/a	1.21 A/a	0.1268	0.6378	0.0125	0.8024
1.5	1.20 A/b	1.18 A/b				
2	1.20 A/ab	1.19 A/ab				
	SEM = 0.016	SEM = 0.004				

Estimates, average standard errors of the mean (SEM) and p-values from F-tests are based on model (2); j_a Annual effect; b_{aj} Block effect; τ_k Treatment effect (sowing density); $(j\tau)_{ak}$ Interaction between treatment and annual effect; Letter display: Estimates at a constant level of the treatment factor that share a capital letter do not differ significantly between years at α = 0.05. Estimates within a year that share a lowercase letter do not differ significantly between treatment factor levels at α = 0.05; ‡ For the marked traits model (2) was extended allowing heterogeneous error variances for each year.

This is in line with results of a field experiment conducted in Southern Italy by Bochicchio et al. [12] who stated that growth and yield of chia were positively influenced by high sowing densities (125 plants m^2). In regard to plant density, and sowing density, Yeboah et al. [32] also observed that the variations in seed yield under Ghanaian conditions are often related to different planting methods and plant densities leading to higher seed yields at highest planting densities 40,000 plants/ha (0.5 m × 0.5 m). In its countries of origin like Argentina, Bolivia and Mexico sowing rates of 6–8 kg per hectare are often recommended [36]. Once the first pair of leaves has completely unfolded, plants are often thinned to a density of 80–90 plants m^2 [26].

As no statistical difference was detected in our study between the two sowing rates of 1.5 and 2 kg ha^{-1}, but significantly higher seed yields were obtained than in the countries of origin, sowing densities around 1.5 kg ha^{-1} can be recommended for the given environment in Germany.

Thousand seed mass was significantly influenced by sowing densities, varying from 1.18 to 1.22 g ($p = 0.0125$, Table 4). High sowing rates are associated with excessive numbers of plants resulting in severe interplant competition and a reduction in TSM on rapeseed and mustard according to Kayaçetin et al. [34] and Mamun et al. [41]. The present study showed a similar trend being in line with these findings as the highest TSM was obtained at the lowest sowing density of 1 kg ha^{-1} while lower TSMs were obtained at sowing densities of 1.5 and 2 kg ha^{-1} (Table 4).

3.2.3. N-Fertilizer Trial

Chia seed yields ranged from 745.67 to 847.00 kg ha^{-1} and were not significantly influenced by N-fertilization, year nor year × fertilization interaction even though an increase in nitrogen fertilizer rate led to higher chia seed yields in both years (Table 5). As nitrogen represents the most essential nutrient, being involved in a variety of metabolic processes strongly influencing plant growth and yield, this is remarkable [42].

Table 5. Estimated seed yield (kg ha^{-1}) and thousand seed mass (TSM) (g) of chia (Genotype G8) based on model (2) cultivated at three different fertilizer rates (0, 20, 40 kg N ha^{-1}) at Ihinger Hof in 2016 and 2017, along with standard error of the mean (SEM) and *F*-tests ($n = 3$, $\alpha = 0.05$).

Trait	**Year**		***F*-Test**			
Fertilizer Rate [kg N ha^{-1}]	**2016**	**2017**	j_a	b_{aj}	τ_k	$(j\tau)_{ak}$
	Seed yield (kg ha^{-1})					
0	745.67 A/a	768.33 A/a	0.4333	0.0020	0.4036	0.8123
20	751.67 A/a	841.33 A/a				
40	838.00 A/a	847.00 A/a				
	SEM = 66.10					
	TSM [g] ‡					
0	1.19 A/a	1.14 B/a	0.0319	0.3148	0.1297	0.1561
20	1.20 A/a	1.17 B/a				
40	1.20 A/a	1.19 B/a				
	SEM = 0.004	SEM = 0.012				

Estimates, average standard errors of the mean (SEM) and *p*-values from *F*-tests are based on model (2); j_a Annual effect; b_{aj} Block effect; τ_k Treatment effect (fertilizer rate); $(j\tau)_{ak}$ Interaction between treatment and annual effect; Letter display: Estimates at a constant level of the treatment factor that share a capital letter do not differ significantly between years at $\alpha = 0.05$. Estimates within a year that share a lowercase letter do not differ significantly between treatment factor levels at $\alpha = 0.05$; ‡ For the marked traits model (2) was extended allowing heterogeneous error variances for each year.

Thousand seed mass varied from 1.14 to 1.20 g and decreased significantly from 2016 to 2017 (p = 0.0319, Table 5). An increase in TSM was observed alongside increased nitrogen fertilization although no significant effect of the N-fertilization was apparent for thousand seed weight.

Results indicated that the applied N-fertilization rates did not affect chia seed yield significantly which is in line with the results of the indicative data presented by Bochicchio et al. [12]. Bilalis et al. [13] in this regard pointed out the lack of response to nitrogen topdressing with sheep manure and commercial organic fertilizer (fertilizer 6–8–10). This, however, contradicts the findings of Coates [38] who stated that chia seed yields up to about 2500 kg ha^{-1} could be achieved under high input conditions with irrigation and fertilization in some experimental trials in Argentina, and Ayerza and Coats [36] who indicated that low nitrogen content represents a significant barrier for an adequate chia seed yield production.

The high residual N_{min} content in 2017 has to be considered in regard to this fertilizer trial. It is very likely that the N_{min} content practically superimposed the levels of N treatments. In fact, no statistical difference in the three different fertilizer levels in 2017 was observed. However, an interaction between year of cultivation and treatment (fertilizer rate) would have been expected if the N_{min} content had a major influence on chia seed yield performance. Additionally, a statistical difference in 2016 would have been expected. However, as in 2016 too, no statistical difference was apparent, it seems likely that the N_{min} content/fertilizer rate did not have a substantial impact on seed yield and TSM. This is in line with studies of Bochicchio et al. [12] and Bilalis et al. [13], who also found no response of chia to nitrogen application.

Mean yields of the different treatments presented in this trial exceeded reported commercial seed yields (500 to 600 kg ha^{-1}) of low input conditions according to Coates [38]. Sosa et al. [43] indicated that the plasticity of chia to adapt and produce under low-input systems has led to the misconception of chia being a low input plant, therefore, underestimating its yield potential significantly. Trials of Sosa et al. [43] showed that applications of 100 kg N ha^{-1} produced 2.21 to 3.0 t ha^{-1} chia seed yield. This is in line with Mary et al. [33] who showed that the highest fertilizer level (90:60:75 kg ha^{-1} NPK) resulted in significantly increased chia seed yields.

The mentioned fertilization recommendations are based on studies conducted in countries with different climatic conditions (and different soils) which are not transferable to southwestern Germany. Even though the results of the present study showed no significant difference between the different fertilization treatments, chia seed yields increased proportionally with increased fertilization rates; therefore an N-fertilizer rate of 20 to 40 kg N ha^{-1} can be recommended for southwestern German conditions. Further field trials need to be conducted in order to define the level of nitrogen up to which this trend would reach its peak, keeping in mind that fertilization management needs to be economically and environmentally efficient; minimizing nutrient losses while optimizing seed yields [9]. As N top-dressing had a detrimental effect on yield and led to higher lodging and lower maturation percentage of seeds in a study of Bochicchio et al. [12], an adapted and optimal fertilization management is crucial in order to maximize commercial chia seed yields under the conditions given in southwestern Germany [38].

3.2.4. Additional Factors Influencing Chia Seed Yield

In contradiction to Pascual-Villalobos et al. [44] who stated that chia was able to be grown without pesticides, flea beetle (*Phyllotreta* spp.) populations imposed a significant problem during the emergence and cotyledon stage of the chia plants in 2017 [45]. If no immediate action would have been taken (see Section 2.2.) plant losses due to the beetle could have led to total crop failure.

Choosing the right time for mechanical harvest, as chia maturation is non-uniform (top-down), presents another vital factor which influences chia seed yield. While the central axis inflorescence was mature, inflorescences on the side branches remained immature and green, not having produced any seeds. However, it is not recommended to wait until all inflorescences are matured as the risk of seed shattering increases by rain, wind, and birds according to Jamboonsri et al. [1]. A trial conducted by

Coates and Ayerza [30] showed that via suboptimal mechanical harvesting conditions yield losses of up to 37% could occur compared to manual harvest. The non-uniformity represented a challenge, especially in the trial conducted in 2017, probably leading to suboptimal harvesting time. Nevertheless, seed yields obtained by the three above mentioned trials were in the range of the genotype G8 also cultivated at IHO in 2016 by Grimes et al. [7], but noticeably lower compared to the results obtained by the same genotype cultivated at Eckartsweier, indicating that the environment plays a significant role in the overall productivity of chia.

As the two trial sites in our study only showed slight alkalinity (7.45) in 2016 and acidity (6.37) in 2017 it was assumed that chia growth and seed yields were not affected by pH, also as no growth abnormalities were detected in both years [46]. If and to which extend chia growth, seed yield, and quality traits might be affected by soil pH needs to be further investigated, as literature on this topic is scarce.

3.3. Quality Traits

As health awareness alongside the demand for regionally produced, health-promoting functional foods is increasing; it is essential to study the effect of different agronomic practices on quality traits of chia seeds grown under the conditions given in southwestern Germany.

In the course of the following section determined chia seed protein, oil, and mucilage contents are displayed as well as the detailed fatty acid results of the row spacing trial as for the other two trials (sowing density and fertilization) no significant influences of the applied agronomic practices were found. The corresponding quality parameter results can be found in the supplementary material (Tables S2 and S3).

3.3.1. Row Spacing Trial

To our knowledge, the impact of row spacing on chia quality traits has not been studied so far. The statistical evaluation showed that different row spacing did not significantly influence chia crude protein content varying from 18.11 to 23.91%. There was no year × treatment interaction. Year, on the other hand, seemed to influence the crude protein content of chia significantly (p = 0.0018, Figure 2a, Table S1). Other oilseed crops did show conflicting results. The protein content of Turkish sesame, for example, was significantly influenced by row spacing, increasing with increasing row spacing from 40, 50, and 60 to 70 cm [47] whereas, according to Eryiğit et al. [48], row spacing did not significantly influence seed protein contents of safflower (*Carthamus tinctorious* L.) but year did. Ayerza and Coates [49] and Grimes et al. [7], in this regard, showed that chia protein contents tend to increase along with increasing mean temperatures, which is in contrast to the results of the present study as protein contents decreased from 2016 to 2017 (Table 2, Figure 2a). A positive relation between chia seed yield and protein content could be observed in this trial (Table 3, Figure 2a). Obtained protein content was in line with existent literature (15–26%) [7,49–51].

Mucilage content was significantly influenced by cultivation year (p < 0.0001, Figure 2b, Table S1) varying from 10.00 to 13.51%. The tested row spacing did show an increased mucilage content in 2017 compared to 2016 which is in line with the findings of Grimes et al. [7] who reported a significant difference in mucilage content between two different cultivation years for two different genotypes (Sahi Alba 914, W13.1). Row spacing effect was not significant, and no interactions were found. Mucilage content appeared to be negatively correlated with chia seed yield (Table 3, Figure 2b). The obtained mucilage content can be assessed as quite high compared to Ayerza and Coates [52], Ixtaine et al. [53], and Silveira Coelho et al. [54]. This characteristic can be considered as favorable as it is broadly applicable in the food industry [55].

Different row spacing significantly influenced chia crude oil content independent from the year of cultivation ranging from 32.20 to 33.37% (p = 0.0216, Figure 2c, Table S1), decreasing with increased row spacing. This is in line with Kayaçetin et al. [34] who observed a significant decrease in the percentage of crude oil in mustard along with an increase in row spacing. The same pattern was observed for sunflower (*Helianthus annuus* L.) and safflower according to El-Satar et al. [56] and Eryiğit et al. [48] who additionally observed a significant effect for year. In contrast, the oil content of sesame cultivated in Turkey was not significantly affected by row spacing in both years of cultivation [47] whereas Rahnama and Bakhshandeh [35] showed that the oil yield of sesame cultivated in Iran significantly decreased with increased row spacing from 37.5 to 60 cm. Indicating that oil content, in general, is highly dependent on environmental conditions, which is in contrast to Grimes et al. [7] who stated that stable oil contents independent from environmental influences could be met. To our knowledge, there is no data available on the direct impact of row spacing on chia crude oil content. Generally, oil contents were in line with current literature, ranging from 30–34% and, therefore, meeting the requirements of the European Novel Food guideline [7,38,57,58].

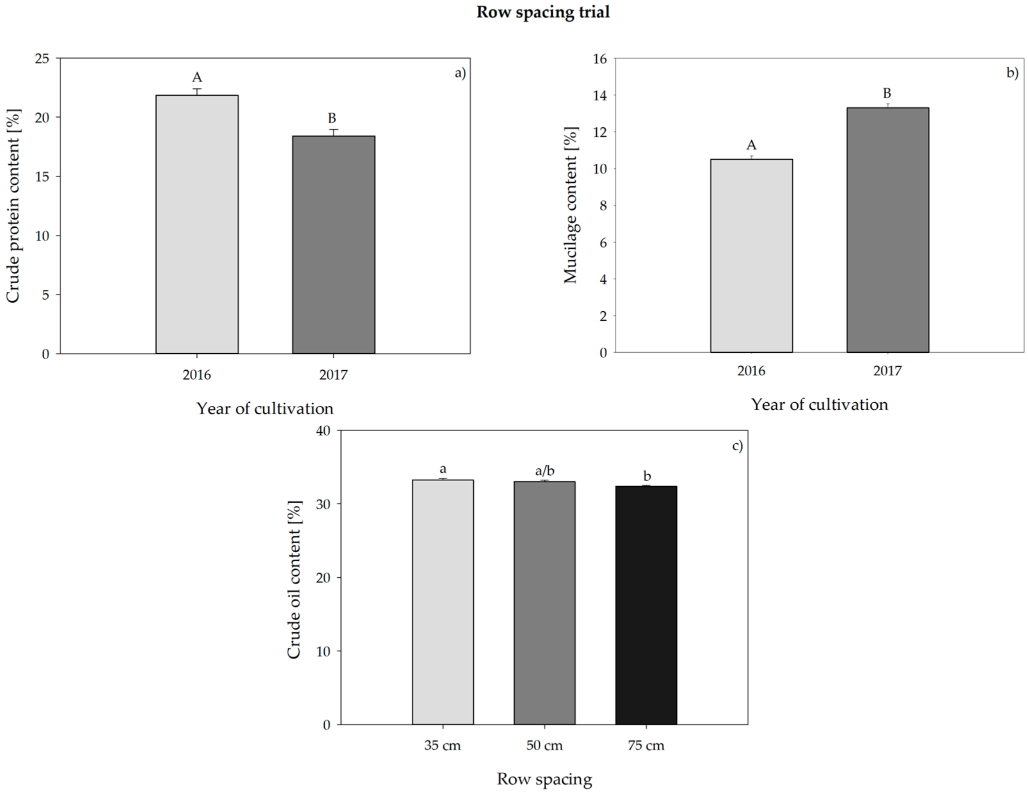

Figure 2. Mean estimates of crude protein (**a**), mucilage (**b**) and crude oil (**c**) content of the genotype G8 cultivated in 2016 and 2017 at Ihinger Hof with different row spacing. Means which share a common capital letter do not differ significantly between years at α= 0.05. Means which share a common lowercase letter do not differ significantly within row spacing at α = 0.05. (estimates, standard error, and Tukey HSD test are based on model (2); for *F*-test results compare Table S1).

As stated by Jamboonsri et al. [1] the high content in polyunsaturated fatty acids is one of the most important characteristics regarding chia seeds, therefore, the results of the examined individual saturated, monounsaturated and polyunsaturated fatty acids (SFAs, MUFAs, PUFAs), as well as their proportions and ratios, are shown in Table 6. Row spacing did significantly influence the individual saturated stearic fatty acid, the monounsaturated oleic and vaccenic fatty acids and the polyunsaturated α-linolenic fatty acid contents ($p = 0.0053$, $p = 0.0052$, $p = 0.0012$ and $p = 0.0125$, respectively, Table 6). This is in line with El-Satar et al. [56], Bellaloui et al. [59] and Boydak et al. [60] who stated that fatty acid composition of sunflower and soybeans are also significantly influenced by row spacing.

Table 6. Mean estimates of fatty acid composition (% of total fatty acid) of chia (genotype G8) cultivated in three different levels of row spacing (35, 50, 75 cm) at Ihinger Hof in 2016 and 2017, along with standard error of the mean (SEM) and *F*-tests.

Trait	**Year 2016**			**Year 2017**				***F*-Test**			
Row Spacing [cm]	**35**	**50**	**75**	**35**	**50**	**75**	**SEM**	j_a	b_{aj}	τ_k	$(j\tau)_{ak}$
Palmitic acid	6.79 A/a	6.76 A/a	6.80 A/a	7.16 B/a	7.04 B/a	7.18 B/a	0.082	<0.0001	0.0825	0.4589	0.7897
Stearic acid	3.30 A/a	3.12 A/b	3.18 A/b	3.58 B/a	3.23 B/b	3.22 B/b	0.061	0.0306	0.0587	0.0053	0.1824
Oleic acid	6.78 A/a	6.30 A/b	6.57 A/ab	6.56 B/a	6.14 B/b	6.13 B/ab	0.195	0.0386	0.0318	0.0052	0.6164
Vaccenic acid	0.75 A/a	0.76 A/a	0.77 A/b	0.81 B/a	0.82 B/a	0.85 B//b	0.005	<0.0001	0.1480	0.0012	0.4652
Linoleic acid	20.53 A/a	19.93 A/ab	19.79 A/b	19.91 B/a	19.98 A/a	20.14 A/a	0.157	0.5521	0.0013	0.2234	0.0389
α-Linolenic acid	61.85 A/a	63.13 A/b	62.88 A/ab	61.98 A/a	62.80 A/b	62.50 A/ab	0.308	0.4329	0.0004	0.0125	0.6697
SFA	10.09 A/a	9.88 A/b	9.98 A/ab	10.74 B/a	10.27 B/b	10.40 B/ab	0.108	0.0002	0.0193	0.0273	0.4359
MUFA	7.53 A/a	7.06 A/b	7.35 A/b	7.37 A/a	6.96 A/b	6.97 A/b	0.193	0.0880	0.0332	0.0070	0.6380
PUFA	82.38 A/a	83.06 A/b	82.67 A/ab	81.89 A/a	82.78 A/b	82.63 A/ab	0.212	0.1304	0.0233	0.0093	0.5911
PUFA/SFA	8.18 A/a	8.41 A/b	8.29 A/ab	7.63 B/a	8.07 B/b	7.95 B/ab	0.098	0.0004	0.0065	0.0164	0.5179
ω6:ω3	0.33 A/a	0.32 A/a	0.32 A/a	0.32 A/a	0.32 A/a	0.32 A/a	0.004	0.8434	0.0042	0.1309	0.1019

Results in g 100 g^{-1} of oil. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; ω6:ω3 ratio (Linoleic: α-Linolenic acid ratio). Mean estimates, standard errors of the mean (SEM) and *p*-values are based on model (2); j_a Annual effect; b_{aj} Block effect; τ_k Treatment effect (row spacing); $(j\tau)_{ak}$ Interaction between treatment and annual effect; Letter display: Means at a constant level of row spacing that share a common capital letter do not differ significantly between years at $\alpha = 0.05$. Means within a year that share a common lowercase letter do not differ significantly between treatment factor levels at $\alpha = 0.05$.

Mean values obtained for stearic, oleic and linoleic (only in 2016) fatty acids were lower when cultivated at a row spacing of 50 and 75 cm compared to values obtained when cultivated at 35 cm row spacing. Vaccenic and α-linolenic fatty acid contents were inversely proportional, increasing along with increased row spacing.

Thus the contents of total SFAs and MUFAs, which decreased with increasing row spacing as well as the PUFAs and PUFA/SFA ratio, which increased alongside increased row spacing were also significantly influenced by row spacing as the main effect (p = 0.0273, p = 0.0070, p = 0.0093 and p = 0.0164, respectively, Table 6).

Saturated (palmitic and stearic) and monounsaturated (oleic and vaccenic) fatty acid contents were significantly influenced by year of cultivation ($p < 0.0001$, p = 0.0306, p = 0.0386 and $p < 0.0001$, respectively, Table 6). Palmitic, stearic and vaccenic acid contents increased from 2016 to 2017, whereas oleic fatty acid content decreased from 2016 to 2017. Total SFA content (increase) and PUFA/SFA ratio (decrease) from 2016 to 2017 were also significantly influenced by year of cultivation (p = 0.0002 and p = 0.0004, respectively, Table 6).

Ayerza and Coates [31], who reported that there was a negative correlation between α-linolenic fatty acid content and mean temperatures, could not be verified by the present study (Table 2, Table 6). Linoleic acid content was significantly influenced by the interaction of year and row spacing (p = 0.0389, Table 6).

Marcinek and Krejpcio [61] displayed the content of individual fatty acids of chia by different studies showing that the results presented in Table 6 are generally in line with existing literature [53,54,62,63]. This is also valid for the following sowing density and fertilizer trials (Tables S2 and S3). Nevertheless, more studies need to be conducted in order to verify the findings that row spacing significantly affects the quality traits of chia seeds as current data on this issue is non-existent.

3.3.2. Sowing Density Trial

Statistical evaluation showed that different sowing densities did not significantly influence chia crude protein content which is in contrast to Bilalis et al. [13] who showed that crude protein of chia significantly increased as sowing density increased. Year of cultivation, on the other hand, significantly influenced crude protein yield ($p < 0.0001$, Table S1) of the present study, ranging from 18.41 to 22.84% decreasing from 2016 to 2017 (Figure 3a).

Mucilage content ranged from 10.20 to 13.74% and the interaction of year × treatment was significant (p = 0.0003, Figure 3b, Table S1). Mucilage content decreased with increasing sowing density in 2016 whereas it increased parallel to sowing density in 2017 (Figure 3b).

Crude oil content ranged between 32.24 and 33.24%. Again the interaction of sowing density and year was significant (p = 0.0281, Table S1): Oil content did not differ significantly between sowing densities in 2016 but decreased with increasing sowing densities in 2017 (Figure 3c).

Crude protein content seemed to be negatively correlated with chia seed yield whereas mucilage and crude oil content appeared to be positively correlated with it (Table 4, Figure 3). Therefore, it can be assumed that the observed effect might rather be a combination of reduced yields in the 1 kg ha^{-1} sowing density, going along with slightly increased protein contents and, thus, lower mucilage and oil contents due to a trade-off between oil content and crude protein in oilseeds [64,65].

The lower p-values of the year main-effect in the model (2) compared to the main effect of sowing density might point to a higher impact of environmental factors (temperature, precipitation, N_{min}, etc.) on crude oil contents (Table S1).

To our knowledge, the present study is the first evaluation of the impact of sowing densities on the fatty acid compositions in chia. The individual SFAs (palmitic and stearic), MUFAs (oleic and vaccenic) and PUFAs (linoleic and α–linolenic acid), as well as the PUFA/SFA and ω6:ω3 ratios did not differ significantly between sowing densities. An interaction of sowing density and year was always absent (Table S2).

Research of Bellaloui et al. [59] showed that sowing density can alter soybean seed constituents and that this effect depends on cultivar and environmental factors, especially temperature and drought. Being in line with this finding, fatty acid contents of the mentioned individual SFAs, MUFAs and PUFAs, except for vaccenic acid, significantly differed between years ($p = 0.0009$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$ and $p < 0.0001$, respectively, Table S2). Palmitic, stearic, oleic and linoleic acid contents decreased from 2016 to 2017, while the α–linolenic acid content increased (Table S2). SFA, MUFA contents and $\omega 6$:$\omega 3$ ratios decreased from 2016 to 2017, while PUFA contents and the PUFA/SFA ratio increased as year of cultivation also influenced fatty acid contents and ratios highly significantly ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$ and $p < 0.0001$ and $p < 0.0001$ respectively, Table S2).

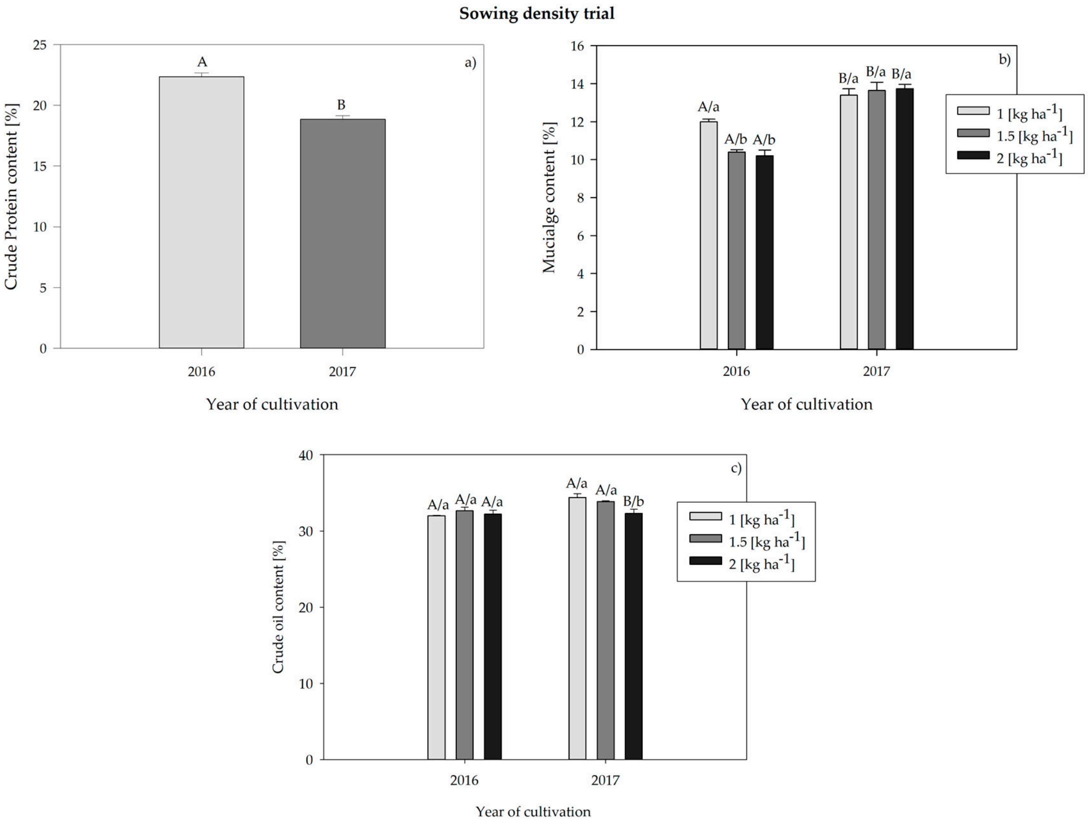

Figure 3. Mean estimates of crude protein (**a**), mucilage (**b**) and crude oil (**c**) content of the genotype G8 cultivated in 2016 and 2017 at Ihinger Hof at different sowing densities. Means at a constant level of sowing density that share a common capital letter do not differ significantly between years at $\alpha = 0.05$. Means within a year that share a common lowercase letter do not differ significantly between sowing densities at $\alpha = 0.05$ (estimates, standard errors, and Tukey HSD test are based on model (2); for *F*-test results compare Table S1).

3.3.3. Fertilizer Trial

Crude protein yields ranged from 18.14 to 23.09, being significantly influenced by year of cultivation ($p < 0.0001$, Table S1), decreasing from 2016 to 2017 (Figure 4a). This is in line with Ayerza and Coates [66] who stated that the protein content of chia seed varies depending on the geographical location and corresponding growing conditions. Bilalis et al. [13] found a positive response of crude protein contents in chia to increased fertilization. The mean estimates of the present study display a proportional increase of crude protein content with increasing fertilizer application. However, the data basis did not allow rejecting the null-hypothesis of the fertilizer effect.

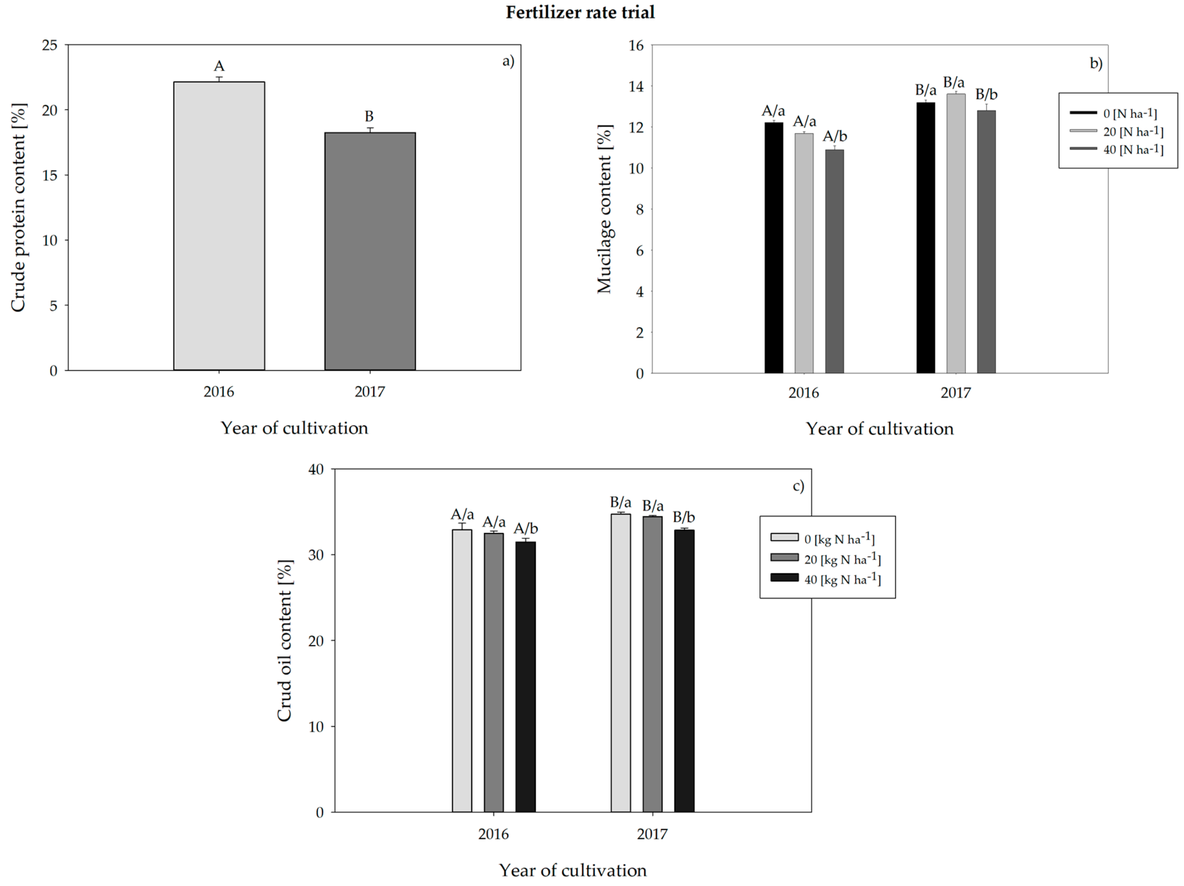

Figure 4. Mean estimates of crude protein (**a**), mucilage (**b**) and crude oil (**c**) content of the genotype G8 cultivated in 2016 and 2017 at Ihinger Hof at different fertilizer rates. Means at a constant level of fertilization rate that share a common capital letter do not differ significantly between years at $\alpha = 0.05$. Means within a year that share a common lowercase letter do not differ significantly between fertilization levels at $\alpha = 0.05$. (estimates, standard errors, and Tukey HSD test are based on model (2), for *F*-test results compare Table S1).

Mucilage content varied from 11.81 to 12.75% being significantly affected by the fertilizer rate and year of cultivation ($p = 0.0018$ and $p < 0.0001$, respectively, Figure 4b, Table S1) but an interaction was absent. Mucilage content increased from 2016 to 2017 and decreased by increased rates of fertilizer (Figure 4b). De Falco et al. [67] were able to show that fertilization lowers the content of carbohydrates and the corresponding metabolites. As chia mucilage is mainly composed of polysaccharides, this finding could be verified by the present study. According to Capitani et al. [68], the correlation between the presence of mucilage and specific environmental conditions has not yet been established. The mean estimates of the present study seem to display a positive relation between chia seed yield and mucilage content (Table 5, Figure 4b).

Statistical analysis of data revealed that different fertilization rates and the year of cultivation significantly influenced crude oil content ranging from 32.16 to 33.78% ($p = 0.0009$ and $p < 0.0001$, respectively, Figure 4c, Table S1). Decreasing alongside the increased fertilizer rate and increasing from 2016 to 2017 (Figure 4c). This contradicts the finding of Amato et al. [40] and de Falco et al. [67], stating that nitrogen supply did not affect chia seed oil content. As presented by Ayerza [50] and Ayerza and Coates [49] oil contents depend on environmental factors such as climatic conditions and the region of provenance in which the plants are cultivated. In this regard, Ayerza [50] was able to show that the oil content of a single chia line varied from 25.93% to 33.50% in five different locations in South America.

Increased N-fertilization resulted in higher yields, which led to a slight decrease in crude protein content and thus to an increase in mucilage and crude oil contents due to a trade-off between oil content and crude protein in oilseeds which was already mentioned in Section 3.3.2 [64,65].

Fertilizer rates had no significant influence on the examined individual saturated (palmitic and stearic), monounsaturated (oleic and vaccenic) and polyunsaturated (linoleic and α–linolenic) fatty acid contents as well as on their corresponding proportions and ratios (Table S3). All above-mentioned fatty acid contents ($p = 0.0003$, $p < 0.0001$, $p < 0.0001$, $p = 0.0182$, $p < 0.0001$, and $p < 0.0001$, respectively), thus the corresponding SFA, MUFA and PUFA contents as well as the PUFA/SFA and ω6:ω3 ratios ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, and $p < 0.0001$, respectively) differed significantly between years (Table S3).

Palmitic, stearic, oleic and linoleic acid plus SFA, MUFA contents, as well as the ω6:ω3 ratio, decreased from 2016 to 2017. Vaccenic and α–linolenic, as well as the PUFA content and the PUFA/SFA ratio, increased from 2016 to 2017.

Corresponding to the results of the present study Amato et al. [40] and de Falco et al. [67] stated that nitrogen fertilization did not affect the fatty acid composition of chia seeds.

4. Conclusions

The research aimed at evaluating yield and quality traits of chia depending on different (i) row spacing (35, 50 and 75 cm), (ii) sowing densities (1, 1.5 and 2 kg ha^{-1}) and (iii) N-fertilization rates (0, 20 and 40 kg N ha^{-1}) in order to adapt management practices to maximize chia seed yields and its associated seed quality traits [38].

The results presented within this study verified the salient contention that given environmental conditions affected seed yield and the nutrient composition of chia significantly, whereas the applied agronomic management practices have shown limited impact [31,49,69].

Nevertheless, with regard to maximizing yield and quality traits of chia cultivated under southwestern German conditions, a row spacing of 50 cm, a sowing density of 1.5 kg ha^{-1} and an N-fertilization rate of 20 to 40 kg N ha^{-1} can be recommended.

In general, it was shown that yield and quality traits were similar to that of commercially available seeds from traditional and new growth areas while simultaneously meeting the given EU requirements of the Novel Food Guideline [40,58]. Based on first results, pursuing agronomical trials for *Salvia hispanica* L. in southwestern Germany seems worthwhile as genotypes either day length insensitive (Sahi Alba 914) or adapted to day lengths greater than 12 h (G8, W13.1) are available in order to obtain earlier flowering [7,12]. Nevertheless, the results of the present study demonstrate that more field trials are required in order to provide unambiguous information regarding the influence of agronomic management practices on overall chia performance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/3/136/s1. Table S1. Standard error of the mean (SEM) and *F*-test results of the crude protein, mucilage and crude oil content of chia (Genotypes G8) cultivated at three different row spacing (35, 50, 75 cm), sowing densities (1, 1.5, 2 kg ha^{-1}) and fertilizer rates (0,20 40 kg N ha^{-1}) at Ihinger Hof in 2016 and 2017 ($n = 3$, $\alpha = 0.05$)., Table S2. Mean estimates of fatty acid composition (% of total fatty acid) of chia (Genotypes G8) cultivated at three different levels of sowing density (1, 1.5, 2 kg ha^{-1}) at Ihinger Hof in 2016 and 2017, along with standard error of the mean (SEM) and *F*-tests, Table S3. Mean estimates of fatty acid composition (% of total fatty acid) of chia (genotype G8) cultivated at three different fertilizer rates (0, 20, 40 kg N ha^{-1}) at Ihinger Hof in 2016 and 2017, along with the standard error of the mean (SEM) and *F*-tests.

Author Contributions: Conceptualization, S.J.G. and S.G.-H.; Formal analysis, S.J.G. and F.C.; Funding acquisition, S.G.-H.; Investigation, S.J.G.; Supervision, S.G.-H.; Validation, S.J.G. and F.C.; Visualization, S.J.G.; Writing–original draft, S.J.G.; Writing–review & editing, S.J.G., T.D.P., F.C. and S.G.-H.

Funding: This research was funded by the German Federal Ministry for Economic Affairs and Energy within the Central Innovation Program for SMEs (16KN050524).

Acknowledgments: The authors would like to thank the University of Kentucky (USA) which generously provided the mutant genotype G8 within the framework of an agreement between the University of Hohenheim and the University of Kentucky. In addition, the authors would like to thank the technical staff of the experimental station Ihinger Hof for the agronomic management of the trials and the technical assistance of the crop science institute.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Jamboonsri, W.; Phillips, T.D.; Geneve, R.L.; Cahill, J.P.; Hildebrand, D.F. Extending the range of an ancient crop, Salvia hispanica L.—A new ω3 source. *Genet. Resour. Crop Evol.* **2012**, *59*, 171–178. [CrossRef]
2. Mohd Ali, N.; Yeap, S.K.; Ho, W.Y.; Beh, B.K.; Tan, S.W.; Tan, S.G. The promising future of chia, Salvia hispanica L. *J. Biomed. Biotechnol.* **2012**, *2012*, 1–9. [CrossRef] [PubMed]
3. Us-Medina, U.; Julio, L.M.; Segura-Campos, M.R.; Ixtaina, V.Y.; Tomás, M.C. Development and characterization of spray-dried chia oil microcapsules using by-products from chia as wall material. *Powder Technol.* **2018**, *334*, 1–8. [CrossRef]
4. Fernández-Ferrína, P.; Calvo-Turrientes, A.; Bande, B.; Artaraz-Miñóna, M.; Galán-Ladero, M.M. The valuation and purchase of food products that combine local, regional and traditional features: The influence of consumer ethnocentrism. *Food Qual. Prefer.* **2018**, *64*, 138–147. [CrossRef]
5. Grimes, S.; Phillips, T.; Claupein, W.; Graeff-Hönninger, S. Chia^4: Is a new tetraploid chia (Salvia hispanica L.) genotype able to revolutionize European agriculture? In *The Food System Approach: New Challenges for Education, Research and Industry*; ISEKI Food Association: Stuttgart, Germany, 2018; p. 79.
6. Hildebrand, D.; Jamboonsri, W. Phillips Early Flowering Chía and Uses Thereof. U.S. Patent 8586831, 19 October 2013.
7. 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. [CrossRef]
8. Bochicchio, R.; Phillips, T.D.; Lovelli, S.; Labella, R.; Galgano, F.; Marisco, A.D.; Perniola, M.; Amato, M. Innovative Crop Productions for Healthy Food: The Case Chia (Salvia hispanica L.). In *The Sustainability of Agro-Food and Natural Resource Systems in the Mediterranean Basin*; Vastola, A., Ed.; Springer International Publishing: Basel, Switzerland, 2015; pp. 29–45. ISBN 9783319163574.
9. Souza, R.S.; Chaves, L.H.G. Initial growth of chia (Salvia hispanica L.) submitted to nitrogen, phosphorus and potassium fertilization. *Aust. J. Crop Sci.* **2017**, *11*, 610–615. [CrossRef]
10. Thiago, F.d.F.; Bruna, B.R.; Alexandre, L.M.; Dyogo, B.B.; Sidiane, C.-R.; Omari, D.F.D.; Guilherme, P.d.F.; Heloisa, F.C.M.-M. Development and production of chia (Salvia hispanica L.) in different space arrangements. *Afr. J. Agric. Res.* **2016**, *11*, 4384–4388. [CrossRef]
11. Wojahn, R.E.; Bortolotto, R.P.; Zamberlan, J.F.; Koefender, J.; Tragnago, J.L.; Camera, J.N.; Pasini, M.P.B.; Salazar, R.F.S.; Damiani, F. Agronomic feasibility of growing chia in northwestern rio grande do sul. *Holos* **2018**, *3*, 112–122. [CrossRef]
12. Bochicchio, R.; Rossi, R.; Labella, R.; Bitella, G.; Perniola, M.; Amato, M. Effect of sowing density and nitrogen top-dress fertilisation on growth and yield of chia (Salvia hispanica L.) in a mediterranean environment: First results. *Ital. J. Agron.* **2015**, *10*, 163–166. [CrossRef]
13. Bilalis, D.; Tabaxi, I.; Zervas, G.; Tsiplakou, E.; Travlos, I.S.; Kakabouki, I.; Tsioros, S. Chia (Salvia Hispanica) Fodder Yield and Quality as Affected by Sowing Rates and Organic Fertilization. *Commun. Soil Sci. Plant Anal.* **2016**, *47*. [CrossRef]
14. Karkanis, A.C.; Kontopoulou, C.K.; Lykas, C.; Kakabouki, I.; Petropoulos, S.A.; Bilalis, D. Efficacy and selectivity of pre- and post-emergence herbicides in chia (Salvia hispanica L.) under mediterranean semi-arid conditions. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2018**, *46*, 183–189. [CrossRef]
15. Mcmaster, G.S.; Wilhelm, W.W. Growing degree-days: One equation, two interpretations. *Agric. For. Meteorol.* **1997**, *87*, 291–300. [CrossRef]

16. IUSS Working Group WRB. World reference base for soil resources 2006, first update 2007 A framework for international classification, correlation and communication. In *World Soil Resources Reports No. 103*; FAO: Rome, Italiy, 2007; pp. 1–128, ISBN 9789251055113.
17. Bassler, R.; Hoffmann, G. Bestimmung von mineralischem, (Nitrat-) Stickstoff in Bodenprofilen, Nmin-Labormethode. In *VDLUFA Methodenbuch Band I.*; Deller, B., Ed.; VDLUFA-Verlag: Darmstadt, Germany, 1997; ISBN 978-3-941273-21-4.
18. Muñoz, L.A.; Cobos, A.; Diaz, O.; Aguilera, J.M. Chia seeds: Microstructure, mucilage extraction and hydration. *J. Food Eng.* **2012**, *108*, 216–224. [CrossRef]
19. Dumas, A. Stickstoffbestimmung nach Dumas. In *Die Praxis des org. Chemikers*; Gattermann-Wieland, Ed.; Walter de Gruyter: Nürnberg, Germany, 1962.
20. AOAC. Micro-Kjeldahl Method. In *Official Methods of Analysis*; Cunniff, P., Ed.; Association of Official Analytical Chemists: Arlington VA, USA, 1995.
21. European Commission. Regulation (EC). No. 152/III H procedure B laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* **2009**, *54*, 1–130.
22. Metcalfe, L.D.; Schmitz, A.A.; Pelka, J.R. Rapid preparation of fatty acid esters from lipids for gas chromatographic Analysis. *Anal. Chem.* **1966**, *38*, 514–515. [CrossRef]
23. DGF Fettsäuremethylester. In *Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen*, 2nd ed.; Deutsche Gesellschaft für Fettwissenschaft e.V. (Ed.) Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart, Germany, 2002.
24. Thurnhofer, S.; Vetter, W. A gas chromatography/electron ionization-mass spectrometry-selected ion monitoring method for determining the fatty acid pattern in food after formation of fatty acid methyl esters. *J. Agric. Food Chem.* **2005**, *53*, 8896–8903. [CrossRef] [PubMed]
25. Roy, J. SAS for Mixed Models. In *Journal of Biopharmaceutical Statistics*; Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., Eds.; Taylor & Francis: London, UK, 2007; Volume 17, pp. 363–365, ISBN 1054340060100.
26. Baginsky, C.; Arenas, J.; Escobar, H.; Garrido, M.; Valero, N.; Tello, D.; Pizarro, L.; Valenzuela, A.; Morale, L.; Silva, H. Growth and yield of chia (Salvia hispanica L.) in the Mediterranean and desert climates of Chile. *Chil. J. Agric. Res.* **2016**, *76*, 255–264. [CrossRef]
27. Ayerza, R.; Coates, W. New industrial crops: Northwestern Argentina Regional Project. In *Progress in New Crops*; Janick, J., Ed.; ASHS Press: Alexandria, Egypt, 1996; pp. 46–51.
28. Allen, R.G.; Pereira, L.S.; Raes, D.; Smith, M. *Crop Evapotranspiration—Guidelines for Computing Crop Water Requirements*; FAO: Rome, Italy, 1998; Volume 56.
29. ESRL Solar Calculation Details. Available online: https://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html (accessed on 15 January 2018).
30. Coates, W.; Ayerza, R. Commercial production of chia in Northwestern Argentina. *JAOCS J. Am. Oil Chem. Soc.* **1998**, *75*, 1417–1420. [CrossRef]
31. Ayerza, R.; Coates, W. Some quality components of four chia (Salvia hispanica L.) genotypes grown under tropical coastal desert ecosystem conditions. *Asian J. Plant Sci.* **2009**, *8*, 301–307. [CrossRef]
32. Yeboah, S.; Owusu Danquah, E.; Lamptey, J.N.L.; Mochiah, M.B.; Lamptey, S.; Oteng-Darko, P.; Adama, I.; Appiah-Kubi, Z.; Agyeman, K. Influence of Planting Methods and Density on Performance of Chia (Salvia hispanica) and its Suitability as an Oilseed Plant. *Agric. Sci.* **2014**, *2*, 14–26. [CrossRef]
33. Mary, J.; Veeranna, H.K.; Girijesh, G.K.; Dhananjaya, B.C.; Gangaprasad, S. Effect of Different Spacings and Fertilizer Levels on Growth Parameters and Yield of Chia (Salvia hispanica L.). *Int. J. Pure Appl. Biosci.* **2018**, *6*, 259–263. [CrossRef]
34. Kayaçetin, F.; Önemli, F.; Yilmaz, G.; Kinay, A.; Hatipoğlu, H.; Kivilcim, M.N.; Kara, N.; Köse, A.; Sefaoğlu, F. Effect of Row Spacing on Yield, Yield Components and Crude Oil of Autumn and Spring Sowed Mustard (Sinapis arvensis L.) in Eight Locations of Turkey. *J. Agric. Sci.* **2018**, *24*, 483–499.
35. Rahnama, A.; Bakhshandeh, A. Determination of Optimum Row-Spacing and Plant Density for Uni-branched Sesame in Khuzestan Province. *J. Agric. Sci. Technol.* **2006**, *8*, 25–33.
36. Ayerza, R.; Coates, W. *Chia: Rediscovering a Forgotten Crop of the Aztecs*; University of Arizona Press: Tucson, AZ, USA, 2005; ISBN 978-0-8165-2488-4.
37. Pozo Pozo, S.A. *Alternativas Para el Control Químico de Malezas Anuales en el Cultivo de la Chía (Salvia hispánica) en la Granja ECAA, Provincia de Imbabura*; Pontificia Universidad Católica del Ecuador: Ibarra, Ecuador, 2010.

38. Coates, W. *Whole and Ground Chia (Salvia hispanica L.) Seeds, Chia Oil—Effects on Plasma Lipids and Fatty Acids*; Elsevier Inc.: Amsterdam, The Netherlands, 2011; ISBN 9780123756886.
39. Deligios, P.A.; Carboni, G.; Farci, R.; Solinas, S.; Ledda, L. Low-input herbicide management: Effects on rapeseed production and profitability. *Sustainability* **2018**, *10*, 2258. [CrossRef]
40. Amato, M.; Caruso, M.C.; Guzzo, F.; Galgano, F.; Commisso, M.; Bochicchio, R.; Labella, R.; Favati, F. Nutritional quality of seeds and leaf metabolites of Chia (Salvia hispanica L.) from Southern Italy. *Eur. Food Res. Technol.* **2015**, *241*, 615–625. [CrossRef]
41. Mamun, F.; Ali, M.H.; Chowdhury, I.F.; Hasanuzzaman, M.; Matin, M.A. Performance of Rapeseed Mustard Varieties Grown Under Different Plant Density. *Sci. Agric.* **2014**, *4*. [CrossRef]
42. Raghuvanshi, N.; Kumar, V. Advances in agronomical manipulation on Indian mustard (Brassica juncea Cezern and Coss.): An review. *J. Pharmacogn. Phytochem.* **2018**, *7*, 833–837.
43. Sosa-Baldivia, A.; Ruiz Ibarra, G. Inadequate Nitrogen Fertilization: Main Cause of the Low Seed Yield on the Chia Crop (Salvia Hispanica l.). *Biomed. J. Sci. Tech. Res.* **2018**, *2*, 2–4. [CrossRef]
44. Pascual-Villalobos, M.; Correal, E.; Molina, E.; Martínez, J. *Evaluación y Selección de Especies Vegetales Productoras de CompuestosNaturales con Actividad Insecticida*; Centro de Investigación y Desarrollo Agroalimentario (CIDA): Murcia, Spain, 1997.
45. Muñoz, L.A.; Cobos, A.; Diaz, O.; Aguilera, J.M. Chia Seed (Salvia hispanica): An Ancient Grain and a New Functional Food. *Food Rev. Int.* **2013**, *29*, 394–408. [CrossRef]
46. Ouzounidou, G.; Skiada, V.; Papadopoulou, K.K.; Stamatis, N.; Kavvadias, V.; Eleftheriadis, E.; Gaitis, F. Effects of soil pH and arbuscular mycorrhiza (AM) inoculation on growth and chemical composition of chia (Salvia hispanica L.) leaves. *Braz. J. Bot.* **2015**, *38*, 487–495. [CrossRef]
47. Alpaslan, M.; Boydak, E.; Hayta, M.; Gerçek, S.; Simsek, M. Effect of row space and irrigation on seed composition of Turkish sesame (sesamum indicum L.). *JAOCS J. Am. Oil Chem. Soc.* **2001**, *78*, 933–935. [CrossRef]
48. Eryiğit, T.; Yıldırım, B.; Kumlay, A.M.; Sancaktaroğlu, S. The Effects of Different Row Distances and Nitrogen Fertilizer Rates on Yield and Yield Components of Safflower (Carthamus tinctorious) Under Micro-Climate Conditions of Iğdır Plain—Turkey. In Proceedings of the 3rd International Conference on Biological, Chemical & Environmental Sciences, Kuala Lumpur, Malaysia, 21–22 September 2015; pp. 21–22.
49. 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]
50. 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]
51. Capitani, M.I.; Spotorno, V.; Nolasco, S.M.; Tomás, M.C. Physicochemical and functional characterization of by-products from chia (Salvia hispanica L.) seeds of Argentina. *LWT Food Sci. Technol.* **2012**, *45*, 94–102. [CrossRef]
52. Ayerza, R.; Coates, W. Omega-3 enriched eggs: The influence of dietary α-linolenic fatty acid source on egg production and composition. *Can. J. Anim. Sci.* **2001**, *81*, 355–362. [CrossRef]
53. Ixtaina, V.Y.; Martínez, M.L.; Spotorno, V.; Mateo, C.M.; Maestri, D.M.; Diehl, B.W.K.; Nolasco, S.M.; Tomás, M.C. Characterization of chia seed oils obtained by pressing and solvent extraction. *J. Food Compos. Anal.* **2011**, *24*, 166–174. [CrossRef]
54. Silveira Coelho, M.; de las Mercedes Salas-Mellado, M. Chemical Characterization of CHIA (Salvia hispanica L.) for Use in Food Products. *J. Food Nutr. Res.* **2014**, *2*, 263–269. [CrossRef]
55. Tavares, L.S.; Junqueira, L.A.; de Oliveira Guimarães, Í.C.; de Resende, J.V. Cold extraction method of chia seed mucilage (Salvia hispanica L.): Effect on yield and rheological behavior. *J. Food Sci. Technol.* **2018**, *55*, 457–466. [CrossRef] [PubMed]
56. El-Satar, M.A.A.; Ahmed, A.A.; Hassan, T.H.A. Response of seed yield and fatty acid compositions for some sunflower genotypes to plant spacing and nitrogen fertilization. *Inf. Process. Agric.* **2017**, *4*, 241–252. [CrossRef]
57. Ixtaina, V.Y.; Nolasco, S.M.; Tomás, M.C. Physical properties of chia (Salvia hispanica L.) seeds. *Ind. Crops Prod.* **2008**, *28*, 286–293. [CrossRef]

58. European Commission. Commission implementing decision authorizing the placing on the market of chia oil (Salvia hispanica) as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. *Off. J. Eur. Union* **2014**, *353*, 15–16.
59. Bellaloui, N.; Mengistu, A.; Walker, E.R.; Young, L.D. Soybean seed composition as affected by seeding rates and row spacing. *Crop Sci.* **2014**, *54*, 1782–1795. [CrossRef]
60. Boydak, E.; Alpaslan, M.; Hayta, M.; Gerçek, S.; Simsek, M. Seed Composition of Soybeans Grown in the Harran Region of Turkey As Affected by Row Spacing and Irrigation. *J. Agric. Food Chem.* **2002**, *50*, 4718–4720. [CrossRef] [PubMed]
61. Marcinek, K.; Krejpcio, Z. Chia Seeds (Salvia Hispanica): Health Promoting Properties and Therapeutic Applications—A Review. *Rocz Panstw Zakl Hig* **2017**, *68*, 123–129. [PubMed]
62. Álvarez-Chávez, L.M.; Valdivia-López, M.D.L.A.; Aburto-Juárez, M.D.L.; Tecante, A. Chemical characterization of the lipid fraction of Mexican chia seed (Salvia hispanica L.). *Int. J. Food Prop.* **2008**, *11*, 687–697. [CrossRef]
63. Ciftci, O.N.; Przybylski, R.; Rudzińska, M. Lipid components of flax, perilla, and chia seeds. *Eur. J. Lipid Sci. Technol.* **2012**, *114*, 794–800. [CrossRef]
64. Peltonen-sainio, P.; Jauhiainen, L.; Hyövelä, M.; Nissilä, E. Trade-off between oil and protein in rapeseed at high latitudes: Means to consolidate protein crop status ? *Field Crop Res.* **2011**, *121*, 248–255. [CrossRef]
65. Wang, N.; Daun, J.K. Effects of variety and crude protein content on nutrients and anti-nutrients in lentils (Lens culinaris). *Food Chem.* **2006**, *95*, 493–502. [CrossRef]
66. Ayerza, R.; Coates, W. *Chia Seeds: New Source of Omega-3 Fatty Acids, Natural Antioxidants, and Dietetic Fiber*; Southwest Center for Natural Products Research & Commercialization, Office of Arid Lands Studies: Tucson, AZ, USA, 2001.
67. de Falco, B.; Incerti, G.; Bochicchio, R.; Phillips, T.D.; Amato, M.; Lanzotti, V. Metabolomic analysis of Salvia hispanica seeds using NMR spectroscopy and multivariate data analysis. *Ind. Crops Prod.* **2017**, *99*, 86–96. [CrossRef]
68. Capitani, M.I.; Ixtaina, V.Y.; Nolasco, S.M.; Tomás, M.C. Microstructure, chemical composition and mucilage exudation of chia (Salvia hispanica L.) nutlets from Argentina. *J. Sci. Food Agric.* **2013**, *93*, 3856–3862. [CrossRef] [PubMed]
69. 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]