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Characterization and Evaluation of *Salvia hispanica* L. and *Salvia columbariae* Benth. Varieties for Their Cultivation in Southwestern Germany

Samantha Jo Grimes ^{1,*}, Filippo Capezzone ² , Peteh Mehdi Nkebiwe ¹ and Simone Graeff-Hönniger ¹

¹ Cropping Systems and Modelling, Institute of Crop Science, University of Hohenheim, 70599 Stuttgart, Germany; mehdi.nkebiwe@uni-hohenheim.de (P.M.N.); simone.graeff@uni-hohenheim.de (S.G.-H.)

² Department of Biostatistics, Institute of Crop Science, University of Hohenheim, 70599 Stuttgart, Germany; filippo.capezzone@uni-hohenheim.de

* Correspondence: s.grimes@uni-hohenheim.de; Tel.: +49-0711-459-24184

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Abstract: Rising consumer attraction towards superfoods and the steadily increasing demand for healthy, environmentally sustainable, and regionally produced food products has sharpened the demand for chia. Over the course of 4 years, two early flowering chia varieties belonging to *Salvia hispanica* L., and *Salvia columbariae* Benth. Species were identified to complete their phenological development and, therefore, able to reach maturity under a photoperiod >12 h, thus enabling the cultivation of chia in central Europe—more specifically, in southwestern Germany—consistently for the first time. Results obtained by the conducted field trial in 2018 showed that chia seed yields and thousand-seed mass ranged from 284.13 to 643.99 kg ha⁻¹ and 0.92 to 1.36 g, respectively. Further, the statistical analyses showed that the protein content of the cultivated chia varieties ranged from 22.14 to 27.78%, the mucilage content varied from 10.35 to 20.66%, and the crude oil content amounted up to 28.00 and 31.73%. Fatty acid profiles were similar to previously reported data with α -Linolenic acid being the most prominent one, ranging from 60.40 to 65.87%, and we obtained ω 6: ω 3 ratios between 0.2 and 0.3. In conclusion, chia could represent a promising raw material from a nutritional point of view, while being able to diversify the local food basis of southwestern Germany.

Keywords: *Salvia hispanica* L.; *Salvia columbariae* Benth.; yield traits; quality traits; regional cultivation

1. Introduction

The human food supply relies on an increasingly dwindling number of species [1]. This dependence is especially critical in the context of climate change and the related environmental problems [2]. Diversifying agricultural production and therefore broadening the food base will inevitably become an essential task to adjust to future climate change [2]. In this context, the cultivation of chia species (*Salvia hispanica* L. and *Salvia columbariae* Benth.) could play a key role in meeting the steadily increasing demand for healthy, environmentally sustainable, and regionally produced food products in Europe [3,4]. Being able to provide food, feed, and industrial products/raw materials for the pharmaceutical and cosmetic industry would make this low-input, multi-purpose crop a valuable and beneficial addition to the present agricultural landscape [5].

Chia (*Salvia hispanica* L.), also known as the gold of the Aztecs, a summer annual herbaceous plant belonging to the *Lamiacea* family, is a crop of high economical value and has gained popularity in Europe in recent years due to its exceptional nutritional composition and the ever increasing demand

for so-called superfoods [6–8]. Besides the three main benefits mentioned by Baginsky et al. [6], i.e., (i) high amount of extractable essential fatty acid, (ii) favorable omega-6 to omega-3 fatty acid ratio, and (iii) high mucilaginous fiber content, Ayerza and Coates [1] stated that chia has more protein, lipids, energy, and fiber—but fewer carbs—than rice, barley, oats, wheat, or corn, while its protein is gluten-free. In addition, chia was mentioned to be an excellent source of calcium, phosphorus, magnesium, potassium, iron, zinc, and copper [1]. It contains significantly less saturated fatty acids compared to marine food sources and has a high α -linolenic acid content, whilst being odor- and tasteless, which constitutes an advantage, particularly in regard to enriching foods [1]. Furthermore, chia seeds represent a concentrated source of polyphenolic compounds such as flavonoids (myricetin, quercetin, kaempferol, and chlorogenic and caffeic acids), which were found to be comparatively higher in quantity than in many other grains, cereals, and oily seeds [1,5,9]. In this regard, Hrnčič et al. [5] underlined the possible anti-cancerogenic, anti-hypertensive, and neuron-protective effects of the flavonoids. Because of its nutraceutical and physiochemical properties, chia has been widely used as a whole seed, flour, seed mucilage, gel, and oil for developing various enriched food products, such as bread, pasta, cakes, cookies, chips, cheese, yoghurt, meat, fish, and poultry [1,9,10]. Providing an unadulterated, herbal source of omega-3 fatty acids, antioxidants, and dietary fiber, chia represents a possibility to improve human nutrition, as its supplementation has potential to lower incidence of cardiovascular disease, obesity, hypertension, cancer, diabetes, pruritus, and celiac disease [1,5,11].

Salvia columbariae Benth., also known as Golden Chia or desert chia, holds great potential from a nutritional point of view as well [12]. It contains similar high levels of α -linolenic acid and vitamin-E-active compounds, such as tocopherols, tocotrienols, and 8-plastochromanol [12]. Additionally, it was proven that Golden Chia contains considerable amounts of cryptotanshinone and tanshinone, which effectively prevent clotting and restore blood flow in stroke [12–15]. Both *Salvia* species could, therefore, represent a new and sustainable sources for fats and oils in the food and pharmaceutical industries, while meeting the ever-increasing demand for new and possibly health-promoting superfoods [12].

Cultivation of *Salvia hispanica* L. under the given environmental conditions in southwestern Germany would have not been possible a few years ago as chia is a short-day flowering plant, being frost intolerant in all developmental stages [16,17]. The induction of flower formation, the transition from vegetative to generative plant growth, is, therefore, highly dependent on day length (<12 h), limiting its cultivation to latitudes lower than 25° near the equator [18–20]. Breeders did overcome the photoperiodic sensitivity, paving the way for chia cultivation and seed formation under day length conditions of 12 to 15 h [18,19,21]. This breakthrough enabled Grimes et al. [22,23] to demonstrate the feasibility of chia cultivation under the given environmental conditions in southwestern Germany.

The objective of this study was to identify and evaluate new, suitable daylength-adapted chia varieties from a pool of varieties unknown in Europe with regard to their daylength sensitivity and their ability to mature under long day conditions while obtaining satisfactory agronomical and quality traits. Those varieties were compared to a patented variety which has already been cultivated under local conditions in previous years and had produced correspondingly satisfactory results [22,23].

2. Materials and Methods

2.1. Preliminary Trial Part 1: Climatic Chamber

In order to select new chia varieties which could possibly be cultivated under the long day conditions of southwestern Germany, a climate chamber trial was conducted at the University of Hohenheim in 2015/2016 where, in total, 13 *Salvia hispanica* L. varieties and one *Salvia columbariae* Benth. variety were cultivated (Table 1).

Table 1. 13 *Salvia hispanica* L. varieties and one *Salvia columbariae* Benth. variety were cultivated during a climate chamber trial at the University of Hohenheim in 2015/2016.

Variety Name/Abbreviation	Species	Country of Origin	Provided by
W13.1	<i>Salvia hispanica</i> L.	USA	University of Kentucky
Sahi Alba 914	<i>Salvia hispanica</i> L.	Argentina	Agustin Sorondo
G8	<i>Salvia hispanica</i> L.	USA	University of Kentucky
07015ARG	<i>Salvia hispanica</i> L.	Argentina	Genebank
06915ARG	<i>Salvia hispanica</i> L.	Argentina	Genebank
06815BOL	<i>Salvia hispanica</i> L.	Bolivia	Genebank
BOL	<i>Salvia hispanica</i> L.	Bolivia	Genebank
KOL	<i>Salvia hispanica</i> L.	Columbia	Genebank
Chia nigra	<i>Salvia hispanica</i> L.	Nicaragua	Genebank
SALV66	<i>Salvia hispanica</i> L.	NA	IPK Gatersleben
Golden Chia	<i>Salvia columbariae</i> Benth.	NA	IPK Gatersleben
UGA	<i>Salvia hispanica</i> L.	Uganda	Genebank
Sachia white	<i>Salvia hispanica</i> L.	Mexico	Genebank
Sachia black	<i>Salvia hispanica</i> L.	Mexico	Genebank

The pot experiment was arranged in a completely randomized block design with six replicates. In total, 360 pots (Ø 11 cm) were filled with two-thirds fertile loam topsoil (Filderlehm) and one-third sand. No additional fertilizer was applied. Sowing took place manually by hand on 4 December 2015. Two seeds were sown per pot. Sowing depth was about 1 cm. Plants were thinned to one plant per pot on 19 December 2015. Twelve trays of 15 pots were placed on top of the tables in each of the two climatic chambers.

One chamber was set at 16 h of illumination to mimic long day conditions whereas the other chamber was set at 8 h of illumination in order to mimic short day conditions. During the whole experiment, which lasted from 100 to 191 days from sowing to harvest (depending on the development of each variety), the plants were illuminated by six 400-watt high-pressure sodium lamps Son-T-Agro (Philips, Amsterdam, The Netherlands), which were mounted at a height of about 80 cm above the two tables. The temperature was first set to 30 °C during the illuminated hours and 25 °C during the non-illuminated hours. On 16th December 2015, the temperature settings were changed to 25 °C during the illuminated hours and 18 °C during the non-illuminated hours. The temperature settings were switched off on 14th March 2016. Temperatures cycled sinusoidally during the day, ranging from 27 to 35 °C [24]. The humidity was kept constant at about 58%.

Through the above-outlined climate chamber trial, two previously unknown chia varieties, able to produce inflorescences and mature under the mimicked long day conditions, could be selected to be cultivated under field conditions in subsequent years, namely Golden Chia and SALV66.

2.2. Preliminary Trial Part 2: Field Trials

In 2016 and 2017, single-row field trials took place at the experimental station Ihinger Hof and solely served the purpose of seed multiplication of SALV66 and Golden Chia, which were identified through the previously conducted climate chamber trial. In 2018, a field trial was set up to test the overall performance of the selected varieties shown in Figure 1 in comparison to G8, which has already been cultivated for several years at Ihinger Hof [22,23].



Figure 1. Inflorescences of *Salvia hispanica* L. ((left) “G8”, (right) “SALV66”) and *Salvia columbariae* Benth. ((middle) “Golden Chia”) varieties.

2.3. Site Description

The field trial was conducted in 2018 at the experimental station Ihinger Hof (University of Hohenheim, Upper Neckarland, 48°44′40,70″ N 8°55′26,36″ E). Precipitation during the experimental period amounted to 208.5 mm. Mean temperature during the respective growing season was 17.6 °C. Meteorological data were obtained by the weather station at Ihinger Hof (Figure 2).

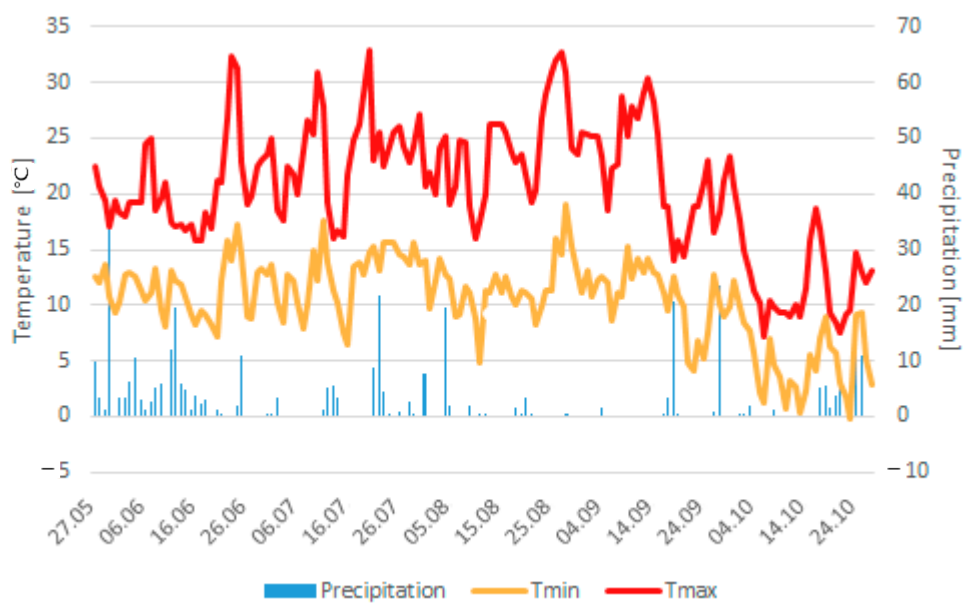


Figure 2. Precipitation (mm; bars) and minimum and maximum temperature (°C; lines) during the experimental period at Ihinger Hof in 2018 (May–October).

Growing degree days were calculated using the growing degree-day Equation (1) of McMaster and Wilhelm [25], where T_{MAX} is defined as the daily maximum air temperature, T_{MIN} is defined as the daily minimum air temperature, and T_{BASE} as the crop base temperature. The crop base temperature of 10 °C according to Baginsky et al. [6] was subtracted from the daily average air temperature.

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

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

The soil on the trial site was characterized as Pelosol brown soil according to the IIUSS Working Group WRB [26]. The soil characteristics are shown in Table 2.

Table 2. Soil characteristics of the experimental site at Ihinger Hof in 2018.

Year	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH	N _{min} ^a (kg ha ⁻¹)
2018	30	13.9	52.3	33.9	7.37	22.99
	60					9.63
	90					7.58

N_{min}^a = soil mineral nitrogen content.

2.4. Trial Setup

The field trial was set up as a randomized complete block design with three replications for each variety with a single plot size of 4 × 15 m. Triticale (*Triticosecale* Tscherm.-Seys. ex Müntzing) was cultivated as a previous crop. Harrowing for seedbed preparation took place shortly before sowing with the help of a mechanical combination seed drill (Rau Landtechnik GmbH; Brigachtal, Germany) in order to loosen the topsoil layer and achieve a good crumbling. Prior to sowing, one pass of seedbed preparation was conducted with a roller (Güttler GmbH, Kirchheim/Teck, Germany) representing the device equipped with a 724 rotary harrow (Kuhn Maschinen-Vertrieb GmbH, Schopisdorf, Germany) to a depth of 5 cm. Mechanical sowing took place on 28th May 2018 using a Fendt 207 (AGCO GmbH, Duluth, GA, USA) equipped with a 121 Deppe D82 Sower (Agrar Markt DEPPE GmbH, Rosdorf, Germany) and a roller (Güttler GmbH, Kirchheim/Teck, Germany). Chia was sown at a row spacing of 50 cm to a depth of 1 cm at a rate of 1.5 kg ha⁻¹.

A universal fertilizer spreader “UKS 230” (RAUCH Landmaschinenfabrik GmbH, Sinzheim, Germany), which was attached to a Massey Ferguson (AGCO GmbH, Duluth, GA, USA), was used to fertilize the plots with 20 kg N ha⁻¹, broadcasted as calcium ammonium nitrate 39 days after sowing. Manual weed control was performed 37 days after sowing on 3rd July 2018. To eliminate flea beetle infestation, 15 days after sowing, Bulldock (Nufarm Deutschland GmbH, Köln, Germany) was applied at a rate of 300 mL ha⁻¹ with β-Cyfluthrin as an active ingredient dissolved in 300 L H₂O ha⁻¹ with the help of a spraying system (Dammann, Buxtehude-Hedendorf, Germany) mounted on a Fendt equipment device (AGCO GmbH, Duluth, GA, USA). In order to control biting and sucking insects, Biscaya (Bayer CropScience Deutschland GmbH, Langenfeld, Germany) was applied 54 and 68 days after sowing at a rate of 300 mL ha⁻¹ with thiacloprid dissolved in 300 L H₂O ha⁻¹.

2.5. Data Collection

2.5.1. Phenological and Agronomic Traits

In 2018, three plants per plot were recorded and assessed in detail every week starting nine days after sowing. Days until flowering and maturity were monitored. Single plant height of the matured plants was measured. Additionally, the number of non-senescent first-degree branches at the main stem, the number of inflorescences (>5 cm for *Salvia hispanica* L.), and the length and the width of the central axis inflorescence of three plants per plot were recorded. Finally, the number of seeds produced per single plant was counted, and the respective seed weight per single plant was assessed.

2.5.2. Yield Traits

Manual harvest of Golden Chia took place on 29th August 2018. Mechanical harvest of the G8 and SALV66 took place on 5th October 2018 with a plot combine Classic (Wintersteiger AG, Ried, Austria). The plot seed yield was determined on the basis of absolute dry matter (0% grain moisture) and thousand

seed mass (TSM) was determined by weighing and counting (Contador, Pfeuffer GmbH, Kitzingen, Germany) one thousand seeds per plot in order to calculate the mean thousand seed mass.

2.5.3. Quality Traits

Quality traits and the following mentioned procedures were determined and amended according to Grimes et al. [22]. Mucilage extraction took place according to Muñoz et al. [27] and total seed nitrogen content was determined according to Dumas [28]. In order to estimate the crude protein content, the total seed nitrogen content values were multiplied by the conversion factor 5.71 [29]. The determination of the crude oil content took place according to the European Commission's Regulation 152/III H procedure B [30]. Fatty acid methyl esters (FAMES) were generated by rapid saponification and esterification in order to identify the fatty acid profile [31–33].

2.6. Statistical Analysis

For agronomical traits including plant height, plant weight, and number of inflorescences, branches, and seeds, as well as the lengths of the main inflorescences, single-plant data of three plants per plot were available. The following linear mixed model was used to detect variety differences among these traits. Each trait was used as a response variable in the subsequent model:

$$y_{ijk} = \mu + b_j + \tau_i + p_{ij} + e_{ijk}, \quad (2)$$

where y_{ijk} is the observation taken on plant k in block j with variety i ; μ is the constant term; b_j is the fixed effect of the j -th block; τ_i is the fixed effect of the i -th variety; p_{ij} is the random effect of the ij -th plot with $p_{ij} \sim N(0, \sigma_p^2)$; and e_{ijk} are the residual errors with $e_{ijk} \sim N(0, \sigma_e^2)$. In the case of some variables, separate error variances for each variety were estimated if residuals plots suggested strong, variety-specific heterogeneity of variance. This was the case with the following traits: length of main inflorescence and number of branches. Variance components were estimated by the method of restricted maximum likelihood through the MIXED procedure in SAS v9.4 (SAS Institute, Cary, NC, USA). The model assumption normal distribution of residuals and homogeneity of variance were graphically assessed by the inspection of quantile-quantile plots for the former assumption and scatter plots of residuals versus predicted values for the latter assumption. Plots of studentized residuals were used. If necessary, response variables were transformed to meet assumptions. Number of inflorescences and number of seeds were log-transformed. Seed weight was transformed by the fourth root. The null hypothesis of equal variety effects was tested using sequential Wald-type F-tests. Denominator degrees of freedom were adjusted using the method of Kenward and Roger. This method additionally corrects the standard errors of means and differences. If the null hypothesis was rejected, the three variety means were compared by pairwise t-tests. Throughout all statistical analyses, a significance level of $\alpha = 5\%$ was used. Estimates of traits, which were transformed for analysis, were back-transformed for presentation. Standard errors were back-transformed using the delta method.

For the quality traits mucilage content, protein content, oil content, fatty acids (palmitic, stearic, oleic, vaccenic, linoleic, and α -linolenic acids), polyunsaturated fatty acid to saturated fatty acid (PUFA:SFA) and omega-6 to omega-3 ratios, as well as for the agronomical traits yield and thousand seed mass, only one observation per plot was available. However, the number of plants per plot varied strongly. To adjust for varying number of plants, (a) the number of plants per m^2 were included as a weighting variable into statistical analysis and (b) the number of plants was, in addition, included as a covariate into the model. The following model was used:

$$y_{ij} = \mu + b_j + \tau_i + \beta_1 k_{ij} + \beta_2 k_{ij} + e_{ij}, \quad (3)$$

where y_{ij} is the observation in block j with variety i , i.e., the plot observation; k_{ij} is the corresponding number of plants per plot, which is used as a covariate and weighting variable; μ is the constant term;

b_j is the fixed effect of the j -th block; τ_i is the fixed effect of the i -th variety; β_1 is the common slope in a regression on the covariate k_{ij} ; β_{2i} is the variety-specific slope for the regression on the covariate; e_{ij} are the residual errors with $e_{ij} \sim N(0, k_{ij}^{-1} \sigma_e^2)$. Parameter estimation, adjustment of denominator degrees of freedom, and the F-test followed the same method as described for agronomical traits. First of all, in the interaction of covariate and treatment, the factor $(\beta_{2i}k_{ij})$ was tested for significance. Only without such interaction, the covariate adjustment of the treatment variable was performed. Non-significant effects were removed from the model, and in case of a rejection of the null hypothesis of equal variety effects, the effects were compared by pairwise t -tests.

3. Results and Discussion

The general feasibility of chia cultivation in southwestern Germany was proven by Grimes et al. as varieties, either adapted or insensitive to photoperiod, have been made available [22]. On this basis, a suitable cropping system was developed for southwestern Germany [23]. Within the genus *Salvia*, two varieties, namely SALV66 (*Salvia hispanica* L.) and Golden Chia (*Salvia columbariae* Benth.), were characterized and examined. Both varieties were compared to G8 (*Salvia hispanica* L.) for their performance under the given climatic conditions since G8 has already been cultivated for several years and was therefore considered as the reference. As reported in more detail in the subsequent sections, the two *Salvia hispanica* L. varieties G8 and SALV66 differed strongly in (i) growth habitus, (ii) photosensitivity reaction, (iii) flower induction/harvest maturity, and (iv) seed yield compared to the *Salvia columbariae* Benth. variety Golden Chia. To our knowledge, this is the first time the agronomical and quality traits of SALV66 and Golden Chia have been reported.

3.1. Plant Development

Both *Salvia hispanica* L. varieties (G8, SALV66) accumulated almost twice as many growing degree days (451.85, 58 days after sowing (DAS)), flowering simultaneously, compared to Golden Chia (247.15, 34 DAS) in order to induce (Table 3). Radiation, average day length, and average mean temperature until flower induction amounted to 10,399 Wh/m², 15.74 h, and 18.1 °C, respectively, for G8 and SALV66 and were found to be similar to Golden Chia at 10,563 Wh/m², 15.84 h, and 17.7 °C, respectively (Table 3).

Previously conducted field trials by Grimes et al. [22,23] showed that cultivated *Salvia hispanica* L. varieties required 65 to 74 days in order to induce flowering, while the radiation and day length obtained were similar (~10,200 Wh/m²; 15.8 h) to the present study. In addition, it could be shown that during those previously conducted field trials, cultivated *Salvia hispanica* L. varieties accumulated more growing degree days (463–545), and the average mean temperature until flower induction tended to be lower (16.8–18.0 °C) compared to the present study [22,23]. The results of the present study therefore verify the findings of Grimes et al. [22] and Baginsky et al. [6], which identified the threshold of 15.8 h of daylength for flower induction to be adequate for the two newly cultivated varieties (SALV66 and Golden Chia), being of higher importance for their development than the accumulated growing degree days. Both *Salvia hispanica* L. varieties, G8 and SALV66, reached harvest maturity 131 DAS, after having accumulated 1006.85 growing degree days (GDD), slightly more compared to previously conducted trials in southwestern Germany [22,23]. Golden Chia, on the other hand, reached harvest maturity after 93 DAS, while having accumulated 802.1 GDD. Average mean temperature from flower induction to harvest maturity was 17.4 °C (G8, SALV66) and 20.5 °C (Golden Chia).

Table 3. Sowing dates, accumulated growing degree days (GDD), radiation, day length, and mean temperatures at Ihinger Hof (IHO) in 2018 for varieties G8, SALV66, and Golden Chia.

Sowing Date	Flower Induction				Radiation (Wh/m ²) ^d	Day Length (h) ^e	Harvest Maturity *			
	DAS ^a	Date	GDD ^b	Mean Temperature (°C) ^c			DAS	Date	GDD	Mean Temperature (°C) ^f
G8 and SALV66										
28th May 18	58	24th July 18	451.85	18.1	10,399	15.74	131	5th October 18	1006.85	17.4
Golden Chia										
28th May 18	34	30th June 18	247.15	17.7	10,563	15.84	93	23rd August 18	802.1	20.5

^a DAS: Days after sowing. ^b GDD: Accumulated growing degree days at a base temperature of 10 °C [25]. ^c Average mean temperature until flower induction. Obtained by the weather stations at IHO. ^d 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). ^e Average day length until flower induction [29], available from: <http://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html> (accessed 15 January 2018). ^f Average mean temperature from flower induction to harvest maturity obtained by the weather stations at IHO. * Harvest maturity is equivalent to vegetation period.

From the results obtained in this present study, it is reasonable to assume that temperature led to an accelerated plant development of G8 and SALV66 accompanied with an earlier beginning of flowering, as already stated by Grimes et al. [22]. The relatively short vegetation period of Golden Chia compared to the results of the trial conducted by Ayerza and Coats [34] in the Tucuman/Bolivian Forest implies that the dry and hot climatic conditions in southwestern Germany (2018) were more favorable as Golden Chia is, in general, more adapted to desert climates, preferring dry and sandy soils (Table 3).

As Grimes et al. [22] discussed previously, sowing date is of great significance in regard to plant development, as it affects day length, GDD, temperature, and vegetation period. Sowing dates in later May limit the risk of frost damage to the emerging *Salvia hispanica* L. plants as the last spring frost often occurs at the end of April/beginning of May (ice saints), while simultaneously exposing plants to the risk of early frost damage occurring in October/November in case of late ripening under the given climatic conditions of southwestern Germany. Therefore, the relatively short vegetation period of *Salvia columbariae* Benth. compared to *Salvia hispanica* L. constitutes an advantage, as maturity is most certainly reached prior to partially unfavorable climatic conditions in late autumn. Furthermore, the short vegetation period of *Salvia columbariae* Benth. enables a greater range of subsequent crops as the necessary fieldwork (soil tillage, etc.) can be conducted earlier during the year. In general, it can be stated that the cultivated varieties (G8, SALV66, Golden Chia) completed their phenological development and were able to reach maturity, leading to the assumption that the thermal and photoperiod requirements were fulfilled [22,23,35,36].

In terms of germination, we could not detect any problems regarding Golden Chia compared to former studies conducted in California (greenhouse, laboratory, and field experiments), which indicated that the ambient field temperatures seemed to be adequate to alleviate the seed dormancy barrier for better germination to achieve a uniform number of plants per m² [37].

3.2. Agronomical Traits

3.2.1. Plant Growth

The statistical analysis of the data indicated a significant difference between the *Salvia hispanica* L. and *Salvia columbariae* Benth. varieties concerning the obtained plant height, number of branches and inflorescences, and the length of the central axis main inflorescence at harvest time ($p = 0.0005$, $p = 0.0002$, $p = 0.0145$, $p = 0.0020$) (Table 4).

Table 4. Mean plant height, number of branches, number of inflorescences per plant and length/width of central axis inflorescence per single plant of three chia varieties (G8, Golden Chia, SALV66) cultivated at Ihinger Hof in 2018 ($n = 3$, $\alpha = 0.05$, mean \pm standard error).

Factor	Plant Height [cm]	Number of Branches Per Plant	Number of Inflorescences Per Plant	Length/Width * of Central Axis Inflorescence [cm]
Varieties				
G8	74.23 \pm 2.1 a	7.77 \pm 0.3 a	7.98 \pm 1.2 a	10.40 \pm 0.7 a
Golden Chia	37.48 \pm 2.3 b	2.08 \pm 0.2 b	29.42 \pm 4.9 b	* 2.76 \pm 0.4 b
SALV66	78.60 \pm 2.1 a	8.27 \pm 0.2 a	15.97 \pm 2.4 b	12.26 \pm 0.9 a
p-values				
Variety	0.0005	0.0002	0.0145	0.0020

* Width of the central axis inflorescence of Golden Chia was measured whereas the length of the inflorescence of G8 and SALV66 have been measured due to the differences in morphology (Figure 1). Means which share a common lowercase letter are not significant at $\alpha = 0.05$.

The morphological differences shown in Table 4 can be attributed to the different growth habitus of the cultivated varieties as shown in Figure 1. To state only some observed differences, which are in accordance with literature, *Salvia columbariae* Benth., for example, grows 10 to 50 cm tall. It develops a tap rooted, rosetted, and unbranched or sporadically branched stem. The stems emerge singly or in several branches at the base and form terminal inflorescences, subtended by a few leaves; one or

more spherical clusters, each containing one dozen to several dozen flowers reaching 8 to 20 mm in diameter, also appear [38,39]. *Salvia hispanica* L., on the other hand, reaches growth heights of up to 1.75 m, has shallow roots, and its stem is sparsely branched and forms terminal pseudo whorl with blue or white flowers which can be more than 20 cm long [1].

Golden Chia produced significantly shorter plants (37.48 cm) in comparison to the other two *Salvia hispanica* L. varieties, G8 (74.23 cm) and SALV66 (78.60 cm), due to the difference in growth habitus as already indicated (Table 4). With a mean of 2.08 branches per plant, Golden Chia produced significantly less branches compared to G8 (7.77) and SALV66 (8.27), which were in the range (7.9–9.3) of what Souza and Chavez [40] observed under greenhouse conditions in Brazil (Table 4). [22]. In regard to the number of branches developed, Sosa Baldivia and Ibarra [41] pointed to the fact that *Salvia hispanica* L. is subject to high plasticity as it is directly influenced by row spacing and seeding density, which has to be matched to the specific, given environmental conditions.

G8 (7.98) produced a significantly lower number of inflorescences compared to Golden Chia (29.42) and SALV66 (15.97), decreasing from Golden Chia > SALV66 > G8. The number of inflorescences produced by *Salvia hispanica* L. varieties in 2016 cultivated at Ihinger Hof amounted to 11.8–13.3 [22]. As for the number of branches, the number of developed inflorescences is most probably also subject to the plasticity of chia [41]. G8 and SALV66 formed pseudo whorl inflorescences whereas Golden Chia developed compact, round inflorescences as shown in Figure 1.

Therefore, the length of the inflorescence of the central axis of SALV66 (12.26) and G8 (10.40) was measured whereas the width of the central axis inflorescence of Golden Chia (2.76) was recorded. The length of the central axis inflorescence of *Salvia hispanica* L. was in the range (7.92–14.82 cm) of what Hüller Goergen et al. [42] observed during a field trial conducted in Brazil but was considerably shorter compared to the results (16.8–19.2 cm) obtained at Ihinger Hof in 2016 by Grimes et al. [22]. The difference in length is most probably due to environmental influences and weather conditions, crop management, or a combination of those influences and specific species responses [43]. To our knowledge, the above-mentioned data of Golden Chia were presented for the first time; therefore, it would be of great interest to examine different environmental and crop management effects in regard to the cultivation of Golden Chia under the given conditions in southwestern Germany.

3.2.2. Yield Traits

Seed weight per single plant was not significantly influenced by variety ($p > 0.05$) (Table 5). It ranged from 3.11 ± 0.6 (G8) to 3.63 ± 0.8 (Golden Chia) and 4.46 ± 0.7 (SALV66) respectively (Figure 3). The same applied for the number of seeds produced per single plant, which varied from 2996 ± 540 (G8) to 3260 ± 695 (Golden Chia) and 3162 ± 525 (SALV66) (Figure 3).

Table 5. Results of the statistical evaluation of seed weight (g) per single plant, number of seeds per single plant, yield (kg ha^{-1}) †, and thousand seed mass (TSM) (g) ‡ of three chia varieties (G8, Golden Chia, SALV66) cultivated at Ihinger Hof in 2018 ($n = 3$, $\alpha = 0.05$).

Factor	Seed Weight (g) Per Single Plant <i>p</i> -Value	Number of Seeds Per Single Plant <i>p</i> -Value	Yield (kg ha^{-1}) † <i>p</i> -Value	TSM (g) ‡ <i>p</i> -Value
Variety	ns	ns	**	***
Plants per m^2	-	-	**	ns
Variety \times Plants per m^2	-	-	ns	ns
Block	**	**	ns	ns

ns = $p > 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, † Plants per m^2 as a covariate and weighting variable. – Factors were not part of the model [2].

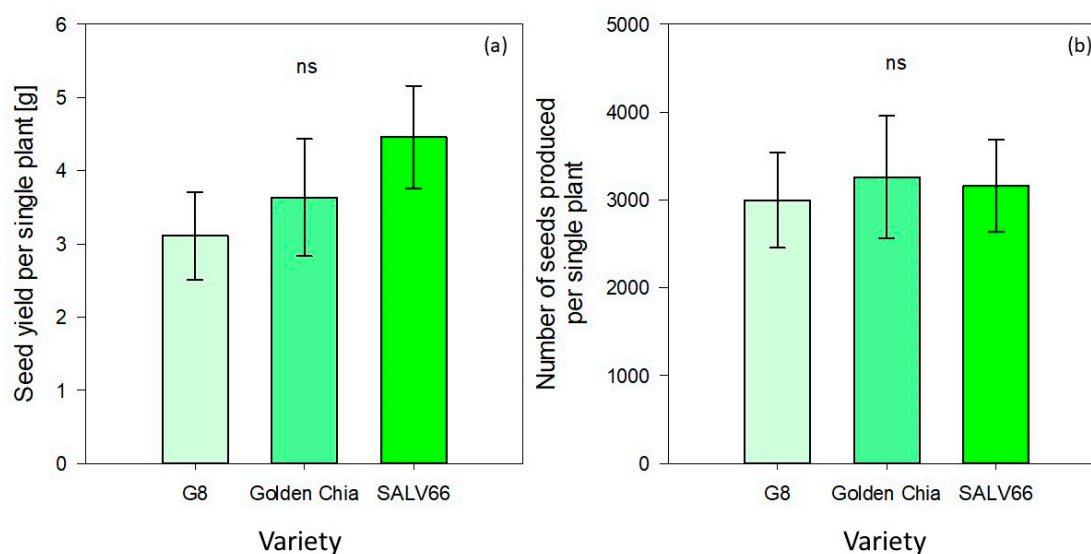


Figure 3. Seed yield (g per plant) (a) and number of seeds produced per single plant (b) of three chia varieties (G8, Golden Chia, SALV66) cultivated and manually harvested at Ihinger Hof in 2018. Means which share a common lowercase letter are not significant at $\alpha = 0.05$. ($n = 3$, $\alpha = 0.05$, means \pm standard error bars based on model [2]).

Reported manually obtained seed yields and amounts of seeds produced per single plant were in the range of 6.3 to 6.4 g and 4051 to 4289 for *Salvia hispanica* L., respectively, being distinctly higher compared to the values obtained by the present study [22]. It is, in general, stated that obtained seed yields per single plant and seeds produced per single plant in different plant species could be influenced by various factors, such as plant density, genotypic differences, climatic conditions, and mineral nitrogen content [44–47]. For both SALV66 and Golden Chia, it can be assumed that single plant yield and seeds produced were most likely predominantly influenced by the environmental conditions of the respective growing season. The assumption which was made in regard to the high plasticity of *Salvia hispanica* L. enabling the compensation of lower plant densities with higher seed numbers per single plant could not be verified by the present study [22,41]. To our knowledge, this is the first time these specific yield traits of *Salvia columbariae* Benth. were published. Further field trials would have to be carried out in order to make well-founded statements about the possible specific effects influencing seed yields and numbers of seeds produced per single plant.

Golden Chia obtained a significantly lower mean yield ($284.13 \pm 74.44 \text{ kg ha}^{-1}$) compared to SALV66 ($643.99 \pm 42.92 \text{ kg ha}^{-1}$), whereas the obtained yield of G8 ($491.91 \pm 67.45 \text{ kg ha}^{-1}$) did not differ significantly from Golden Chia and SALV66, as shown in Figure 4. As presented in Table 5, the statistical analysis showed that the number of plants per m^2 ($p = 0.0372$) and variety ($p = 0.0354$) significantly influenced obtained yields (kg ha^{-1}). Mean number of plants per m^2 of the present study varied from 34 (G8) to 20 (Golden Chia) and 26 (SALV66), being roughly in the range of the plants per m^2 obtained in 2016 (17–30) and considerably lower compared to the obtained number of plants per m^2 in 2017 (41–47) at Ihinger Hof. In this regard, it must be stated that three days after sowing, a storm with downpour (35 mm) took place, followed by a dry period (Figure 2), leading to soil crusting and a partially irregular germination, which probably significantly influenced the obtained number of plants per m^2 and yields of the cultivated varieties [48]. The myxocarpic characteristic of the *Salvia* genus generally constitutes an advantage to seeds coping with restricted and irregular water availability during germination and early seedling development, as its mucilage may aid in supplying water to the seed [49,50]. However, the potential advantage of the mucilaginous chia seeds might have been overruled by the susceptibility of the seedlings, which could not penetrate the soil crust [51].

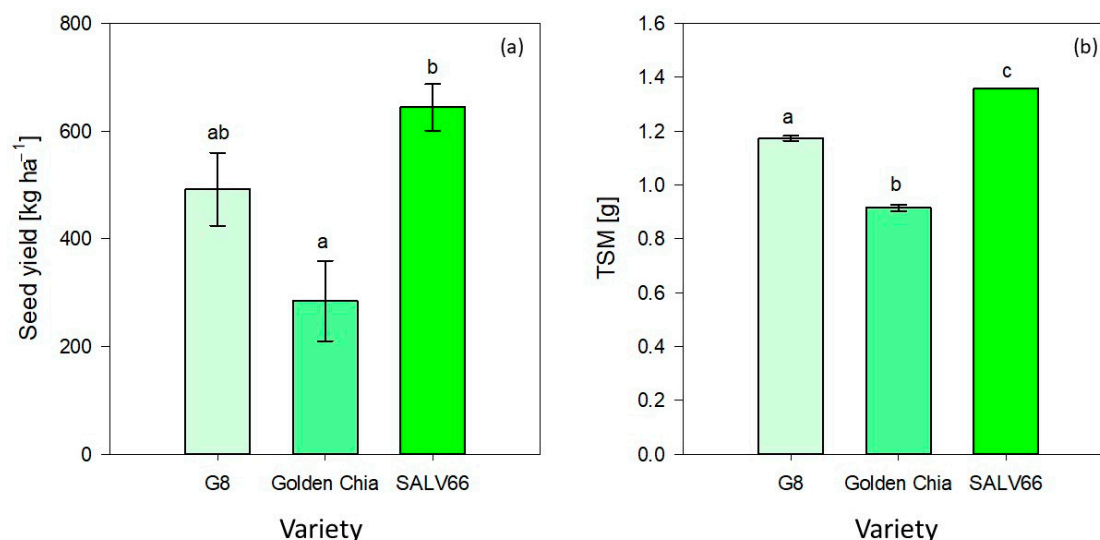


Figure 4. Seed yield (kg ha^{-1}). (a) and thousand seed mass (TSM) (g) (b) of the three *Salvia* varieties (G8, Golden Chia, and SALV66) cultivated at Ihinger Hof in 2018. Means which share a common lowercase letter are not significant at $\alpha = 0.05$. ($n = 3$, $\alpha = 0.05$, means \pm standard error bars based on model [3]).

In this present study, G8 obtained a noticeably lower seed yield compared to previously obtained seed yields of G8 at Ihinger Hof in 2016 and 2017 ($752\text{--}1170 \text{ kg ha}^{-1}$) cultivated at the same sowing rate, row spacing, and nitrogen fertilization rate. The relatively low seed yields obtained by both of the *Salvia hispanica* L. varieties in this study could be explained first and foremost due to unfavorable field conditions and the accelerated plant development resulting from them, as addressed in Section 3.1 and shown in Table 3. In this regard, Mathan et al. stated that flower induction, which marks the transition from vegetative to generative plant growth, is, inter alia, decisive for optimal yield in crop plants [52]. The average mean temperature until flower induction was slightly higher ($0.1\text{--}1.3 \text{ }^\circ\text{C}$) compared to the previous studies conducted at Ihinger Hof in 2015 to 2017; furthermore, the average mean temperature from flower induction to harvest maturity was considerably higher ($3\text{--}5.5 \text{ }^\circ\text{C}$). Those high temperatures during the grain filling phase probably led to an increase in the photosynthesis rate above its optimum, leading to reduced metabolic activity during grain filling and maturation and, thus, to yield losses [6,16,22]. Alongside the relatively high temperatures, precipitation was partly substantially lower (between 38–193 mm) compared to the studies conducted at Ihinger Hof between 2015 and 2017, which certainly contributed to the comparably low seed yields obtained. Even though it is stated that *Salvia hispanica* L. is able to grow in dry conditions and is semi-tolerant to drought, this only applies to already established plant stocks [35,53–55]. It is indicated that *Salvia hispanica* L. profits from precipitation and general water availability ranging from 300 to 1000 mm during the entire vegetation period, having a higher water demand during its vegetative phase and while establishing the reproductive organs, whereas drier conditions are required during the generative phase and seed maturity [56–58]. In this regard, Steduto et al. stated two possible negative effects on yield in general: (1) the inhibition of water stress on pollination and successful formation of the embryo, which could have led to a reduced number of set fruits (or grains) and, therefore, limited harvest index and seed yield, respectively, and (2) the under-filling and abortion of younger fruits resulting from a lack of photosynthetic assimilates available to be used for fruit developing [58].

In contrast to that, the seed yield of Golden Chia could, in comparison to the obtained seed yield reported by Ayerza and Coats (133 kg ha^{-1}), be considered as quite high. The disparity between the seed yield (g) produced per single plant and the seed yield per kg ha^{-1} of Golden Chia and the significantly lower seed yield (kg ha^{-1}) compared to the other *Salvia hispanica* L. varieties can be explained by the degree of domestication. Domestication of *Salvia hispanica* L. began, according to Ayerza and Coats, more than 2000 years ago, whereas Golden Chia remains highly undomesticated to

date [1]. The high seed shattering level is a distinct attribute of the wild stage (lack of domestication), leading to enormous seed yield losses due to threshing [34,59]. Further breeding approaches towards reduced seed shattering should, therefore, be developed in order to be able to cultivate Golden Chia on an economically profitable basis. Based on our results, it can be stated that Golden Chia holds great potential, especially with regard to its photosensitive reaction, as flower induction and harvest maturity were reached significantly earlier, reducing the risk of frost damage due to late harvests. One general factor possibly leading to lower obtained seed yields is the choice of harvesting time and harvest intensity (manually/mechanically). It has been reported that sub-optimal combine operations and harsh weather conditions could lead to yield losses of up to 37% as chia is highly susceptible to seed shattering [60,61]. In this context, the non-uniform (top-down) maturation of *Salvia hispanica* L. should be mentioned, posing an issue in regard to optimal harvest time as the central inflorescence matures and ripens while inflorescences on side branches are still immature [23,62]. The same applies to *Salvia columbariae* Benth., as it could be observed that the flowers generally ripen from the inside out in addition to a delayed ripening of the side branches. In addition, the short vegetative period could, in this regard, negatively influence the potential seed yield of *Salvia columbariae* Benth.

Thousand seed mass as another yield determining trait seemed to be influenced exclusively by variety ($p < 0.0001$). As illustrated in Figure 4, the TSM of G8 was 1.17 ± 0.01 g, while the TSM of Golden Chia was 0.92 ± 0.01 g, and SALV66 obtained a TSM of 1.36 ± 0.01 g. TSM differed significantly between each of the varieties. The TSMs for G8 and SALV66 were in the range of current literature (1.1–1.4 g) [19,22,63]. In regard to obtained *Salvia hispanica* L. TSMs, it could be shown that a high number of plants per m^2 leads to interplant competition and therefore to a decrease in TSM [23]. Other studies related to wheat (*Triticum aestivum* L.) showed that plant density and nitrogen fertilization influenced TSM significantly [64–66]. Identifying factors which influence the TSM of the cultivated *Salvia* varieties could be advantageous in order to further improve agronomic traits of the cultivated varieties in general and especially under the given climatic conditions in southwestern Germany.

With regard to Golden Chia, it should be mentioned that there might be a need to develop a specific cultivation system which differs in row distance, seeding density, and nitrogen fertilizer rates, as well as a specific harvest process from that established for *Salvia hispanica* L. in order to exploit its full potential, leading to higher seed yields and TSM [23].

3.3. Quality Traits

3.3.1. Protein, Mucilage and Oil content

Variety, numbers of plants per m^2 , and the interaction of variety and numbers of plants per m^2 had no significant effect on crude protein content ($p > 0.05$). Crude protein content was $24.78 \pm 0.9\%$ (G8), $22.14 \pm 1.1\%$ (Golden Chia), and $24.46 \pm 1.0\%$ (SALV66) (Table 6).

Table 6. Protein, mucilage, and oil content (expressed as a percentage) of three chia varieties (G8, Golden Chia, SALV66) cultivated at Ihinger Hof in 2018 ($n = 3$, $\alpha = 0.05$, mean \pm standard error).

	Crude Protein (%)	Mucilage (%)	Crude Oil (%)
Varieties			
G8	24.8 \pm 0.87 a	10.8 \pm 0.25 a	30.5 \pm 0.98 a
Golden Chia	22.1 \pm 1.12 a	20.7 \pm 0.29 b	28.0 \pm 1.08 a
SALV66	24.5 \pm 1.00 a	10.4 \pm 0.17 a	31.7 \pm 0.62 a
Factor		<i>p</i> -values	
Varieties	ns	***	ns
Plants per m^2	ns	**	ns
Varieties \times Plants per m^2	ns	ns	ns
Block	ns	ns	ns

Mean \pm SEM, ns = $p > 0.05$ * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ Plants per m^2 as a covariate and weighting variable. Means which share a common lowercase letter are not significant at $\alpha = 0.05$.

The results of this present study are in line with data obtained in previous field trials conducted in southwestern Germany and current literature [22,23]. The decrease in protein content due to high temperatures and due to an increase in altitude and elevation could not be verified, as obtained protein contents can be classified as high even though the average mean temperatures until flower induction (0.1–1.3 C) and harvest maturity (3–5.5 C) were higher compared to previously conducted studies at Ihinger Hof between 2015 and 2017 as mentioned in Section 3.2.2 [22,23,63,67]. *Salvia hispanica* L. and *Salvia columbariae* Benth. species do not only exceed the protein content quantitatively but also qualitatively, compared to other starch crops such as wheat, corn, rice, oats, and barley. It was shown that chia can be incorporated into human diets as a plant-based protein enrichment in order to produce more balanced protein food sources [1,16].

The statistical analysis indicated significant differences in the mucilage content between the examined varieties ($p \leq 0.001$). Additionally, it seemed that the numbers of plants per m^2 did influence the mucilage content ($p \leq 0.01$). As presented, the total mucilage content of the seeds was $10.78 \pm 0.3\%$ (G8), $20.66 \pm 0.3\%$ (Golden Chia), and $10.35 \pm 0.2\%$ (SALV66) (Table 6). The mucilage content of Golden Chia was displayed and statistically evaluated although the values are not representative since it could be demonstrated that the applied mucilage extraction method was only suitable for G8 and SALV66, being in line with the values obtained by current literature [22,23,68]. Seeds of Golden Chia crumbled during the extraction process, which led to a major surface enlargement. The statistically significant difference which was found regarding the mucilage content between the *Salvia hispanica* L. varieties G8 and SALV66 and the *Salvia columbariae* Benth. variety Golden Chia can be explained by this fact. The mucilage extraction method for Golden Chia could be adapted by decreasing the temperature and hydration time. According to Tavares et al., first attempts examining the applicability of cold extraction method for *Salvia hispanica* L. seemed to be promising [69]. Further analysis should be conducted in order to make well-founded statements about the possible content of mucilage extracted from Golden Chia. In regard to the mucilage content, chia could be used inter alia as a vegan thickener, foam stabilizer, suspending agent, emulsifier, adhesive, or binder due to its water-holding capacity and viscosity [5,70]. Another field of application that is of particular interest in terms of environmental protection is the constant and recurring theme around packaging waste. In the packaging industry, mucilage obtained from chia seeds may be used for functional coating and edible films, representing a promising alternative to synthetic packaging [5,71].

The crude oil content of the seeds was not significantly affected by variety, plants per m^2 , or the interaction between variety and plants per m^2 ($p > 0.05$, Table 6). Crude oil content was within the range stated in recent literature, varying from $30.52 \pm 1.0\%$ (G8) to $28.00 \pm 0.6\%$ (Golden Chia) and $31.73 \pm 1.1\%$ (SALV66), though still being lower compared to the crude oil contents obtained by G8 in previous trials conducted at Ihinger Hof in southwestern Germany (30.9–33.8%) [6,12,22,23,34,72]. In this regard, it must be pointed out that the non-uniform maturation of *Salvia hispanica* L. (top-down) and *Salvia Columbariae* Benth. (inside-out) makes it difficult to determine the optimal harvest time and, hence, may have contributed to the low oil content due to a possible high number of immature seeds. Generally, the variation in chia seed oil yields depend on factors such as climatic conditions [67,73].

As summarized by Hrnčič et al., chia oil obtained from *Salvia hispanica* L. is one of the most valuable oils on the market today and is used in the pharmaceutical and food industries [5]. In this regard, Hrnčič et al. draw particular attention to nano-emulsion-based delivery systems based on chia seed oil from *Salvia hispanica* L. for prospective applications to encapsulate lipophilic bioactive components in food, cosmetics, and pharmaceuticals [5]. In order to meet the given EU requirements of the Novel Food guideline, crude oil contents of *Salvia hispanica* L. must range from 30 to 34%, which is also in accordance to current literature [22,63,74,75]. As the crude oil content of Golden Chia obtained by this present study was below 30%, it would not meet those requirements yet. Subsequent trials would be necessary in this respect in order to validate or falsify the obtained results, as seeds of Golden Chia would also constitute a promising raw material for the production of edible, high-quality oil and for the previously mentioned applications [12].

3.3.2. Fatty Acids

Results of the ANOVA showed that the fatty acid composition of the investigated *Salvia* varieties was significantly affected by variety ($p \leq 0.01$, Table 7). As shown in Table 2, contents of all individual fatty acids, except for vaccenic acid, and respective ratios of Golden Chia differed significantly from the *Salvia hispanica* L. varieties G8 and SALV66. All samples were characterized by a high content of ω -3 and ω -6 polyunsaturated fatty acids, namely α -linolenic acid as the main fatty acid (60.4 to 65.9 g/100 g) and linoleic acid (15.1 to 20.4 g/100 g), being in line with values obtained by previously conducted field trials in southwestern Germany [12,22,23,75–79]. We also verified the findings of Matthäus and Özcan showing that within the genus *Salvia*, the amount of α -linolenic acid is highest in *Salvia columbariae* Benth., making it highly interesting from a nutritional point of view [12].

The high seed lipid concentration (~30%), especially of omega-3 and omega-6 fatty acids, is due to proteins connected to the production and storage of plant lipids [80]. In accordance with Ayerza and Coats [34], the study could demonstrate that the amount of α -linolenic acid obtained by Golden Chia was higher compared to that of the *Salvia hispanica* L. varieties G8 and SALV66, whereas it was the opposite for linoleic acid, as presented in Table 7. The amounts of monounsaturated fatty acids obtained from G8 and SALV66, namely oleic (6.9 to 9.1 g/100 g) and vaccenic (0.8 to 0.9 g/100 g), were in line with current literature [7,22,23,81,82]. The same applied to the amounts of saturated fatty acids, which varied between 5.7 and 7.7 g/100 g for palmitic acid and between 3.2 and 3.8 g/100 g for stearic acid. The only published article to date which examines the lipid fraction of seeds from *Salvia columbariae* Benth. grown in Arizona obtained slightly lower mean contents of vaccenic (1.0 g/100 g), palmitic (4.8 g/100 g), and stearic (2.1 g/100 g) fatty acids and a slightly higher amount of oleic acid (9.8 g/100 g) [12].

In the context of fatty acid composition, it should be pointed out that a high amount of polyunsaturated fatty acids determines the low oxidative stability of chia oil, which represents a solvable challenge as respective production processes are available [83].

According to current literature, chia fatty acid compositions are highly dependent on environmental conditions and, therefore, are affected by location [67,73,84,85]. In addition, it was shown that the relationship between elevation, fatty acid composition, and oil saturation for chia is most likely related to a temperature–elevation interaction since elevation is strongly negatively related to temperature, which, as such, affects oil content variation during seed development [67,73,84,85]. This applies to individual fatty acids, which are influenced by irrigation levels, climate conditions including temperature, elevation, and proteins [67,73,80,86,87].

In this context, various approaches are discussed in current literature. Herman et al. [86], for example, mentioned that water stress contributes to an increased production of α -linolenic acid, whereas Amato et al. showed inter alia that mineral fertilization increased free acidity in seeds while it reduced oxidative stability.

The importance of qualitative analysis regarding fatty acids becomes clear when looking at the changes in Western diets during the last decades. Total fat intake in general has continuously decreased, shifting towards a highly carbohydrate-focused diet [88]. The enormous decrease in omega-3 fatty acid intake in addition to the tremendous increase in omega-6 fatty acid intake has resulted in an increased omega-6 to omega-3 ratio up to 20:1 or even higher as Simopoulos stated [88]. This shift in the composition of fatty acids has led to a rise in overweight and obesity as well as pathogenesis of other diseases such as cardiovascular illnesses, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6 to omega-3 ratio) exert suppressive effects [88,89]. Therefore, a balanced omega-6 to omega-3 ratio must be emphasized in regard to general health and in the prevention and management of obesity [88].

The results of the present study indicate that due to the composition of fatty acids and vitamin E active compounds of *Salvia hispanica* L. and *Salvia columbariae* Benth., an increased intake of chia seeds and seed oil could contribute to a more balanced omega-6 to omega-3 diet and high-value food products, possibly leading to an overall improved nutrition [12,22].

Table 7. Fatty acid composition (% of total fatty acid) of three chia varieties (G8, Golden Chia, SALV66) cultivated at IHO in 2018 ($n = 3$, $\alpha = 0.05$, mean \pm standard error).

	Palmitic Acid	Stearic Acid	Oleic Acid	Vaccenic Acid	Linoleic Acid $\omega 6$	α -Linolenic Acid $\omega 3$	PUFA:SFA	$\omega 6$: $\omega 3$
Varieties								
G8	7.4 \pm 0.08 ^a	3.8 \pm 0.05 ^a	7.3 \pm 0.17 ^b	0.8 \pm 0.01 ^b	20.2 \pm 0.21 ^a	60.5 \pm 0.49 ^b	7.2 \pm 0.09 ^b	0.3 \pm 0.01 ^a
Golden Chia	5.7 \pm 0.10 ^b	3.2 \pm 0.06 ^b	9.1 \pm 0.22 ^a	0.9 \pm 0.01 ^a	15.1 \pm 0.27 ^b	65.9 \pm 0.62 ^a	9.0 \pm 0.12 ^a	0.2 \pm 0.01 ^b
SALV66	7.7 \pm 0.09 ^a	3.8 \pm 0.06 ^a	6.9 \pm 0.19 ^b	0.9 \pm 0.01 ^a	20.4 \pm 0.24 ^a	60.4 \pm 0.56 ^b	7.1 \pm 0.10 ^b	0.3 \pm 0.01 ^a
Factor					<i>p</i> -values			
Varieties	***	**	***	**	***	**	***	***
Plants per m ²	ns	ns	ns	ns	ns	ns	ns	ns
Varieties \times Plants per m ²	ns	ns	ns	ns	ns	ns	ns	ns
Block	ns	ns	ns	ns	ns	ns	ns	ns

Results in g 100 g⁻¹ of oil. Estimated *p*-values and standard errors based on model (3). The percentage of saturated and polyunsaturated fatty acids (S/PUFA) was calculated from the total quantity of identified FAs. Mean \pm SEM, ns = $p > 0.5$, ** $p \leq 0.01$, *** $p \leq 0.001$ Plants per m² as a covariate and weighting variable. Means which share a common lowercase letter are not significant at $\alpha = 0.05$.

4. Conclusions

It can be concluded that within the given biodiversity of *Salvia* species, there are existing *Salvia hispanica* L. and *Salvia columbariae* Benth. varieties adapted to day lengths greater than 12 h. Besides intensive breeding approaches, the given range in biodiversity enables the cultivation of chia under the existing day length conditions of southwestern Germany. With seed yields obtained being more or less in line with those of their countries of origin, the selected chia varieties SALV66 and Golden Chia represent very promising raw materials from a nutritional point of view. Chia cultivation in Germany would fulfill the steadily increasing demand for healthy, environmentally sustainable, and regionally produced food products while simultaneously representing a profitable economic source of income for local farmers, potentially leading to an advantageous local, economically and environmentally profitable way of broadening the food chain and food base under the given conditions in southwestern Germany. However, seed shattering of Golden Chia is a significant drawback in regard to its commercial production. Further breeding efforts and scientific studies on agronomic management practices are therefore necessary.

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