Screening and cultivation of chia (Salvia hispanica L.) under Central European conditions: The potential of a re-emerged multipurpose crop

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List of abbreviations and acronyms

BC	before christ
%	percent
°E	degree east, longitude
°N	degree north, latitude
°C	degree centigrade
µmol	micromole
a.s.l.	above sea level
ANOVA	Analysis of variance
CAGR	compound annual growth gate
CO_2	Carbon dioxide
DAS	days after sowing
DGE	Deutsche Gesellschaft für Ernährung e. V.
e.g.	exampli gratia (for example)
et. al.	et alii, and others
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure
GHG	Greenhouse Gas
L.	Linné
NPK	nitrogen-phosphorus-potassium fertilizer
N	nitrogen
N_{min}	soil mineral nitrogen
ssp.	Subspecies
TKW	thousand kernel weight
TSM	thousand seed mass
T_{max}	maximum air temperature
T_{min}	minimum air temperature
US/USA	United States of America
var.	variety
WRB	World Reference Base for Soil Resources

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1. Introduction

1.1 "Super Food": Demand and challenges

Human nutrition has always adapted to adverse food situations and has been historically and culturally subject to constant changes (Holler, 2002; Ortner, 2018). Because nutrition is dependent on economic opportunities and is influenced by socialization, nutritional forms and possibilities have never been as multifaceted as today (Schröder, 2016). Therefore, especially middle- and high-income health-conscious consumers in developed regions of the world demand for functional food or so-called "superfoods" associated with multitudinous health benefits (Ali et al., 2012; Magrach & Sanz, 2020). Strongly influenced by current medical and other scientific topics nutritionally conscious consumers give rise to the ever-increasing number of food trends like "paleo", "free from", "detox", "clean eating", "vegan" etc. (Arenas-Jal et al., 2020; Salanță, 2020). The sports nutrition trend for example went from a niche market to mainstream over the last decade as more and more health-conscious consumers are adopting an active and sportive lifestyle (Arenas-Jal et al., 2020; Schmidt, 2014). Along with these trends, it becomes apparent that nutrition in modern days serves as a strong expression of socio-metric status in society and of one's own individuality to an unprecedented extend (Schröder, 2016).

To meet those growing and changing demands of health and lifestyle orientated consumers "superfoods" which are "considered especially nutritious or otherwise beneficial to health and well-being" gained more and more attention in recent years (Graeff-Hönninger & Khajehei, 2019; Oxford Englisch Dictionary, n.d.). It should be noted that until today there is no scientifically or legally established definition of "superfoods". At present, food is considered a "superfood", if it contains high levels of desirable nutrients that are associated with disease-preventive properties or are believed to provide a variety of health benefits beyond their regular nutritional value (Harvard Chan School, n.d.).

However, achieving a sustainable diverse diet of the ever-increasing population without jeopardizing biodiversity poses an enormous challenge (Magrach & Sanz, 2020). Increased consumer awareness regarding food origin, social and environmental impacts of their food choices opened up new research approaches and areas (Campbell et al., 2018; Magrach & Sanz, 2020). A regional, sustainable production of "superfoods" represents an opportunity to meet those consumer demands while taking into account the ecological and socioeconomic consequences in the countries of origin (Dumont et al., 2017; Rejman et al., 2019). The "quinoa fever" linked to its excessive demand can be quoted as a prime example. Price explosions in

Bolivia and Peru seemed to be beneficial for quinoa farmers as their income increased. On the other side of the coin the local population had to switch to cheaper, often nutrient-poor substitute foods potentially leading to nutrient deficiencies and unbalanced diets in the affected regions (Qiii Media, n.d.). Also, the increased quinoa demand led to the industrialization of cultivation: a larger production volume was achieved through monoculture, heavy use of machinery, and intensive use of resources (Qiii Media, n.d.; P. M. Silva et al., 2020). The loss of biodiversity, soil fertility, water shortages, and land degradation happening also in other super-food producing countries – threatens the longevity of farmer's businesses and their source of income (Qiii Media, n.d.; P. M. Silva et al., 2020).

As chia is one of the most well-accepted superfoods by consumers in Germany, representing a part of Central Europe, a closer look at the general cultivability, possible production systems, and genotype selection under prevailing temperate conditions seemed worthwhile (Statista Research Department, n.d.).

1.2 Chia: A General Overview

Chia, also known as the gold of the Aztecs, is an ancient crop native to northern Guatemala and southern Mexico (Busilacchi et al., 2013). In pre-Columbian tropical and subtropical Mesoamerican countries, it was used as a staple crop representing a basic energizing dietary element before the conquest by the Europeans and the associated near extinction (Ayerza & Coates, 2005; Grancieri et al., 2019; Kulczyński et al., 2019).

Geographically chia cultivation was limited to the tropics (0°- 20°) and subtropics (20° - 40°) as chia is a short-day plant with a threshold of 12–13 h daylength for flowering (Ayerza, 2014; Busilacchi et al., 2013; Jamboonsri et al., 2012). Therefore, its period of growth and fruiting depends on the latitude where it is grown (Baginsky et al., 2016; Grimes et al., 2018). In subtropical areas of the northern (e.g. California, Egypt, Turkey, and China) and southern (e.g. Argentina, Chile, South Africa, and Australia) hemisphere, including European countries in the Mediterranean areas like Portugal, Spain, France, Italy, and Greece, chia cultivation is theoretically possible with the existing short day genotypes as the temperature and day length requirements of chia are met during the winter months.

Yet cultivation expansion of chia to temperate regions (40°-60°) as it prevails for example in Germany however, requires adaptations of genotypes regarding day length sensitivity as cultivation is only feasible during the summer months as the temperature conditions are only fulfilled in these months.

In cold temperate climates, however, apart from the day length adaption, chia would also need to be further improved regarding its low tolerance towards cold temperatures and its severe frost intolerance.

Figure 1 shows the dispersal of chia cultivation from early pre-Columbian till modern times.

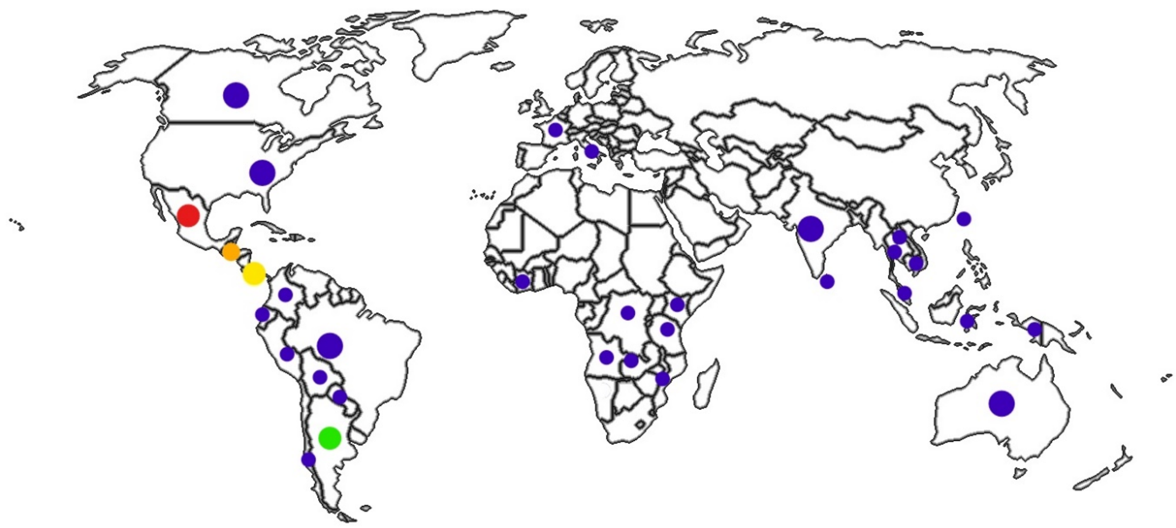

Figure 1 The agricultural areas presented represent the areas of chia cultivated in early pre-Columbian times (red and orange, 3500 BC-1000 AC); late pre-Columbian times (yellow, 1000AC-1500 AC); post-Columbian times (green, 1500 AC- 2000 AC); and modern times (blue, 2010-present), respectively amended according to Baldivia (2018), Jamboonsri et al. (2012) and Šilc et al. (2020).
World map available online at https://en.wikipedia.org/wiki/File:Black_and_white_political_map_of_the_world.png

1.2.1 Botanical and Morphological Characteristics

Chia is a summer annual herbaceous plant that belongs to the *Lamiaceae* family from the genus *Salvia* L. and is classified as species *Salvia hispanica* L. (Ayerza & Coates, 2005; Cahill, 2004; United States Department of Agriculture, n.d.). The diploid chia plant (2n=2x=24 chromosomes) (Figure 2) is about 1 to 1.5 m high, having quadrangular branched stems with short hairs (Ayerza & Coates, 2005). It has opposite leaves, which are 4 to 10 cm long and 3 to 6 cm wide, oval-elliptical, pubescent, with acute apex and a petiole up to 4 cm (Ayerza & Coates, 2005; Grancieri et al., 2019). Chia forms long inflorescences up to 34 cm (Baginsky et al., 2016). The calyx is persistent, tube-shaped, bulky, and striated, having three acute teeth, one of them slightly longer and double the width compared to the others (Ayerza & Coates, 2005). The corolla is tubular and blue or white, with four stamens, two of them larger and sterile (Ayerza & Coates, 2005). The ovary is discoid and the stigma is bifid (Ayerza & Coates, 2005). The seeds are present in groups of four quasi-oval, 1 to 2 mm long, 0.8 to 1.4 mm wide, and 0.8 and 1.3 mm in diameter (Ayerza & Coates, 2005; Grancieri et al., 2019). The sleek and glossy surface color varies from white, gray, brown, black-spotted, to black (Ayerza & Coates, 2005; Grancieri et al., 2019; Orona-Tamayo et al., 2017). Mucilage of chia seeds consists of high molecular weight complex polysaccharides and is released immediately by the outer cell wall of the epidermal cells by rupturing the primary cell layer when immersed in water giving it its characteristic gel-like appearance (Campos et al., 2016; Muñoz et al., 2012; Tavares et al., 2018).

Figure 2 Botanical and morphological attributes of chia (*Salvia hispanica* L.) (own photo).

1.2.2 Chia Cultivation and its hindrances

After chia was almost extinct, its cultivation is now spreading rapidly from its tropical and subtropical center of origin in Central America to new areas of cultivation as shown in Figure 1 (Grimes et al., 2018). Its exceptional nutritional composition, characterized by a high proportion of polyunsaturated fatty acids, vitamins, minerals, and antioxidants, fuels increasing market shares and profits (Amato et al., 2015; Bochicchio et al., 2015). European growers looking into materializing their share of profits by growing chia face inherent hindrances which are to overcome. Chia itself is considered to be a frost intolerant short-day plant, with a maximum threshold of 12–13 h day length to turn from vegetative into the generative phase (Bochicchio et al., 2015; Jamboonsri et al., 2012). Hence, its phenological plant development strongly depends on the cultivation area's latitude. At a latitude of 48 ° and a day length of 12-13 hours, Germany might be considered as a border region in which chia could be grown. However, initiating the transition from vegetative to generative growth - formation of flowers, fruits, and seeds, would not be reached before September/October. Therefore, from a climatic point of view, the most critical point is the timing of sowing as chia itself is highly frost intolerant, which limits the cultivation period under the given climatic conditions in Germany from mid-May to mid-October, after the ice saints and prior to the first autumn and early winter frosts, respectively (Grimes et al., 2020; Grimes et al., 2018).

While only about 25% of the total fruit weight was made of ripe fruits in trials conducted in Italy, neither, ripening nor seed harvest maturity could be reached in trials carried out in Greece, While only about 25% of the total fruit weight was made of ripe fruits in trials conducted in Italy, neither, ripening nor seed harvest maturity could be reached in trials carried out in Greece, thus representing a major drawback regarding chia seed production in Europe (Bochicchio, Rossi, et al., 2015; Karkanis et al., 2018). Under the given Central European conditions, cultivation of chia was simply impossible and thus no cultivation practices were available (Karkanis et al., 2018).

As breeders did overcome the photoperiodic sensitivity new long-day flowering species can induce flower formation under day lengths of 12 to 15 hours, enabling plant maturation and seed formation outside its latitudes of origin allowing its cultivation in places greater than 25 degrees north and therefore at a broader range of environmental conditions (Jamboonsri et al., 2012; Sorondo, 2014).

1.2.3 Agronomic Management

The breakthrough in overcoming the photoperiodic sensitivity enabled the cultivation of chia for its seed production in Europe and made the development of a holistic cultivation system for chia under the given climatic conditions essential. Abroad from day length additional climate requirements and agronomic management practices need to be fulfilled regarding chia seed yield quantity and quality according to Bochicchio, Phillips, et al. (2015). Scientific literature reports of agronomic management of chia focus mainly on trials and observations conducted in their countries of origin of South and Central America (Ayerza, 2010, 2013, 2019; Ayerza & Coates, 2011, 1996).

Generally, it is mentioned that chia prefers sandy, well-drained soils with moderate salinity and pH values between 6 and 8.5, while being highly adaptable to soils of other textures as long as they are drained well and not too wet (Baginsky et al., 2014; Muñoz et al., 2013; Yeboah et al., 2014; Zavalía et al., 2010). Chia is considered as being drought-tolerant and having an optimal growth temperature between 16 and 26 °C (Ayerza & Coates, 2009a, 2009b). It benefits from precipitation during vegetative growth, while drier conditions are beneficial during its generative phase, especially seed maturation (Yeboah et al., 2014). There are contradicting findings regarding chia's response to fertilization. Studies conducted outside its countries of origin, namely in Italy and Greece indicated that there is no response to nitrogen application (D. Bilalis et al., 2016; Bochicchio, Rossi, et al., 2015). Nitrogen application was even associated with lodging (Bochicchio, Rossi, et al., 2015). On the other hand, a study conducted under edaphoclimatic conditions in México showed that the productive potential of chia is six times higher than the world average (0.36 t ha^{-1}) when improved genotypes were combined with adequate nitrogen fertilization (Sosa-Baldivia & Ruiz Ibarra, 2018). The generally prevailing opinion however is that given environmental conditions affected seed yield and the respective nutrient composition of chia significantly, whereas the influence of the applied agronomic management practices was limited (Ayerza & Coates, 2004, 2009b, 2011). As for any other crops too, chia growth and yield are also influenced by sowing density and row spacing, which depends on environmental factors such as water availability, temperature, soil condition, etc. Therefore, there is no universal recommendation. In South America for example, chia is sown at rates of 5 to 6 kg ha^{-1} with row spacing usually varying from 0.7 to 0.8 m whereas in Kentucky a sowing rate of 2 to 3 kg ha^{-1} obtained better yields with less lodging (Bochicchio, Phillips, et al., 2015; Coates, 2011). In Ghana field experiments with 40,000 plants ha^{-1} and 0.5 m row spacing reached the highest yields. This is in line with field experiments conducted in southern Italy where first results indicated that seed yield increased with plant density (Bochicchio, Rossi, et al., 2015; Yeboah et al., 2014). Under low-input conditions, the average yield of commercial seeds is around 500 to 600 kg ha^{-1} (Ayerza & Coates, 2005; Orona-Tamayo et al., 2017). However, some growers have obtained up to 1200 kg ha^{-1} and 2500 kg ha^{-1} under optimal agronomic conditions (Cahill, 2003; Coates, 2011; Ullah et al., 2015). Considering weed control, the early growing stage is, as for many crops, critical as the growth rate of chia is slow compared to common weeds (Bochicchio, Phillips, et al., 2015; Coates, 2011). Till canopy closure is reached weed control needs to be conducted manually or mechanically (Bochicchio, Phillips, et al., 2015). For large-scale chia cultivation systems, chemical weed control becomes essential, hence the development of a suitable herbicide is necessary. Few trials have been conducted in Ecuador and Chile in this regard (Pozo Pozo, 2010; Villegas Rojas, 2013).

A recently published study examined the efficacy and selectivity of pre -and post-emergence herbicides in chia under Mediterranean semi-arid conditions in central Greece (Karkanis et al., 2018). As little to no information is available about its cultivation practices in Europe they concluded that “further studies have to be conducted to evaluate the selectivity of herbicides in chia crop under different application rates, soil types and environmental conditions to make safe suggestions for chemical control of weeds” (Karkanis et al., 2018). Concerning insect and disease control it is stated on the one side that chia can be grown without pesticides and other chemical compounds as inter alia the essential oils of the leaves act as repellents against insects (Muñoz et al., 2013; Pascual-Villalobos et al., 1997). On the other side trials conducted in Germany, Ghana, Italy, and Kentucky (personal communication, October 7, 2020) showed that flea beetles, foliage beetles, whiteflies, and aphids posed a threat as well Fusarium wilt and

*Corynespora cassiicola i*nfections and two viruses (*Sida mosaic Bolivia virus 2* and *Tomato yellow spot virus*), which are causing severe disease symptoms (Celli et al., 2014; Grimes et al., 2019; Yeboah et al., 2014).

As chia, in general, matures non-uniform, from the top of the inflorescence to the bottom, and starting from its central axis inflorescence gradually to its side branches, time is the defining key factor in terms of its harvest and therefore maximum seed yield potential. Due to the occurrence of seed shattering, which poses an enormous drawback, it is nevertheless not recommended to wait until all inflorescences are matured (Jamboonsri et al., 2012).

1.2.4 Quality Traits

The chemical composition of chia seeds and their health benefits was examined and highlighted by a large number of scientists (Ali et al., 2012; Kulczyński et al., 2019; Ullah et al., 2015). The following section, therefore, intends to provide a general and condensed overview of this raw material, which gained considerable interest in recent years due to its high nutritive value associated with the possible promotion of health and well-being, while preventing or even treating certain diseases (Kulczyński et al., 2019; Vorster & Gibney, 2013).

Its crude protein content consists of complex organic compounds, which are essential to the human body (Food and Agriculture Organization of the United Nations, n.d.; Grancieri et al., 2019). Protein content varies between 12 to 26 % and is rich in plenty of exogenous amino acids (Cahill, 2003; B. P. da Silva et al., 2016; da Silva Marineli et al., 2014; Kulczyński et al., 2019; Reyes-Caudillo et al., 2008). In this regard, it should be pointed out that the quality of chia protein refers to its ability to meet the nutritional needs of the organism by means of essential amino acids and nonessential nitrogen, for protein synthesis (Jood & Singh, 2001). Besides the greater protein content compared to other cereals (corn: 9.4 %, rice: 6.5 %, quinoa: 14.1 %, wheat: 12.6 %) it is free from gluten representing an enormous advantage especially regarding celiac disease treatment and the disproportionate increase in growth of the gluten-free food industry (Reilly, 2016; Ullah et al., 2015). A review about chia seeds as being a source of protein and bioactive peptides with health benefits concluded that the identified proteins "and their peptide sequences have auspicious biological potentials, mainly antioxidative, antihypertensive, and hypoglycemic properties" (Grancieri et al., 2019).

Fat constitutes an essential source of energy supply in human nutrition. For this reason among others, chia seeds gained particular attention due to the higher proportion of polyunsaturated fatty acids, especially that of the non-synthesizable α-linolenic acid, which has been associated with a large number of positive physiological functions like: lowering cholesterol levels, anti-inflammatory activity, cardioprotective and hepatoprotective activities, antidiabetic action, and protection against cancer, arthritis, and autoimmune in the human body (Ali et al., 2012; de Falco et al., 2018; Grancieri et al., 2019; Grand View Research, 2019; Orona-Tamayo et al., 2017; Ullah et al., 2015). An obvious advantage compared to marine α-linolenic acid (ω 3) sources, like algae or fish in the family Clupeidae, is the fact that chia contains noticeably less saturated fatty acids and a tremendously higher α-linolenic acid content and therefore suits the requirement of a plant-based fatty acid source in vegetarian or vegan diets (Ayerza & Coates, 2005). Furthermore, it is odor- and tasteless which is an essential benefit particularly regarding enriching foods (Ayerza & Coates, 2005). The concentration of fatty acids in chia seed oil, in general, can be classified in the following frequency of occurrence: α-linolenic acid (C18:3, ω3) > linoleic acid (C18:2, ω6) > palmitic acid (C16:0) > oleic acid (C18:1) > stearic acid (C18:0) > vaccenic acid (C18:1) (Amato et al., 2015; Bochicchio, Phillips, et al., 2015; Ixtaina, Martínez, et al., 2011; C. Silva et al., 2016). Containing a remarkable amount of about 65 % of the essential ω-3 α-linolenic acid (ALA) chia is considered a 'powerhouse of ω-3 fatty acids' (Ali et al., 2012; Nadeem et al., 2017; Ullah et al., 2015).

In Western diets, the percentage of total calories consumed from fat decreased in the past decades, while the intake of ω-6 fatty acid rose and the ω-3 fatty acid dwindled, resulting in ω-6 to ω-3 ratio from1:1 during evolution to 16-20:1 today or even higher (Simopoulos, 2016, 2020). Therefore, the ω-6/ω-3 ratio is substantially more important than the absolute amounts of ω-6 and ω-3 fatty acid taken in (Simopoulos, 2016). ω-6/ω-3 ratios of 1–2/1 are recommended as one of the most critical dietary element to prevent obesity, while ratios of 5/1 are considered desirable by the German Nutrition Society (DGE) as a balanced ω-6/ω-3 ratio is a crucial health contributor during the entire life cycle (Deutsche Gesellschaft für Ernährung e. V., 2003; Simopoulos, 2016).

Table 1 Nutritional data of major staple cereal grains and four underutilized (pseudo) cereal grains (amended according to Bekkering & Tian, 2019).

Nutritional data (per 100 g grain or grain flour)		Wheat	Maize	Rice	Buckwheat	Amaranth	Chia	Quinoa
Form Consumed		Wheat flour, white, all–purpose, unenriched	Whole grain, yellow	White, long–grain, unenriched	Whole–groat flour	Whole grain	Whole grain	Whole grain
Calories (kcal)		364	365	365	335	371	486	368
Carbohydrate	(g)	76.31	74.26	79.95	70.59	65.25	42.12	64.16
Protein	(g)	10.33	9.42	7.13	12.62	13.56	16.54	14.12
Total lipid	(g)	0.98	4.74	0.66	3.1	7.02	30.74	6.07
Dietary Fibre	(g)	2.7	7.3	1.3	10	6.7	34.4	7
Vitamin A, IU	IU	0	214	0	0	2	54	14
Vitamin B-6	(mg)	0.044	0.622	0.164	0.582	0.591	–	0.487
Vitamin C	(mg)	0	0	0	0	4.2	1.6	–
Vitamin E	(mg)	0.06	0.49	0.11	0.32	1.19	0.5	2.44
Folate	(µg)	26	19	8	54	82	49	184
Phosphorus	(mg)	108	210	115	337	557	860	457
Potassium	(mg)	107	287	115	577	508	407	563
Iron	(mg)	1.17	2.71	0.8	4.06	7.61	7.72	4.57
Calcium	(mg)	15	7	28	41	159	631	47
Zinc	(mg)	0.7	2.21	1.09	3.12	2.87	4.58	3.1
Magnesium	(mg)	22	127	25	251	248	335	197

Also, chia seed oil is used as a source of fiber and contains minerals including iron, calcium, magnesium, zinc, and natural antioxidants such as carotenoids, phytosterols, tocopherols, and phenolic compounds, including quercetin, myricetin, caffeic acid, chlorogenic acid, and kaempferol (Amato et al., 2015; D. J. Bilalis et al., 2020; da Silva Marineli et al., 2014; de Falco et al., 2018; Grand View Research, 2019; Reyes-Caudillo et al., 2008). Compared to other cereal grains (wheat, rice, maize) chia contains more micronutrients (vitamins and minerals) and phytonutrients (nutraceuticals and phytomedicines) and higher caloric values because anatomically it contains less endosperm (accumulating starch) and a higher proportion of embryos (accumulating proteins and lipids) (Table 1) (Bekkering & Tian, 2019; Prego et al., 1998; Valdivia-López & Tecante, 2015). For the sake of completeness, it should be mentioned that bioactive peptides have also been found in chia seeds (Grancieri et al., 2019).

Looking at the quality characteristics of chia, it must be pointed out that the Regulation (EU) 2015/2283 on novel foods is applicable for chia (European Commission, 2015). Novel Foods are described as “foods originating from plants, animals, microorganisms, cell cultures, minerals, etc., specific categories of foods (insects, vitamins, minerals, food supplements, etc.), foods resulting from production processes and practices, and state of the art technologies (e.g. intentionally modified or new molecular structure, nanomaterials), which were not produced or used before 1997” (European Commission, 2015). Quality criteria defined by the Union list of novel foods as displayed in Table 2 have to be fulfilled (European Commission, 2015).

Table 2 Specification of chia seeds (*Salvia hispanica* L.) according to the Union list of novel foods (European Commission, 2015).

Parameter	Range
Dry matter	90–97%
Protein	15–26%
Fat	18–39%
Carbohydrate	18–43%
Crude fiber	18–43%
Ash	3–7%

Currently, no other chia species are considered under the Commission Decision 2009/827/EC (European Commission, n.d.).

1.3 Outline and Objectives

The present doctoral thesis focused on the screening, selection, and cultivation of chia under Central European climate conditions. Major aims were to (i) assess agronomic and quality traits of new day length insensitive or adapted chia genotypes; (ii) develop a cultivation system for chia adapted to the climatic conditions given in Central Europe; and, (iii) to select and evaluate new chia genotypes as scientific literature on crop management, agronomic characterization and physiological aspects outside its center of origin is missing (Bochicchio, Phillips, et al., 2015). The information generated, should lead to a locally adapted and optimized chia cultivation possibly broadening existing crop rotations being ecologically and economically advantageous.

The main body of the present thesis consists of three scientific peer-reviewed publications (Chapters I-III), which are presented in the chapter ‘’Publications’’, followed by a general discussion and a summary.

The objectives of this thesis were:

- to investigate the cultivability of different chia genotypes under Central European climate conditions
- to determine and specify agronomic and quality traits of different chia genotypes cultivated under Central European climate conditions
- to optimize and establish an applicable cultivation system for chia under the climatic conditions given in Central Europe

- to select new chia genotypes that can be cultivated under Central European climatic conditions

To verify the specified objectives, climate chamber and field experiments were conducted between 2015 to 2018 at the University of Hohenheim and its experimental stations 'Ihinger Hof' and 'Eckartsweier'. The detailed description of all field experiments and the related experimental designs are described in Chapters I, II, and III of the present doctoral thesis.

The first publication served to determine whether a regional cultivation of new chia genotypes, which were either day length insensitive (Sahi Alba 914) or adapted to day lengths greater than 12 h (G8, W13.1), would be feasible under Central European conditions and if seeds would meet the quality standards according to the Novel Food guidelines (European Commission, 2014). The following traits, which have been obtained at the two experimental stations Ihinger Hof and Eckartsweiher, were examined:

- Phenological traits: Accumulated growing degree-days (GDD), vegetation period, radiation, day length, and mean temperature until flower induction and maturity
- Morphological traits: Mean plant height, number of branches per plant, number of inflorescences per single plant, and the length of the central axis inflorescence
- Yield traits: Mean number of seeds, seed weight, seed length and seed width per single plant and mean number of plants per square meter, mechanically threshed seed yield [kg ha^{-1}], and corresponding thousand kernel mass (TKM) [g])
- Quality traits: Mean crude protein, mucilage crude oil, fatty acid composition (% of total fatty acid) proportions, and ratios

Based on this, the second publication discusses the impact of (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 chia yield and quality traits.

Climate chamber trials were conducted parallel to the field trials to select new chia genotypes able to induce flower formation at day length about ~ 15 hours, therefore being cultivatable under the conditions given in Central Europe. This allowed the cultivation of the selected genotypes first in single rows and single plots for seed propagation purposes solely. In Chapter III, the differences in phenological, morphological, yield, and quality traits between an approved chia genotype and newly selected genotypes were evaluated potentially representing a new crop for temperate regions in the future.

2. Publications

The present cumulative thesis consists of three articles that have been published in peer-reviewed, international high standard referenced journals. For citation of the three articles, which correspond to the Chapters I-III of the present thesis, please use the references given below.

Chapter I

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.

Chapter II

Grimes, S.J.; Phillips, T.D.; Capezzone, F.; Graeff-Hönninger, S. Impact of Row Spacing, Sowing Density and Nitrogen Fertilization on Yield and Quality Traits of chia (*Salvia hispanica* L.) Cultivated in southwestern Germany. *Agronomy* **2019**, *9*, 136.

Chapter III

Grimes, S. J., Capezzone, F., Nkebiwe, P. M., & Graeff-Hönninger, S. (2020). Characterization and Evaluation of *Salvia hispanica* L. and *Salvia columbariae* Benth. Varieties for Their Cultivation in Southwestern Germany. *Agronomy* **2020**, *10*, 2012.

3. Chapter I: Growth, Yield Performance and Quality Parameters of Three Early Flowering Chia (*Salvia hispanica* L.) Genotypes Cultivated in Southwestern Germany

Publication I

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.

Several studies were conducted examining yield and quality parameters of chia (Salvia hispanica L.) from their tropical and subtropical Mesoamerica countries of origin. Chia, itself originally is a short-day flowering plant. Inflorescences are therefore developed when day lengths fall below a time span of 12 hours. Until recently chia cultivation was limited to latitudes lower than 25 degrees near the equator. Due to the effort and dedication of breeders new genotypes either day length insensitive or adapted to day lengths > 12 h were developed. Flower formation is therefore possible under day length conditions exceeding the 12 hours' threshold enabling its cultivation beyond the currently existing northern and southern borders. These genotypes flower earlier in summer, allowing for maturation before frost sets in at a latitude of 48 degrees.

Chapter I focused on the generation of basic information regarding growth, yield, and quality traits of Salvia hispanica L. under the temperate climate conditions given in Central Europe. It was shown that 1) the thermal and day length requirements, to reach flowering stage and harvest maturity could be fulfilled; 2) seed yields of the three cultivated chia genotypes surpassed yields obtained in their countries of origin and therefore could represent an attractive alternative to local farmers; 3) oil, protein and mucilage content of the three cultivated chia genotypes were in line with current literature and the Novel Food-Regulation (EU) 2015/2283.

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Growth, Yield Performance and Quality Parameters of Three Early Flowering Chia (*Salvia hispanica* L.) Genotypes Cultivated in Southwestern Germany

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Abstract: The combination of consumer's ongoing demand for chia (*Salvia hispanica* L.) alongside the increased demand for regionally produced food products provided the impetus for this study. Its aim was to test if a regional cultivation of new chia genotypes, which were adapted to day lengths greater than 12 h, is feasible under Central European conditions. Therefore, three early flowering chia genotypes (Sahi Alba 914, W13.1, G8) were cultivated in a randomized block design at two experimental stations in Southwestern Germany (Ihinger Hof, Eckartsweier) over the course of two years (2015, 2016). Mean yields ranged from 100 to 1290 kg ha^{-1}. Mucilage content ranged from 9.5% to 12.2%, while the crude protein content ranged from 17.2% to 25.0%. Crude oil content fell in the range of 30.9–33.7% and the PUFA:SAT ratio ranged from 4.0 to 9.4, whereas the omega6:omega3 ratio varied from 0.27 to 0.5. As chia seed yields surpassed yield levels obtained by their countries of origin and as quality parameters obtained, were in line with the genotypes cultivated in their countries of origin, it can be assumed that a regional chia production in Southwestern Germany offers great potential, being ecologically and economically profitable.

Keywords: *Salvia hispanica*; genotypes; regional cultivation; quality parameters; yield parameters

1. Introduction

Chia (*Salvia hispanica* L.) is a summer annual herbaceous plant belonging to the *Lamiaceae* family. Evidence suggests the Aztecs first used chia as a food prior to 3500 BC [1] (p. 63). Later, by 1500 to 900 BC, it was grown in the center of Mexico as a cash crop [1] (p. 63). In pre-Columbian times chia was one of the four basic dietary elements of the Central American civilization besides corn, beans and amaranth [1,2]. It was used as a tithe or form of sacrificial offering, among other things [1] (p. 72). Chia nearly passed into extinction due to the destruction and eradication of everything related to pre-Hispanic religion [1] (p. 65).

As worldwide public health awareness and the demand for functional food with multitudinously health benefits has increased [3], chia seeds have gained popularity in recent years due its numerous nutritional characteristics including high concentrations of extractable fatty acids, large quantities of polyunsaturated fatty acids (ω3 and ω6), extraordinary mucilaginous fiber content, vitamins, minerals and antioxidants [2,4–7]. Further, due to its high adaptability to different pedoclimatic conditions, chia is considered as an alternative crop in terms of food security and climate change [8].

Chia grows naturally in tropical and subtropical environments. Its cultivation is spreading rapidly from its center of origin in Central America to new areas of cultivation such as Africa, Australia, Europe and North America as the market for chia is profitable and favorable [9]. Chia is intolerant to freezing in all development stages [9,10] and has optimum growth temperatures of 16–26 °C [11]. It is considered to be a short-day plant with a threshold of 12–13 h [12,13] and as such, its period of growth and fruiting depends on the latitude where it is grown. Due to the dedication of breeders, new long-day flowering genotypes have been produced, which are able to induce flower formation at 12 h day lengths or more. Unlike regular chia, these genotypes can therefore flower earlier in summer when day length are longer than 12 h, allowing for maturation before frost. This no longer limits chia cultivation to latitudes lower than 25 degrees near the equator, therefore enabling its cultivation to a wider range of environmental conditions [12,14].

In Europe, promising studies have already been conducted in Italy (latitude 40° N) and Greece (latitude 22° N) but as of now, chia has never been cultivated further north from the equator [9,15]. Cultivating chia at a latitude of 48 degrees north, were mean temperatures are lower and day length of 13.86 to 15.12 h are given has never been tested before. Hence, the objective of this study was to examine three early flowering chia genotypes with regard to their growth and yield performance under the given day length and climatic conditions of Southwestern Germany. Additionally, quality parameters like protein and oil content, mucilage content as well as fatty acid profiles were investigated. Based on the results of this research work, suitable chia genotypes for the cultivation in Southwestern Germany should be selected that are in line with the Novel Food-Regulation (EU) 2015/2283 in order to ensure a domestic chia supply [16,17].

2. Materials and Methods

2.1. Site Descriptions

Field trials were conducted in two consecutive growing seasons (2015/2016) at the experimental station Ihinger Hof (IHO) (Upper Neckarland, Lat. N 48°44′40,70′′ Lon. E 8°55′26,36′′) of the University of Hohenheim. An additional field trial was conducted in 2016 at the University's experimental station in Eckartsweier (EWE) (Upper Rhine Valley, Lat. N 48°31′45,24′′ Lon. E 7°51′12,81′′) in order to determine the influence of different environmental conditions on yield and quality parameters of chia.

Precipitation during the experimental period at IHO in 2015 and 2016 amounted to 246.6 and 354.8 mm respectively and the mean temperature during the experimental period was 15.0 and 15.5 °C respectively. At EWE, the precipitation amounted to 212.4 mm during the experimental period in 2016 and the mean temperature was 18.8 °C (Figure 1). Meteorological data was obtained by the weather stations at IHO and EWE.

GDD for both locations was calculated using the growing degree-day formula Equation (1) of McMaster and Wilhelm [18], where T_{max} is defined as the daily maximum air temperature and 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. [2], 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 crop base temperature, $((T_{MAX} + T_{MIN})/2) < T_{BASE}$, then $((T_{MAX} + T_{MIN})/2) = T_{BASE}$

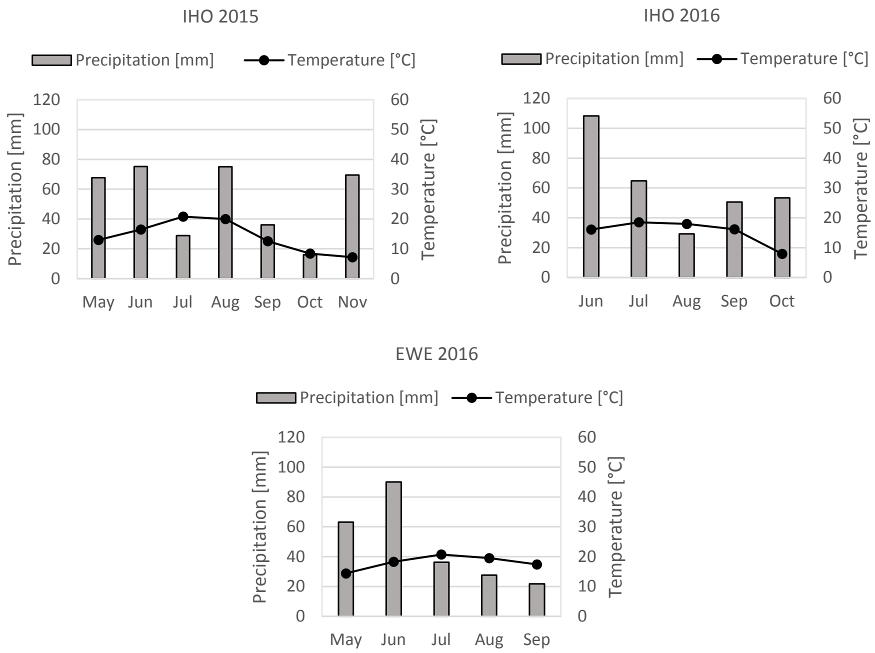

Figure 1. Precipitation [mm, bars] and mean temperature [°C; ●] during the experimental period at Ihinger Hof in 2015, 2016 and EWE in 2016. Jun, January. Jul, July. Aug, August. Sep, September. Oct, October. Nov, November.

According to the World Reference Base [19] experimental soils at IHO can be characterized as Terra Fusca brown soil and Pelosol brown soil in 2015 and 2016, respectively. The composition of clay, sand and silt contents of the top soils in both years were quite similar. On average sand content was about 10%, silt about 64% and clay about 26%. The pH values differed only marginally from each other (7.1 to 7.45). Prior to sowing, soil mineral nitrogen content (Nmin) from 0 to 90 cm amounted to 9.45 kg ha^{-1} (Stockacker) and 31.82 kg ha^{-1} (Dreispitz) in 2015 and 22.96 kg ha^{-1} (Härdtle) in 2016. A soil mineral nitrogen content of 6.30 kg ha^{-1} was identified for EWE in 2016.

2.2. Plant Material

At both locations the three genotypes cultivated, produced inflorescences under long day conditions as they were either day length insensitive (Sahi Alba 914) or adapted to day lengths greater than 12 h (G8, W13.1) (Table 1). Genotype G8 was generated by gamma ray-mutagenesis according to Hildebrand et al. [20]. W13.1 derived from a cross between G8 and a white-flowered, white-seeded commercial chia (wild type). Sahi Alba 914 was obtained by individual selection of Sahi Alba 912 for early flowering plants over a period of 6 years in Argentina [14].

Table 1. Cultivated chia genotypes at IHO (2015, 2016) and EWE (2016).

Genotype	Origin	Breeder	Flower Color	Seed Color
G8	Argentina	University of Kentucky	Blue	Charcoal
Sahi Alba 914	Argentina	Augustin Sorondo	White	White
W13.1	Argentina	University of Kentucky	Blue	White

2.3. Trial Setup

In both years and locations, field trials were carried out as randomized block designs with three replications. Plot size for each cultivar at IHO was 6 m × 5 m in 2015 and 10 m × 3 m in 2016, respectively. At EWE in 2016 plot sizes were 7 m × 1.5 m. Mechanical sowing at IHO took place on 21 May 2015 and 27 May 2016 using a Deppe D82 Sower (Agrar Markt DEPPE GmbH, Rosdorf, Germany). Hand sowing at EWE took place on 11 May 2016. Sowing density amounted to 2.5 kg ha^{-1} in 2015 with a row spacing of 75 cm, and 1.5 kg ha^{-1} in 2016 with a row spacing of 50 cm at IHO, while 2.0 kg ha^{-1} sowing density at 37.5 cm was chosen for EWE. In order to prevent outcrossing during flowering, the two white seeded genotypes W13.1 and Sahi Alba 914 were cultivated spatially separated from G8 in 2015 (different experimental areas), whereas in 2016 a spatial distance was kept on the same experimental area. The different row spacing and sowing densities were accounted for in the statistics later on. In 2015, harrowing at IHO took place one day before sowing, in 2016 it took place 73 days before sowing, and at EWE in 2016 51 and 30 days prior to sowing. Seedbed preparation was conducted at IHO on the same day as sowing in 2015 and eight days prior to sowing in 2016. Seed bed preparation at EWE was done one day before sowing. Nitrogen fertilization (20 kg N ha^{-1}) as calcium ammonium nitrate was applied 23 days after sowing at IHO in 2015 and 15 days after sowing in 2016. In 2016, 31 days after sowing 40 kg N ha^{-1} were applied as 'ENTEC® 26' (EuroChem Agro GmbH, Mannheim, Germany), at EWE. Weed control was performed manually in the plots and mechanically by hoeing around the plots if necessary at both locations in both years.

2.4. Experimental Procedure

2.4.1. Soil Mineral Nitrogen Content

Soil mineral nitrogen content was determined prior to sowing according to Bassler and Hoffman [21]. Soil samples were dried at 100 °C for 24 h in a drying oven. Afterwards the samples were ground with a type SR3 rotor beater mill (Retsch, Haan, Germany) (5 mm sieve). 20 g of soil were weighed in a 250 mL PE bottle with screw cap. The extraction was carried out with 200 mL of 0.025 n $CaCl_2$ solution (extraction ratio 1:10) by shaking for one hour. The nitrate content of the filtrate, which was filtered through a folded filter (15 cm diameter, Type 619 G 1/4) (Macherey-Nagel GmbH & Co. KG, Düren, Germany), was analyzed by using the FIAstar 5000 Analyzer (FOSS Analytical, Hilleroed Denmark).

2.4.2. Phenological and Morphological Data Collection

During the course of the field experiment in 2016, ten plants per plot were recorded and assessed in detail every week. Days until flowering and maturity were monitored, single plant height and final seed weight was measured. Mean length and width of seeds were determined with the seed analyzer MARVIN (GTA Sensorik GmbH, Neubrandenburg, Germany). The number of non-senescent first degree branches at the main stem, number of inflorescences (>5 cm) and seeds produced per single plant were counted and the length of the central axis inflorescence was measured.

2.4.3. Yield Parameters

Ten plants per plot were harvested manually at maturity in order to obtain the mean single plant yield. All inflorescences per single plant were cut off and dried for 24 h at 40 °C. Afterwards the inflorescences were threshed manually and the cleaned seeds were weighed and counted.

Plot harvest took place mechanically with a plot combine Classic (Wintersteiger AG, Ried, Austria). Yield was evaluated on the basis of absolute dry matter (0% grain moisture) and the mean thousand kernel was additionally identified on the basis of the mechanically threshed yield. Thousand seeds per repetition were counted by Contador (Pfeuffer GmbH, Kitzingen, Germany) and weighed in order to determine the mean thousand kernel mass. Additionally, the final number of plants per square meter was ascertained.

2.4.4. Quality Parameters

Mucilage extraction took place according to Muñoz et al. [22]. In order to extract the mucilage, whole chia seeds (5 g) were placed in a 250 mL beaker. Distilled water was added in a ratio of 1:40. At the beginning of soaking and after 2 h of hydration process, the pH was recorded. During extraction, the temperature was maintained at 80 ± 1.5 °C using a temperature controller. The mixtures were stirred by ultrasound bathing and hydrated for 2 h. In deviation to Muñoz et al. [22], extraction took place three times. Afterwards the aqueous suspension was spread on a drying tray and exposed to 50 °C for at least 10 h. By rubbing the dried mucilage over a 0.5 mm mesh screen was separated and the weight was recorded. Mucilage weight was represented by the difference in weight before and after its extraction.

By using the vario MACRO cube CHNS (Elementar Analysensysteme GmbH, Langenselbold, Germany) the total seed N content was determined according to Dumas [23]. The values were multiplied by 5.71 (conversion factors) in order to estimate the crude protein content [24].

Crude oil content was determined by Soxhlet extraction with petroleum benzene as solvent according to the European Commission's Regulation 152/III H procedure B [25].

Producing fatty acid methyl esters (FAMEs) with methanolic BF_3 ($BF_3 \cdot CH_3OH$) by rapid saponification and esterification led to the determination of the fatty acid profile [26–28]. To do so, 20 µL of nonadecylic acid (C19:0) (Merck KGaA, Darmstadt, Germany) (5 mg mL^{-1} hexane), the internal standard, was pipetted into a screw cap (culture tube) (DURAN GL14) and evaporated for 15 min beneath the fume hood. By adding 0.5 mL methanolic KOH (0.5 M) to a tube with 5 mg crude fat sample at 80 °C for 5 min saponification of the triglycerides took place. Subsequent to the cooling process, 2 mL of methanolic BF_3 was added to each sample and heated to 80 °C for 5 min one more time. Samples were put in an ice bath for 10 min to cool before 1 mL of saturated NaCl solution and 2 mL of n-hexane were added. The organic phase, including the FAMEs, which resulted from the above-mentioned procedure, was isolated and stored at −24 °C for the following gas chromatography analysis. In order to calibrate the gas chromatograph, a FAME standard (10 mg/mL hexane, Marine Oil FAME Mix, Restek Corporation, Bellefonte, PA, USA), containing a mixture of fatty acids with even and uneven numbers of C-atoms (nonadecylic acid included), concentrations of 0.25, 0.5 and 1 mg mL^{-1} was used. A GC appliance (Shimadzu GC-2010 Plus, Shimadzu Corp., Kyōto, Japan) equipped with a flame ionization detector (Shimadzu FID-2010 Plus) was used for FAME analysis. By the use of an autosampler (Shimadzu AOC-201) 1.0 µL of sample solution was injected. Coated with 0.25 µm polyethylene glycol a 30 mm × 0.25 mm inner diameter fused silica capillary column (FAMEWAX, Restek Corporation, Bellefonte, PA, USA) was installed in the GC oven. Helium (purity 99.9%) was used as carrier gas at a constant flow rate of 25.7 mL min^{-1}. Total runtime of the continuous process was 26 min. The temperature program of the GC oven began at 180 °C, heated at 5 °C min^{-1} to 220 °C (kept for 1 min). Thereafter it was heated at 5 °C min^{-1} to 240 °C (kept for 8 min). Lastly, it was heated at a rate of 5 °C min^{-1} to 250 °C (kept for 4 min). Analyses were performed in duplicates. Fatty acid contents were determined using their peak area relative to that of the internal standard.

2.5. Statistical Analysis

Statistical analysis was conducted by using the statistical software R version 3.2.1 (The R Foundation for Statistical Computing, Vienna, Austria). The following linear mixed model was fit to different traits, which were determined by single plant measurements (10 per plot), namely plant height, length of central axis inflorescence, number of branches, number of inflorescences, number of seeds, seed weight, seed length and seed width:

$$y_{ijkl} = \mu + l_i + b_{ij} + \tau_k + (l\tau)_{ik} + p_{ijk} + e_{ijkl} \quad (2)$$

where y_{ijkl} is the observation of the traits of the l-th plant from the k-th variety, in the j-th block and the i-th location. μ is the overall intercept, l_i is the effect of the i-th location, b_{ij}. is the effect of the j-th block within the i-th location, τ_k is the effect of the k-th variety, $(l\tau)_{ik}$ is the interaction of location and variety, p_{ijk} is the effect of the ijk-th plot and e_{ijkl} are the errors associated with y_{ijkl}. b_{ij}, p_{ijkl} and e_{ijkl} were considered random effects with mean zero and variances σ_b^2, σ_p^2 and σ_e^2.

The following model was used for analysis of the assessed yield and quality traits such as yield, TKM, crude fat and protein content, mucilage content, fatty acids and the corresponding ratios.

$$y_{aikj} = \mu + l_i + j_a + \tau_k + (j\tau)_{ak} + (l\tau)_{ik} + b_{aij} + e_{aikj} \quad (3)$$

where y_{aikj} is the observation of the k-th variety in the j-th block of the i-th location and the a-th year, μ is the common intercept, l_i is the effect of the i-th location, j_a is the effect of the a-th year, τ_k is the effect of the k-th variety, $(j\tau)_{ak}$ is the interaction of year and variety, $(l\tau)_{ik}$ is the interaction of location and variety, b_{aij} is the effect of the aij-th block and e_{aikj} are the errors associated with y_{aikj}. b_{aij} and e_{aikj} were considered random effects with mean zero variances σ_b^2, and σ_e^2.

Referring to the different sowing rates and distances given in Section 2.3 it should be pointed out that sowing rate and row distance were integral parts of the site and annual effects.

In order to display means and standard errors for the genotype G8, simplified versions of models (2) and (3) were used which did not contain variety effects. Normality of residuals and homogeneity of variance was tested by the inspection of residual plots. If residual plots indicated any violations the response variable was transformed using logarithm or square root transformation. Variance components were estimated by the REML algorithm of the *lmerTest* package [29]. Thousand kernel mass revealed variances-heterogeneity, which could not be corrected by transformation, therefore the analysis was proceeded on the untransformed data, knowing that the sensitivity of some tests might be reduced. In order to account for heterogeneous variances between the different combinations of year, location and variety separate error variances were used for each combination of year, location and variety regarding some of the traits. Where heterogeneous residual variances were necessary to improve the model fit the package *nlme* was used [30]. The fixed effects in the model were tested for significance using F-tests. Denominator degrees of freedom in F-tests were adjusted using the Satterthwaite method [29]. Non-significant results at the α = 5% significance level were removed from the model. The levels of factors found significant were compared with pairwise *Tukey*-tests and results were presented as letter display. For mean comparisons tools from the package *lsmeans* were used [31].

3. Results

3.1. Plant Development and Growth

3.1.1. Growth and Development Conditions

Table 2 shows that plants at IHO in 2015 began to flower 74 DAS, having accumulated 545.45 GDD. Until flower induction radiation, mean day length and mean temperature amounted up to 10200.54 Wh/m^2, 15.82 h and 17.3 °C in 2015. The mean temperature from flower induction to harvest maturity at IHO in 2015 was 11.9 °C. Harvest maturity was reached 170 days after sowing, which corresponded to 948.3 growing degree-days, respectively.

In 2016, beginning of flowering took place 66 (EWE) and 70 (IHO) DAS which corresponded to 500.2 (EWE) and 492 (IHO) GDD. During the growing season of 2016, radiation accumulated to 10,267.59 Wh/m^2 (EWE) and 10,155.02 Wh/m^2 (IHO) until flower induction. Mean day length until flower induction was 15.83 h (EWE) and 15.81 h (IHO), while the mean temperature until flower induction was 16.8 °C (EWE) and 17.5 °C (IHO). From flower induction to harvest maturity the mean temperature at EWE in 2016 was 20.5 °C whereas the mean temperature at IHO in 2016 was 14.1 °C. Maturity was reached 127 (EWE) and 154 (IHO) days after sowing which corresponded to 1143.9 (EWE) and 910 (IHO) GDD.

Table 2. Accumulated growing degree-days (GDD), vegetation period, radiation, day length and mean temperature until flower induction at Ihinger Hof in 2015, 2016 and EWE in 2016.

Location	Sowing Date	Flower Induction			Radiation (Wh/m^2) [c]	Day Length (h) [d]	Mean Temperature (°C) [e]	Mean Temperature (°C) [f]	Harvest Maturity *		
		DAS [a]	Date	GDD [b]					DAS	Date	GDD
IHO	21 May 2015	74	3 August	545.45	10,200.54	15.82	17.3	11.9	170	7 November	948.3
IHO	27 May 2016	70	5 August	492	10,155.02	15.81	16.8	14.1	154	28 October	910.2
EWE	11 May 2016	66	16 July	500.2	10,267.59	15.83	17.5	20.5	127	15 September	1143.9

[a] DAS Days after sowing. [b] GDD Accumulated growing degree days with a base temperature of 10 °C [2]. [c] Average Global Radiation based on Sunshine Hours until flower induction [32]. [d] Average day length until flower induction [33]. [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 and EWE. * Harvest maturity is equivalent to vegetation period.

3.1.2. Vegetative Growth

In 2016, plant heights of 112.7 cm (Sahi Alba 914), 115.6 cm (W13.1) and 103.8 cm were reached at IHO, whereas at EWE plant heights of 102.3 cm (Sahi Alba 914), 117.5 cm (W13.1) and 104.6 cm (G8) were recorded (Table 3). Significant differences were detected between the two genotypes Sahi Alba 914 and W13.1 (p-value 0.0263).

Table 3. Mean plant height, number of branches per plant, number of inflorescences per single plant and the length of the central axis inflorescence, of three chia genotypes (G8, Sahi Alba 914, W13.1,) cultivated at two different locations (IHO/EWE) in 2016 (n = 10, α = 0.05, mean ± standard error).

Factor	Plant Height [cm] ‡		Number of Branches Per Plant ‡		Number of Inflorescences Per Plant ‡		Length of Central Axis Inflorescence [cm] ‡	
	IHO	EWE	IHO	EWE	IHO	EWE	IHO	EWE
Genotype								
G8	103.0 ± 0.5	104.6 ± 0.5	NA	5.7 ± 0.7	NA	8.3 ± 1.1	16.8 ± 1.1	16.0 ± 1.1
Sahi Alba 914	112.7 ± 2.7	102.3 ± 2.5	5.6 ± 1.0	3.5 ± 0.7	11.8 ± 2.5	6.7 ± 1.5	19.2 ± 1.3	16.8 ± 1.1
W13.1	115.6 ± 2.8	117.5 ± 2.8	6.9 ± 1.2	6.5 ± 1.1	13.3 ± 2.8	10.2 ± 2.2	18.8 ± 1.3	19.5 ± 1.3
***p*-values**								
Genotype	0.0263		0.0352		0.2130		0.3222	
Location	0.1724		0.1443		0.1427		0.5272	
Interaction	0.0758		0.2553		0.4486		0.1682	

‡ **Estimates**, p-values and standard errors based on model (2). IHO: Experimental station Ihinger Hof. EWE: Experimental station Eckartsweier. NA: Data not available.

The mean number of branches developed by the genotypes cultivated at IHO were 5.6 (Sahi Alba 914) and 6.9 (W13.1) (Table 3). At EWE, the number of developed branches was 3.5 (Sahi Alba 914), 6.5 (W13.1) and 5.7 (G8), indicating significant differences between the two genotypes Sahi Alba 914 and W13.1 cultivated at EWE (p-value 0.0352).

3.1.3. Generative Growth

At IHO 11.8 (Sahi Alba 914) and 13.3 (W13.1) inflorescences per plant were produced. Genotypes cultivated at EWE produced 6.7 (Sahi Alba 914), 10.2 (W13.1) and 8.3 (G8) inflorescences (Table 3). In general, the number of inflorescences per plant did not vary significantly but it was obvious that G8 produced fewer inflorescences compared to Sahi Alba 914 and W13.1 (Table 3).

The length of the central axis inflorescence was 18.8 cm (W13.1), 19.2 cm (Sahi Alba 914) and 16.8 cm (G8) for the genotypes cultivated at IHO, whereas at EWE it amounted to 16.8 cm (Sahi Alba 914), 19.5 cm (W13.1) and 16.0 cm (G8) (Table 3). No significant difference in length of the central axis inflorescence between Sahi Alba 914 and W13.1 was detected. The interactions between genotypes and locations were not significant.

Genotypes cultivated at IHO produced 4051 (W13.1) and 4289 (Sahi Alba 914) seeds per plant. At EWE seeds per plant amounted to 2073 (Sahi Alba 914), 3519 (W13.1) and 2738 (G8). No significant differences in the amount of seeds produced per plant were recorded for the two locations (Table 4).

Table 4. Mean number of seeds, seed weight, seed length and seed width per single plant of three chia genotypes (G8, Sahi Alba 914, W13.1) cultivated at two different locations (IHO/EWE) in 2016 (n = 10, α = 0.05, mean ± standard error).

Factor	Number of Seeds ‡		Seed Weight [g] ‡		Seed Length [mm] ‡		Seed Width [mm] ‡	
	IHO	EWE	IHO	EWE	IHO	EWE	IHO	EWE
Genotype								
G8	NA	2738 ± 480.9	NA	3.2 ± 0.6	1.9 ± 0.0	1.8 ± 0.0	1.3 ± 0.6	1.3 ± 0.6
Sahi Alba 914	4289 ± 844.2	2073 ± 408.2	6.4 ± 1.3	2.7 ± 0.7	2.0 ± 0.0	1.9 ± 0.0	1.3 ± 0.0	1.3 ± 0.0
W13.1	4051 ± 797.4	3519 ± 692.7	6.3 ± 1.3	4.5 ± 1.0	2.1 ± 0.0	2.0 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
***p*-values**								
Genotype	0.2854		0.2546		0.0008		<0.0001	
Location	0.0982		0.0571		0.0126		0.1939	
Interaction	0.2008		0.2304		0.2594		0.7735	

‡ **Estimates**, *p*-values and standard errors are based on model (2). IHO: Experimental station Ihinger Hof. EWE: Experimental station Eckartsweier. NA: Data not available.

Mean seed weight per plant amounted to 6.3 g (W13.1) and 6.4 g (Sahi Alba 914) for the genotypes cultivated at IHO whereas the seed weights per plant in EWE were considerably lower due to the lower seed numbers, showing weights of 4.5g (W13.1), 2.7 g (Sahi Alba 914) and 3.2 g (G8). Again, no significant difference between genotypes (Sahi Alba 914, W13.1) and locations could be detected (Table 4).

Seed length (Table 4) of genotypes cultivated at IHO was 2.0 mm (Sahi Alba 914), 2.1 mm (W13.1) and 1.9 mm (G8). Differences between Sahi Alba 914 and W13.1 were significant (*p*-value 0.0008). Samples of the genotypes cultivated at EWE were 1.9 mm (Sahi Alba 914), 2.0 mm (W13.1) and 1.8 mm (G8) long. A significant difference was detected between the two locations regarding Sahi Alba 914 seed length (*p*-value 0.0126).

Seed width of Sahi Alba 914 (1.3 mm) was significantly lower compared to that of W13.1 (1.4 mm) at both locations (*p*-value < 0.0001, Table 4). Seeds of G8 cultivated at IHO and EWE were 1.3 mm wide. The above-mentioned data showed no significant genotype-by-location interaction

3.2. Yield Parameters

In 2016 mean plant density per square meter at IHO amounted to 30 (W13.1), 23 (Sahi Alba 914) and 17 plants (G8) (Table 5). At EWE, plant densities amounted to 87 (W13.1), 134 (Sahi Alba 914) and 92 (G8) per square meter in 2016. The study showed that there was a significant difference in plant density between locations (IHO, EWE) and the white seeded genotypes W13.1 and Sahi Alba 914 (*p*-value 0.0028 and 0.0025, respectively). There was no difference in germination capacity identified. Compared to IHO an increased seedling emergence at EWE was obtained probably due to the fact, that the sandy soil type was less susceptible towards soil surface crust formation resulting in a higher number of plants per square meter [34].

In this regard, it should be mentioned that the different seeding rates and the resulting plant densities were considered in the statistical evaluation.

Table 5 shows that at IHO in 2015 genotype W13.1 generated a significantly higher seed yield (832.7 kg ha^{-1}) compared to Sahi Alba 914 (422.8 kg ha^{-1}) (*p*-value 0.0154). With 186.3 kg ha^{-1} G8 obtained a substantially lower yield. In 2016, seed yields at IHO were significantly higher compared to 2015, ranging from 973.4 kg ha^{-1} (W13.1), 865.3 kg ha^{-1} (Sahi Alba 914) to 751.7 kg ha^{-1} (G8) (*p*-value 0.0003). Yields obtained at EWE in 2016 were significantly higher compared to the yields obtained at IHO in 2016 (*p*-value 0.0166). Genotype W13.1 generated a yield of 1274.7 kg, Sahi Alba 914 generated a yield of 1161.4 kg ha^{-1} and G8 obtained a yield of 1286.0 kg ha^{-1}.

The TKM in 2015 at IHO was 1.2 g (Sahi Alba 914), 1.3 g (W13.1) and 1.1 g (G8). In 2016 TKM has increased significantly by 0.1 g each to 1.3 g (Sahi Alba 914), 1.4 g (W13.1) and 1.2 g (G8) (*p*-value 0.0066). In 2016 TKM at EWE was 1.3 g (Sahi Alba 914), 1.4 g (W13.1) and 1.2 g (G8). Significant differences could be observed between the genotypes Sahi Alba 914 and W13.1 cultivated at IHO (p-value 0.0075, Table 5).

A genotype-by-year interaction was found to be significant (p-value 0.0147) for seed yield (Table 5). No further interactions were found to be significant.

Table 5. Mean number of plants per square meter, mechanically threshed seed yield [kg ha^{-1}] and corresponding thousand kernel mass (TKM) [g] of three chia genotypes (G8, Sahi Alba 914, W13.1) cultivated at two different locations (IHO/EWE) in 2015 and 2016 (n = 3, α = 0.05, mean ± standard error).

Factor	Plants per (m^2) ‡	Yield (kg ha^{-1}) †		TKM (g) †	
	2016	2015	2016	2015	2016
Location × Genotype					
IHO × G8	17 ± 4.3	186.3 ± 115.8	751.7 ± 115.8	1.1 ± 0.1	1.2 ± 0.1
EWE × G8	92 ± 22.0	NA	1286.0 ± 115.8	NA	1.2 ± 0.1
IHO × Sahi Alba 914	23 ± 5.5	422.8 ± 37.3	865.3 ± 76.4	1.2 ± 0.0	1.3 ± 0.0
EWE × Sahi Alba 914	134 ± 14.3	NA	1161.4 ± 102.5	NA	1.3 ± 0.0
IHO × W13.1	30 ± 7.4	832.7 ± 73.5	973.4 ± 85.9	1.3 ± 0.0	1.4 ± 0.0
EWE × W13.1	87 ± 9.3	NA	1274.7 ± 138.4	NA	1.4 ± 0.0
***p*-values**					
Location	0.0028	0.0166		0.6254	
Genotype	0.0025	0.0154		0.0075	
Year	-	0.0003		0.0066	
Genotype × Year	-	0.0147		0.8721	
Genotype × Location	0.1060	0.9168		0.4114	

‡ **Estimates**, p-values and standard errors based on model (2). † Estimates, p-values and standard errors based on model (3). IHO: Experimental station Ihinger Hof. EWE: Experimental station Eckartsweier. NA: Data not available.

3.3. Quality Parameters

3.3.1. Protein, Mucilage and Oil Content

Genotypes cultivated at IHO in 2015 showed mean crude protein contents of 24.2% (W13.1), 20.6% (Sahi Alba 914) and 23.0% (G8). Protein content was 22.9% (W13.1), 25.0% (Sahi Alba 914) and 21.7% (G8) for genotypes cultivated at IHO in 2016. Genotypes Sahi Alba 914 and W13.1 cultivated at EWE in 2016 showed significantly lower protein contents compared to those cultivated at IHO in 2016, namely 17.8% (W13.1), 18.1% (Sahi Alba 914) and 17.2% (G8) (p-value < 0.0001) (Figure 2). Significant differences between Sahi Alba 914 and W13.1 were detected for IHO in 2016 (p-value < 0.0001).

Mean mucilage content was 10.8% (W13.1/Sahi Alba 914) and 10.6% (G8) for the genotypes cultivated in 2015 at IHO. In 2016, mucilage content was 10.1% (W13.1), 9.5% (Sahi Alba 914) and 11.7% (G8). Mucilage contents of Sahi Alba 914 and W13.1 cultivated at EWE were significantly higher reaching a mucilage content of 11.8% (W13.1), 10.9% (Sahi Alba 914) and 12.2% (G8) (p-value 0.0003, Figure 2). In 2016, Sahi Alba 914 and W13.1 obtained significantly different mucilage contents at both locations (p-value 0.0016, Figure 2).

No statistically significant difference was identified for total crude oil content. Mean contents shown in Figure 2 were 32.4% (W13.1), 32.7% (Sahi Alba 914) and 32.0% (G8) for genotypes cultivated at IHO in 2015. In 2016, total crude oil content was 30.9% (W13.1), 31.3% (Sahi Alba 914) and 32.5% (G8) for the genotypes cultivated at IHO. Genotypes cultivated at EWE showed slightly higher total crude oil contents, 31.7% (W13.1), 33.7% (Sahi Alba 914) and 33.5% (G8) in 2016. No significant interactions between genotypes, locations and year of cultivation were found for the above-mentioned data.

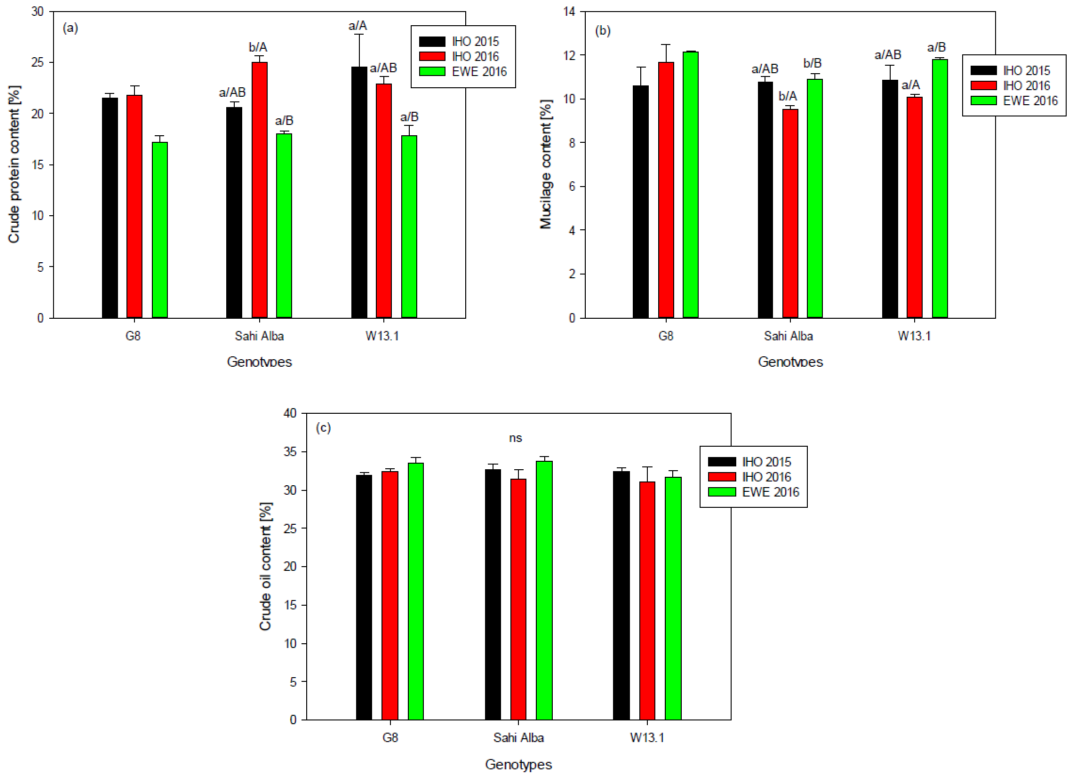

Figure 2. Mean crude protein (**a**), mucilage (**b**) and crude oil (**c**) content of three chia genotypes (G8, Sahi Alba 914, W13.1,) cultivated at two different locations (IHO/EWE) in 2015 and 2016. Genotype-means within an environment which share a common lower-case letter do not differ significantly at α = 0.05. Environment-means which share a common upper case letter do not differ significantly within a genotype at α = 0.05. (Two way ANOVA, *Tukey* test, *n* = 3, means ± standard errors based on model (3)).

3.3.2. Fatty Acid Contents, Proportions and Ratios

The statistical analysis of the identified single fatty acids showed no significant interaction between genotype × year and genotype × location (Table 6). Location and year significantly influenced the content of the saturated palmitic (*p*-value 0.0209 and *p*-value 0.0053, respectively) and stearic (*p*-value 0.0059 and *p*-value 0.0004, respectively) fatty acids (Table 6).

Mean palmitic acid content of the genotypes cultivated at IHO in 2015 was 6.9% (W13.1), 7.3% (Sahi Alba 914) and 6.6% (G8). Significantly, higher contents could be detected in 2016. Mean palmitic acid content amounted to 8.2% (W13.1/Sahi Alba 914) and 7.5% (G8). Mean palmitic acid contents of the genotypes cultivated at EWE in 2016 were significantly higher, varying between 9.7% (W13.1), 11.8% (Sahi Alba 914) and 11.1% (G8).

Genotypes cultivated at IHO showed mean stearic acid contents between 2.0% (W13.1), 2.1% (Sahi Alba 914) and 2.8% (G8) in 2015. Significantly, higher contents could be identified in 2016 varying from 3.4% (W13.1) to 3.8% (Sahi Alba 914/G8). A significant difference in stearic acid content was detected between the genotypes Sahi Alba 914 and W13.1 (*p*-value 0.0254) in 2016. Mean stearic acid contents of the genotypes cultivated at EWE in 2016 were significantly higher, ranging from 4.3% (W13.1) to 6.1% (Sahi Alba 914) and 5.9% (G8).

The monounsaturated oleic fatty acid was also significantly influenced by location (*p*-value 0.0067) and year (*p*-value 0.0009) whereas the monounsaturated vaccenic fatty acid seemed not to be influenced by location, genotype nor year (*p*-value > 0.05) (Table 6).

Table 6. Fatty acid composition (% of total fatty acid) of three chia genotypes (G8, Sahi Alba 914, W13.1) cultivated at two different locations (IHO/EWE) in 2015 and 2016 ($n = 3$, $\alpha = 0.05$, mean ± standard error).

Factor	Palmitic Acid (C16:0) †		Stearic Acid (C18:0) †		Oleic Acid (C18.1) †		Vaccenic Acid (C18:1) †		Linoleic Acid (C18:2) †		α-Linolenic Acid (C18:3) †	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Location × Genotype												
IHO × G8	6.6 ± 0.1	7.5 ± 0.1	2.8 ± 0.1	3.8 ± 0.1	6.2 ± 0.2	8.2 ± 0.2	0.8 ± 0.0	0.8 ± 0.0	19.0 ± 0.7	21.6 ± 0.7	64.7 ± 1.3	58.1 ± 1.0
EWE × G8	NA	11.1 ± 0.2	NA	5.9 ± 0.1	NA	11.8 ± 0.4	NA	1.1 ± 0.0	NA	23.2 ± 0.7	NA	46.7 ± 0.8
IHO × Sahi Alba 914	7.3 ± 0.3	8.2 ± 0.4	2.1 ± 0.2	3.8 ± 0.1	4.6 ± 0.3	6.6 ± 0.2	0.8 ± 0.0	0.8 ± 0.1	18.0 ± 0.5	21.1 ± 0.2	67.2 ± 0.8	59.6 ± 0.7
EWE × Sahi Alba 914	NA	11.8 ± 1.3	NA	6.1 ± 0.7	NA	9.2 ± 0.8	NA	1.0 ± 0.1	NA	23.1 ± 0.9	NA	48.7 ± 3.0
IHO × W13.1	6.9 ± 0.3	8.2 ± 0.4	2.0 ± 0.2	3.4 ± 0.1	5.3 ± 0.4	6.7 ± 0.2	0.8 ± 0.0	0.9 ± 0.1	20.2 ± 0.6	22.0 ± 0.2	64.7 ± 0.8	58.8 ± 0.7
EWE × W13.1	NA	9.7 ± 1.1	NA	4.3 ± 0.5	NA	8.1±0.7	NA	1.0 ± 0.1	NA	21.9 ± 0.9	NA	54.2 ± 3.3
***p*-values**												
Location	0.0209		0.0059		0.0067		0.0616		0.1803		0.0171	
Genotype	0.3410		0.0254		0.3951		0.1054		0.0099		0.0938	
Year	0.0053		0.0004		0.0009		0.0945		0.0010		0.0001	
Genotype × Year	0.6457		0.4231		0.2776		0.3373		0.1041		0.2430	
Genotype × Location	0.2956		0.2329		0.2890		0.3669		0.1659		0.2223	

Results in g 100 g^{-1} of oil. † Estimates, *p*-values and standard errors based on model (3). IHO: Experimental station Ihinger Hof. EWE: Experimental station Eckartsweier. NA: Data not available.

Oleic acid content of the genotypes cultivated at IHO in 2015 ranged from 4.6% (Sahi Alba 914) to 5.3% (W13.1) and 6.2% (G8). In 2016 the oleic acid values showed a significant increase, varying between 6.6% (Sahi Alba 914), 6.7% (W13.1) and 8.2% (G8). The oleic acid content of the genotypes cultivated at EWE in 2016 were significantly higher compared to those cultivated at IHO in 2016, namely 8.1% (W13.1), 9.2% (Sahi Alba 914) and 11.8 % (G8).

Vaccenic acid content obtained by all three genotypes (W13.1, Sahi Alba 914, G8) cultivated at IHO in 2015 was 0.8%. In 2016, genotypes cultivated at IHO showed vaccenic acid contents of 0.8% (Sahi Alba 914, G8) and 0.9% (W13.1) whereas those vaccenic acid contents observed at EWE in 2016 were 1.0% (W13.1, Sahi Alba 914) and 1.1% (G8).

Polyunsaturated linoleic and α-Linolenic fatty acid contents were significantly influenced by year (*p*-value 0.0010 and *p*-value 0.0001, respectively). Further, it was shown that the linoleic content was significantly influenced by genotype (*p*-value 0.0099) whereas the α-Linolenic content was significantly influenced by location (*p*-value 0.0171) (Table 6).

Linoleic acid contents of the genotypes cultivated at IHO in 2015 were 18.0% (Sahi Alba 914), 19.0% (G8) and 20.2% (W13.1). A significant difference was observed between Sahi Alba 914 and W13.1 in that year. In 2016, the linoleic acid content significantly increased compared to the contents recorded in 2015. Contents of 21.1% (Sahi Alba 914), 21.6% (G8) and 22.0% (W13.1) were obtained for linoleic acid. The genotypes cultivated at EWE in 2016 showed linoleic acid contents of 23.1% (Sahi Alba 914), 23.2% (G8) and 21.9% (W13.1).

Contents of α-Linolenic acid were found to be 64.7% (W13.1, G8) and 67.2% (Sahi Alba 914) for the genotypes cultivated at IHO in 2015. In 2016, genotypes cultivated at IHO showed significantly lower α-Linolenic acid contents (Table 6), varying between 58.1% (G8), 58.8% (W13.1) and 59.6% (Sahi Alba 914). α-Linolenic acid contents of genotypes cultivated at EWE in 2016 were significantly lower, namely 46.7% (G8), 48.7% (Sahi Alba 914) and 54.2% (W13.1).

The statistical analysis of the fatty acid proportions and ratios indicated no significant interaction between genotype × year as well as for genotype × location (Table 7).

The percentage of saturated fatty acids (SFA) obtained in 2015 by the genotypes cultivated at IHO was 9.4 (Sahi Alba 914), 9.0 (W13.1) and 9.3 (G8). In 2016, the percentage of SFA was significantly higher for those genotypes cultivated at IHO (*p*-value 0.0006) (Table 7). Genotypes showed 12.0 (Sahi Alba 914), 11.5 (W13.1) and 11.2 (G8) percentage of SFA. Compared to the genotypes cultivated at IHO the percentage of SFA from those of EWE cultivated in 2016 was significantly higher, namely 17.8% (Sahi Alba 914), 14.0% (W13.1) and 17.1% (G8) (*p*-value 0.0169) (Table 7).

Table 7. Fatty acid proportions and ratios of three chia genotypes (G8, Sahi Alba 914, W13.1,) cultivated at two different locations (IHO/EWE) in 2015 and 2016 (n = 3, α = 0.05, mean ± standard error).

Factor	SFA (%) †		MUFA (%) †		PUFA (%) †		PUFA:SFA †		ω6:ω3 †	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Location × Genotype										
IHO × G8	9.3 ± 0.2	11.2 ± 0.2	7.0 ± 0.2	9.0 ± 0.2	83.7 ± 0.7	79.8 ± 0.6	9.0 ± 0.1	7.1 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
EWE × G8	NA	17.1 ± 0.3	NA	12.9 ± 0.3	NA	70.0 ± 0.5	NA	4.1 ± 0.2	NA	0.50 ± 0.0
IHO × Sahi Alba 914	9.4 ± 0.3	12.0 ± 0.5	5.4 ± 0.3	7.3 ± 0.2	85.2 ± 0.6	80.7 ± 0.8	9.1 ± 0.4	6.7 ± 0.4	0.27 ± 0.0	0.35 ± 0.0
EWE × Sahi Alba 914	NA	17.8 ± 2.1	NA	10.2 ± 0.9	NA	72.0 ± 2.5	NA	4.0 ± 0.6	NA	0.48 ± 0.0
IHO × W13.1	9.0 ± 0.3	11.5 ± 0.5	6.1 ± 0.4	7.6 ± 0.2	84.9 ± 0.6	80.8 ± 0.8	9.4 ± 0.4	7.0 ± 0.4	0.31 ± 0.0	0.37 ± 0.0
EWE × W13.1	NA	14.0 ± 1.6	NA	9.1 ± 0.8	NA	76.2 ± 2.7	NA	5.5 ± 0.8	NA	0.41 ± 0.0
***p*-values**										
Location	0.0169		0.0083		0.0172		0.0171		0.0018	
Genotype	0.1637		0.2367		0.6617		0.2316		0.0234	
Year	0.0006		0.0008		0.0009		0.0006		< 0.0001	
Genotype × Year	0.8280		0.3293		0.5309		0.7804		0.2978	
Genotype × Location	0.2622		0.2854		0.3075		0.2714		0.2706	

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). † Estimates, p-values and standard errors based on Model (3). IHO: Experimental station Ihinger Hof. EWE: Experimental station Eckartsweier. NA: Data not available.

Monounsaturated fatty acid (MUFAs) content was 5.4% (Sahi Alba 914), 6.1% (W13.1) and 7.0% (G8) for the genotypes cultivated at IHO in 2015. There was a significant increase in MUFA'S in 2016 (p-value 0.0008) (Table 7). Genotypes cultivated at IHO showed MUFA contents of 7.3% (Sahi Alba 914), 7.6% (W13.1) and 9.0% (G8). Results for the genotypes cultivated at EWE were also significantly higher compared to those cultivated at IHO in 2016. MUFA contents were 10.2% (Sahi Alba 914), 9.1% (W13.1) and 12.9% (G8) (p-value 0.0083) (Table 7).

Contents of polyunsaturated fatty acids (PUFAs) were 84.9% (W13.1), 85.2% (Sahi Alba 914) and 83.7% (G8) for the genotypes cultivated in 2015 at IHO. A significantly lower content of PUFAs was detected for those genotypes cultivated at IHO in 2016 (p-value 0.0009) (Table 7). In this year PUFA contents were 80.8% (W13.1), 80.7% (Sahi Alba 914) and 79.8% (G8). Significant differences were detected between the two locations IHO and EWE in 2016 (p-value 0.0172) (Table 7). PUFAs were significantly lower for the genotypes cultivated at EWE, showing PUFA contents of 76.2% (W13.1), 72.0% (Sahi Alba 914) and 70.0% (G8).

Genotypes cultivated at IHO in 2015 showed PUFA:SFA ratios of 9.4 (W13.1), 9.1 (Sahi Alba 914) and 9.0 (G8) whereas the PUFA:SFA ratios of the genotypes cultivated at IHO in 2016 were significantly lower (p-value 0.0006) (Table 7). Ratios were 7.0 (W13.1), 6.7 (Sahi Alba 914) and 7.1 (G8). Compared to the ratios of the genotypes cultivated at IHO in 2016 the ratios of the genotypes cultivated at EWE in 2016 were significantly lower with 5.5 (W13.1), 4.0 (Sahi Alba 914) and 4.1 (G8) (p-value 0.0171) (Table 7).

ω6:ω3 ratios of genotypes cultivated at IHO in 2015 were 0.31 (W13.1), 0.27 (Sahi Alba 914) and 0.29 (G8). Significantly higher ω6:ω3 ratios were obtained in 2016 showing ratios of 0.37 (W13.1), 0.35 (Sahi Alba 914) and 0.37 (G8) (p-value < 0.0001) (Table 7). Significantly higher ω6:ω3 ratios were obtained by the genotypes cultivated at EWE in 2016, 0.41 (W13.1) 0.48 (Sahi Alba 914) and 0.50 (G8), respectively (p-value 0.0018) (Table 7). This study generally indicated that the fatty acid proportions and ratios were strongly influenced by location and year rather than by genotype.

4. Discussion

Being a frost sensitive short day plant, requiring day length < 12 h to induce flower formation, the cultivation of chia is traditionally limited to latitudes up to about 25 degrees [35]. In temperate regions of the northern hemisphere chia only starts to produce flower buds in short days of late September/October, which are killed by first frost shortly thereafter [35]. According to Grimes et al. [35] the photoperiodic sensitivity can thus be seen as a bottleneck for the expansion of chia cultivation towards more northern and southern latitudes. By overcoming this barrier, chia cultivation could be introduced to a wider range of environmental conditions outside of its Mesoamerican origin [12,14].

4.1. Plant Development

4.1.1. Impact of Day Length and Temperature

In this study, the cultivation of three chia genotypes either day length insensitive (Sahi Alba 914) or adapted to day lengths greater than 12 h (G8, W13.1) were tested under the given climatic and day length conditions of Southwestern Germany. To the best of our knowledge, it was the first attempt to grow these genotypes at latitudes > 48° N.

Jamboonsri et al. [12] was able to proof the existence of new chia genotypes, which were able to induce flower formation in greenhouse (15.9 h) and field (14 h 41 min) experiments exceeding the known threshold. With flower induction occurring 66 to 74 days after sowing by mid-July (EWE, 2016) and early August (IHO, 2015 & 2016) at a mean day length of 15.81 to 15.83 h (Table 2), this study verified the findings of Jamboonsri et al. [12] indicating that these genotypes can cope with the given day length conditions in Southwestern Germany. With 66 (EWE, 2016) to 70 (IHO, 2016) and 74 (IHO, 2015) days until flower initiation, findings of this study were centered between the data reported by Baginsky et al. [2], indicating that flower initiation occurred 40 to 60 (coastal dessert/dessert climate) and 80 to 110 (Mediterranean climate) days after sowing at a latitude of 18°–33° S.

In order to induce flowering, Baginsky et al. [2] stated that chia plants have to accumulate 600 to 700 growing degree-days, if day length is not sufficiently long enough. As within this study, lesser growing degree-days were accumulated until flower induction (Table 2), the identified threshold of 15.8 h day length for flower induction seemed to be adequate for the three cultivated genotypes. It is therefore suggested that an adequate adoption to day length is of higher importance for the development of the tested genotypes than the accumulated growing degree days. Growing degree-days observed in this study (Table 2) fell amongst the data obtained by Baginsky et al. [2] which reported 1549.1 to 1638.8 (coastal dessert and dessert climate) and 610.3 (Mediterranean climate) accumulated growing degree-days, respectively.

Temperature until flower induction was higher (0.7 °C) at EWE in 2016 compared to IHO (2016), which led to an accelerated plant development accompanied with an earlier beginning of flowering. Referring to temperature Ayerza and Coats [11] stated that chia's minimum and maximum growth temperature is 11 °C and 36 °C with an optimum range between 16 °C and 26 °C, hence thermal requirements could be fulfilled within this study (Table 2).

As the mean day length until flowering, did not vary between the two locations in 2016 (Table 2), the early flower induction at EWE is therefore most likely attributed to temperature. In general, it can be stated that all genotypes at both locations completed their phenological development and were able to reach maturity. Nevertheless, the climatic conditions of the upper Rhine Valley can be considered favorable in terms of the limited period of time (frost-free), in which the cultivation of chia under Southwestern German conditions is feasible.

In this regard, it should be mentioned that sowing date is crucial as it affects the above-mentioned findings (day length, GDD, temperature) to a significant extent. Sowing date determines the duration of the crop cycle and therefore its ability to reach maturity depending on the variations in environmental temperature and day length to which it is exposed during cultivation [10]. Chosen sowing dates in May seem to match with the given requirements of chia and can therefore be recommended for chia cultivation in Southwestern Germany. Earlier sowing dates might increase the risk of frost damages to the emerging chia plants, as the region of Southwestern Germany is often exposed to a cooler weather period and the last spring frost often occurs at the end of April/beginning of May (ice saints).

4.2. Vegetative Growth

Identified plant heights of 102.3 to 117.5 cm were in line with studies of Sorondo [14] who measured plant heights of Sahi Alba 914 cultivated in Argentina between 106 and 121 cm (Table 3). In a study by Nyamweha et al. [36] carried out in Uganda (latitude ~0° N) common chia cultivars reached plant heights of 110.6 cm while a study in Ghana (latitude ~10° N) documented heights of

92.9 to 101.1 cm [37]. Chia plant heights decrease with shorter day length according to Baginsky et al. [2]. At latitudes of zero to ten (Uganda/Ghana) shorter plants were developed compared to those grown at the higher latitude (48° N) presented in this study (Table 3) [36,37]. This pattern is reported also for other short day plants such as soybean and *Amaranthus* spp. and could be observed for the chia genotypes, which were adopted to day length greater > 12 h (W13.1, G8), but in consequence not for the day length insensitive genotype (Sahi Alba 914) (Table 3) [38,39].

The mean number of branches identified by the present study was below the values reported so far in literature (Table 3). Souza & Chaves [40] reported 7.9 to 9.3 branches under greenhouse conditions (latitude 7° S, mean temp. 23–34°C) while Yeboah et al. [37] indicated 21.2 to 26.8 branches per plant under field conditions (latitude 6° N, mean temp. 24–28 °C). As the number of branches per plant is directly influenced by row spacing and seeding density, the given variability between the different studies indicated a high plasticity of chia regarding the number of branches, wherefore the chosen density has to be matched to the specific environmental conditions at each growing site [41].

4.3. Yield Parameters and Yield

Under Chilean conditions, Baginsky et al. [2] showed that black and white seeded genotypes produced lesser inflorescences in the Mediterranean climate (6.3, 6.1) compared to the coastal dessert (11.2, 10.8) and dessert climate (8.7, 10.0) conditions. With increased day length the number of inflorescences produced per single plant decreased according to Baginsky et al. [2]. This contradicts the findings of the present study as the number of inflorescences produced by the genotypes cultivated in this study (Table 3) was in line with results of Karim et al. [42] who determined 4.67 to 13.67 inflorescences per single plant in a field trial conducted in Bangladesh (latitude ~25° N). However, as the number of inflorescences as well as the number of branches per plant might also be influenced by agronomic parameters like sowing density, row spacing etc., differences in yield parameters might rather be related to complex interactions between agronomic and environmental parameters instead of being related to a single parameter.

Mean length of the central axis inflorescences produced by the three genotypes cultivated at IHO and EWE in 2016 were between 16.0 to 19.5 cm. Sorondo [14] referred to the mean length of the central axis inflorescence of 22.4 cm for Sahi Alba 914, which could not be reached. Baginsky et al. [2] reached central axis inflorescences length of 13.6 to 34.1 cm, depending on sowing date and location. In this regard, they observed that delayed sowing dates (shorter day length) tend to increase inflorescence length in all locations and that the length of the central axis inflorescences was in general negatively and significantly associated with day length at the beginning of flowering. This would explain why reported lengths of inflorescences in literature could not be reached in our study, but might have been rather compensated by a higher number of inflorescences per plant.

The amount of seeds produced per single plant has not yet been reported in the existing literature. The study conducted at IHO and EWE produced 2073 to 4289 seeds per single plant as shown in Table 4. Regarding the different sowing rates and row distances it should again be mentioned that they were integral parts of the site and annual effects and that the resulting plant densities are hence to be disregarded. Referring to this, the higher seed numbers per single plant at IHO indicate that due to its high plasticity, chia is able to compensate lower plant numbers with higher seed numbers per single plant [41], finally leading to satisfactory yields under the given climatic conditions in Southwestern Germany. However, it has to be stated that the right choice of harvesting time and harvest intensity (manually/mechanically) is of crucial importance in regard to potentially achievable yields, as chia is highly susceptible to seed shattering [43]. Amongst others, it was reported that suboptimal combine operations and harsh weather conditions between manual and mechanical harvest, could led to yield losses of up to 37% [44], which both would explain the observed differences between manually and mechanically harvested yields (Tables 4 and 5).

When speaking of yield, thousand kernel mass is another important yield determining trait. Ixtaina et al. [4] showed average thousand kernel masses of dark and white chia seeds of 1.323 g and 1.301 g, respectively. Sorondo [14] stated thousand kernel masses between 1.1 and 1.4 g for Sahi Alba 914. Amato et al. [45] reported first biometric data on chia seeds produced in Europe showing thousand kernel masses of 1.26 to 1.37 g. Those values are in line with the findings of this study (Table 5). Indicating that genotype and year influenced thousand kernel mass significantly. Therefore, more studies would be beneficial in order to identify the specific influencing factors on chia's thousand kernel mass.

As reported in Table 5, seed yields of the white seeded genotypes Sahi Alba 914 and W13.1 obtained in this study (422.8–1286.0 kg ha^{-1}) are in line with the yields obtained by Baginsky et al. [2] and Sosa et al. [46]. Seed yields observed at EWE were significantly higher compared to those obtained at IHO in 2016. In this regard, it should again be mentioned that the different sowing rates and row distances were integral parts of the site and annual effects and could therefore be disregarded as well as the resulting plant densities. The combination of higher temperature and growing degree day accumulation along with higher radiation (Table 2), which represents the energy source for photosynthesis during the whole crop cycle, very likely influenced seed yield at EWE in 2016 positively. As temperature in general seemed to be more constant at EWE a higher metabolic activity during grain filling and maturation compared to IHO might have occurred. This may have contributed to a possible superior rate of matured seeds, leading to an overall higher yield potential at EWE [2,9].

A field experiment conducted in Southern Italy, at ~40 ° N produced seed yields between 126.5 and 490.4 kg (total dry mass) and increased significantly with plant density according to Bochicchio et al. [9]. This demonstrates the enormous potential of chia cultivation outside its center of origin. Relatively low seed yields are often the result of phenology (late flowering), temperature conditions (peaks of low temperatures, winter kill) and lodging which affected the seed yield negatively [9].

Dependent on the environment and cultivation conditions seed yields of chia seem to vary greatly. In Argentina for example, Sosa et al. [46] reported yields from 150 to 1200 kg ha^{-1} whereas seed yields of about 2600 kg ha^{-1} were obtained under Ghanaian conditions [37]. Another trial conducted by Baginsky et al. [2] resulted in significantly varying seed yields between the Chilean dessert/coastal dessert climate (950–2900 kg ha^{-1}) and the Chilean Mediterranean climate (70–130 kg ha^{-1}). Sowing date is highly relevant as it determines the development period of the crop given the fluctuations in environmental temperature and day length, which according to Ayerza [47,48] are mainly responsible for yield potential and seed quality. Even though the genotypes cultivated in this study were adapted to temperate regions, the cultivation period in Southwestern Germany is highly restricted due to low temperatures and frost occurrences in the first and last quarter of the year, which unfortunately strictly limits chia cultivation from April/May until October/November. In temperate regions, late sowing dates would kill the plants before they reached maturity.

4.4. Quality Parameters

4.4.1. Protein, Mucilage and Oil Content

Chia seeds cultivated at IHO contained between 20.6 and 25% of crude protein which is in line or rather higher compared to findings of Amato et al. [45] and Silva et al. [49]. Ayerza and Coats [50] showed that the protein content tends to decrease as the temperature increases. When looking at the protein contents of genotypes cultivated at EWE (Figure 2), where mean temperature from flower induction to harvest maturity was 6.4 degrees higher this tendency could be verified and a negative correlation between seed yield and protein content as for most plants was observed. In order to realize a higher protein content at EWE, which holds a high yield potential, the level of nitrogen fertilization should therefore be raised.

Mucilage (gel, which surrounds the seeds when hydrated) yields are reported to vary between 5 to 15.1% [24,51–53]. With mean mucilage yield values between 9.51 and 12.15% results of this study can be assessed as quite high and therefore favorable (Figure 2). It has been reported to be essentially composed of complex high molecular weight polysaccharides [9,54,55] and to have exceptional physical properties on the basis of the high soluble to insoluble fiber ratio [54]. In recent years, scientists have been researching chia seeds mucilage and its possible areas of application showing promising functional properties from a technological and physiological perspective [56].

Total crude oil contents varied from 30.9 to 33.7% and were in line with the findings of Ixtaina et al. [52], Silveira Coelho & de las Mercedes Salas-Mellado [53], Amato et al. [45], Baginsky et al. [2] and Silva et al. [49] where oil yields ranged from 20.3% to 34.4%. According to Ixtaina et al. [52] and Silva et al. [49] seed oil yield was crucially affected by extraction technique and solvent used, regarding n-hexane as the solvent yielding the highest oil yields. It was additionally stated that the seed oil yield generally depends on factors, such as climatic conditions and the region of provenance [5,50]. Ayerza [5] was able to demonstrate that oil contents of a single chia line varied from 25.93% to 33.50% in five different ecosystems. As oil contents were not influenced by genotype, location or year in our study, farmers might profit from stable oil contents while meeting the given EU requirements of the Novel Food guideline [57].

4.4.2. Fatty Acid Contents, Proportions and Ratios

The determined fatty acid profiles were in line with the given profiles in literature [49,52]. Within this study it could be shown that the fatty acid composition was influenced by year > location > genotype in descending order. Overall, temperature affects the determined oil quality [58]. Cool climatic conditions are supposed to postpone the maturity of the seeds and thus offer a longer period for oil and fatty acid synthesis leading to an increase in the level of unsaturated fatty acids [5,59]. Taking the mean temperature from flower induction to harvest maturity of both locations in 2016 into account, the location IHO was cooler than EWE, resulting in higher amounts of PUFAs. In addition, mean temperature at IHO in 2015 during grain filling was lower than in 2016 leading to an even higher amount of PUFAs in 2015 at the same location (Table 2).

The reduction of inflammatory conditions, tumor growth, angiogenesis and metastasis and the lower risk of cardiovascular disease and mortality are just some of the health benefits associated with ω-3 PUFAs [60–66]. It is known from the literature that the main components of chia oil are PUFA's with α-linolenic acid (ω3) as the predominant one and linoleic acid (ω6) as the second most common PUFA [45]. The fatty acids of the genotypes cultivated in our study can be classified in the following frequency of occurrence: α-linolenic acid (C18:3) > linoleic acid (C18:2) > palmitic acid (C16:0) > oleic acid (C18:1) > stearic acid (C18:0) > vaccenic acid (C18:1) [49,52]. In comparison to marine α-linolenic acid (ω3) sources, like algae or fish in the family Clupeidae, chia contains significantly less saturated fatty acids and a much higher α-linolenic acid content, while being odor- and tasteless which constitutes an advantage particularly in regard to enriching foods [1].

Humans require the essential polyunsaturated fatty acids ω6 and ω3 from food intake as they lack the endogenous enzymes for ω3 desaturation and therefore cannot be synthesized by the human body [1,67,68]. Modern agriculture has led to a disproportionately high level of ω6 PUFAs parallel to an extreme reduction of ω3 PUFAs in western diets, leading to an unbalanced and unhealthy ω6:ω3 ratio of between 10:1 and 20:1, compared to the 1:1 ratio during the Paleolithic period [68–70]. Balancing the ω6:ω3 ratio plays an important role in the prevention and management of obesity and coronary heart diseases [1,71]. With ω6:ω3 ratios between 2.7:1 and 5:1 as shown in Table 7, chia oil intake could contribute to a more balanced ω6:ω3 uptake therefore.

5. Conclusions

The three chia genotypes cultivated surpassed the yields obtained by their countries of origin and are thus an attractive alternative to local farmers in Southwestern Germany. Oil, protein and mucilage content are in line with current literature and the Novel Food-Regulation (EU) 2015/2283. Location, year and genotype choices appeared to be of equal importance for a successful chia production whereas the fatty acid profile seemed directly impacted by the factors as follows: year > location > genotype. Underlined by the results of this study, Southwestern Germany could be seen as a seminal opportunity to expand the cultivation area of chia to latitudes up to 48° N. Nevertheless, additional studies should be conducted in order to establish an optimized cultivation system for chia under the given conditions of Southwestern Germany.

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

Publication II

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The preceding chapter showed that chia cultivation under Central European conditions was feasible regarding the given relevant agronomic, yield and quality traits. This finding formed the foundation for the following chapter, which focuses on the establishment of a crop management system for chia as so far local information about critical management recommendations is basically non-existent for Europe. To establish a crop management system for chia relevant agronomic techniques need to be developed and adapted to the given environmental conditions. Therefore, field trials with 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}) were conducted. The influence of those management practices on chia seed yields and their associated seed quality traits were evaluated. It could be demonstrated that 1) 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} was recommendable and that, 2) yield and quality traits were similar to that of commercially available seeds from traditional and new growing areas while simultaneously meeting the given EU requirements of the Novel Food Guideline (EU) 2015/2283. The given environmental conditions significantly affected yield traits and the nutrient composition of chia seeds whereas the applied agronomic management practices showed only limited impact.

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

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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).

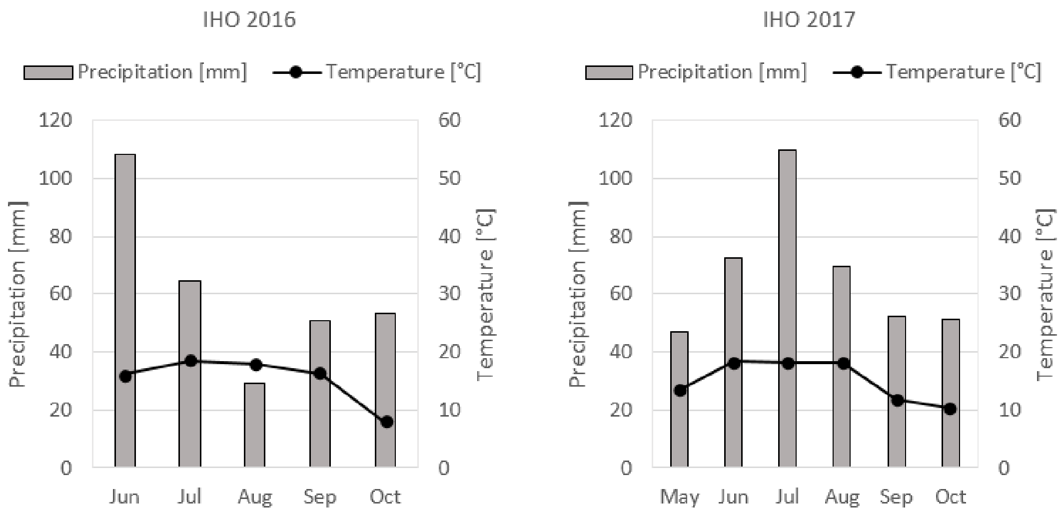

Figure 1. Precipitation [mm, bars] and mean temperature (°C; •) during the experimental period at Ihinger Hof in 2016 and 2017 (June–October, and May–October, respectively).

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.

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

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

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):

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

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, α = 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				
50	1170.33 A/a	844.33 B/a	0.0017	0.0004	0.3809	0.9284
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				
50	1.23 A/a	1.17 B/a	<0.0001	0.6242	0.8895	0.7997
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 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.

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				
1.5	880.01 A/b	844.33 B/b	0.1122	0.0094	0.0114	0.4283
2	748.00 A/ab	704.00 B/ab				
	SEM = 55.12					
	TSM [g] ‡					
1	1.22 A/a	1.21 A/a				
1.5	1.20 A/b	1.18 A/b	0.1268	0.6378	0.0125	0.8024
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, α = 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 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.

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].

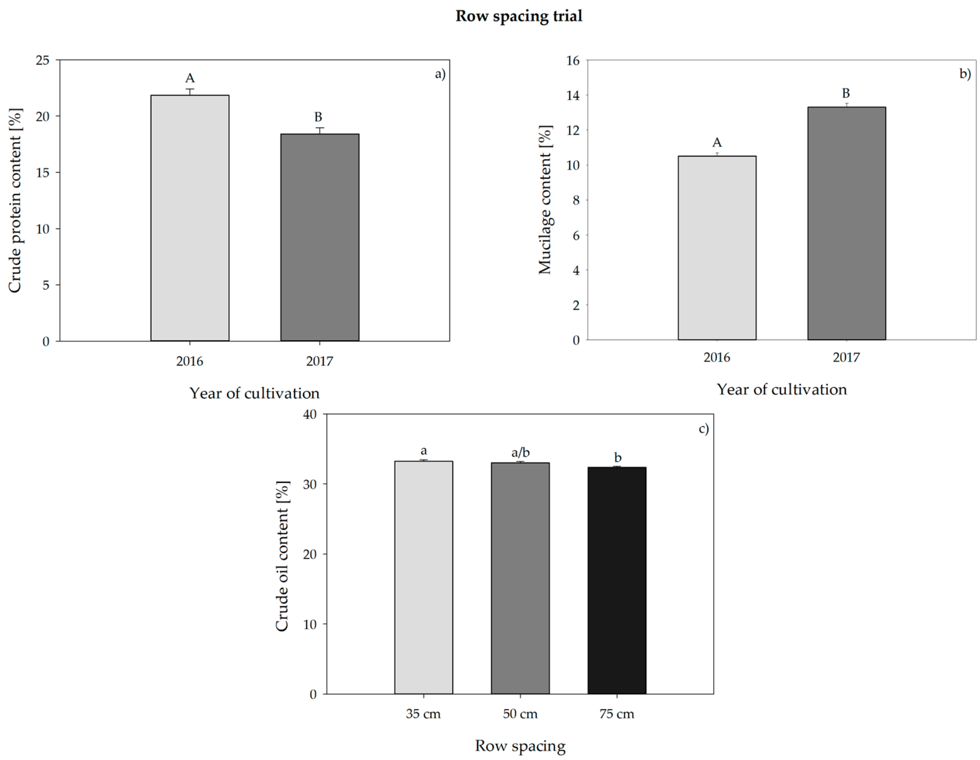

Figure 2. Mean estimates of crude protein (**a**), mucilage (**b**) and crude oil (**c**) content of the genotype G8 cultivated in 2016 and 2017 at Ihinger Hof with different row spacing. Means which share a common capital letter do not differ significantly between years at $\alpha = 0.05$. Means which share a common lowercase letter do not differ significantly within row spacing at $\alpha = 0.05$. (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 ω6:ω3 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).

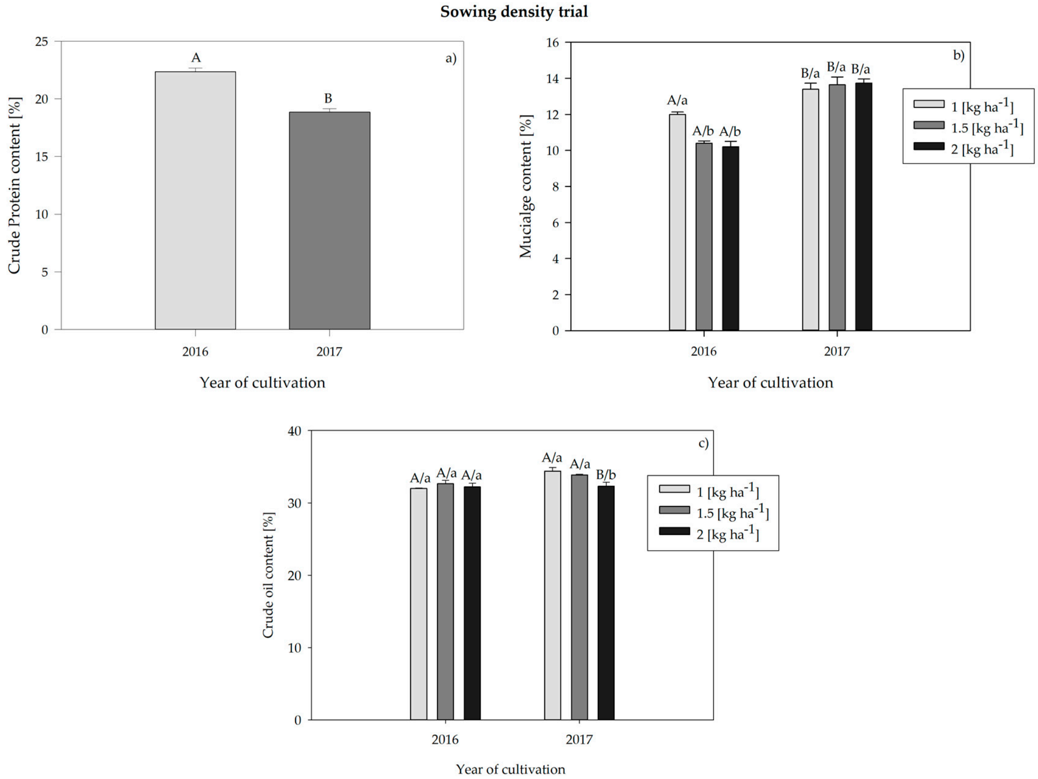

Figure 3. Mean estimates of crude protein (**a**), mucilage (**b**) and crude oil (**c**) content of the genotype G8 cultivated in 2016 and 2017 at Ihinger Hof at different sowing densities. Means at a constant level of sowing density that share a common capital letter do not differ significantly between years at α = 0.05. Means within a year that share a common lowercase letter do not differ significantly between sowing densities at α = 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.

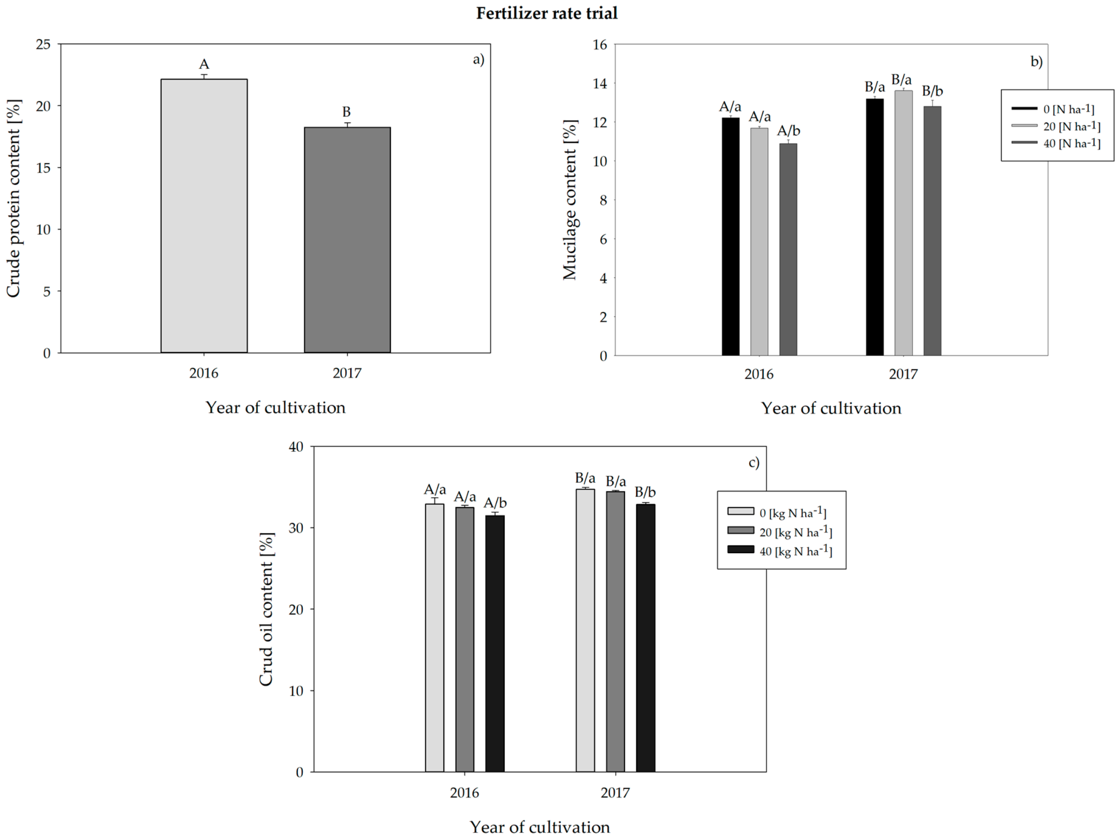

Figure 4. Mean estimates of crude protein (**a**), mucilage (**b**) and crude oil (**c**) content of the genotype G8 cultivated in 2016 and 2017 at Ihinger Hof at different fertilizer rates. Means at a constant level of fertilization rate that share a common capital letter do not differ significantly between years at α = 0.05. Means within a year that share a common lowercase letter do not differ significantly between fertilization levels at α = 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, α = 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.

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

Publication III

Grimes, S. J., Capezzone, F., Nkebiwe, P. M., & Graeff-Hönninger, S. (2020). Characterization and Evaluation of *Salvia hispanica* L. and *Salvia columbariae* Benth. Varieties for Their Cultivation in Southwestern Germany. *Agronomy*, *10*(12), 2012.

Previous chapters demonstrated that i) Salvia hispanica L. *cultivation is feasible under the climatic conditions in Central Europe as new genotypes, either adapted or insensitive to photoperiod, are available and ii) the given environmental conditions impacted the respective seed yields and nutrient composition more than the applied agronomic management practices. Within the following study so far not further described Salvia hispanica* L. *(SALV66) and Salvia columbaria*e Benth*. (Golden Chia) varieties were selected and evaluated over the course of four years, exhibiting necessary phenological growth traits, while simultaneously obtaining yield and quality traits being in line with current literature. To our knowledge, it is the first cultivation of both varieties in Central Europe. Chia in general represents a raw material of incredible interest for the food and pharmaceutical industry with its numerous possible applications. In addition, the demand for healthy superfoods cultivated ecologically friendly, sustainable, and regionally continues unabated in Europe, including Germany. Those aspects, amongst others, could lead to the fact that Central Europe could profit from chia cultivation not only economically, but also in terms of environmental and nutritional sustainability as it could broaden the local food base.*

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Article

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

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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 ω6:ω3 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 omage-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	*Salvia hispanica* L.	USA	University of Kentucky
Sahi Alba 914	*Salvia hispanica* L.	Argentina	Agustin Sorondo
G8	*Salvia hispanica* L.	USA	University of Kentucky
07015ARG	*Salvia hispanica* L.	Argentina	Genebank
06915ARG	*Salvia hispanica* L.	Argentina	Genebank
06815BOL	*Salvia hispanica* L.	Bolivia	Genebank
BOL	*Salvia hispanica* L.	Bolivia	Genebank
KOL	*Salvia hispanica* L.	Columbia	Genebank
Chia nigra	*Salvia hispanica* L.	Nicaragua	Genebank
SALV66	*Salvia hispanica* L.	NA	IPK Gatersleben
Golden Chia	*Salvia columbariae* Benth.	NA	IPK Gatersleben
UGA	*Salvia hispanica* L.	Uganda	Genebank
Sachia white	*Salvia hispanica* L.	Mexico	Genebank
Sachia black	*Salvia hispanica* 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).

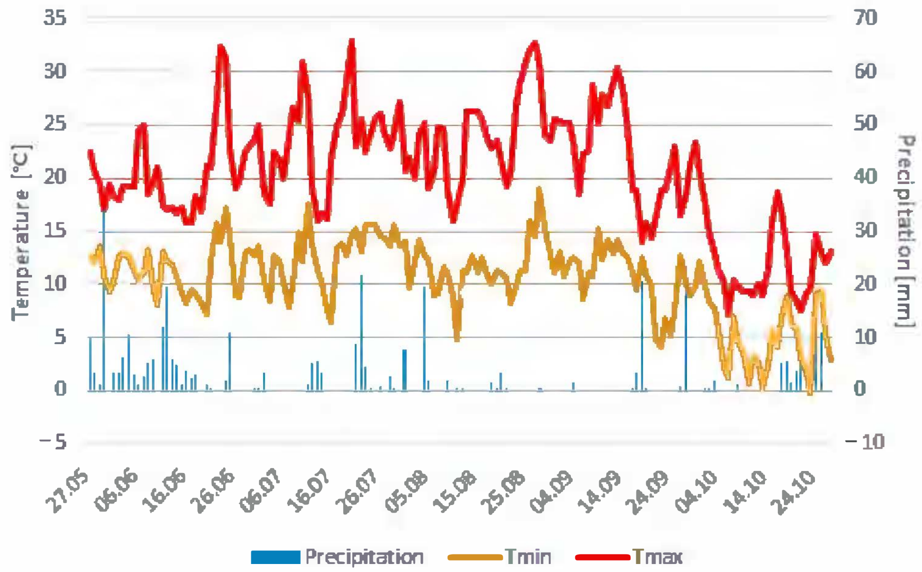

Figure 2. Precipitation (mm; bars) and minimum and maximum temperature (°C; lines) during the experimental period at Ihinger Hof in 2018 (May–October).

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 Machinen-Vertrieb GmbH, Schopsdorf, 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 H_2O 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 H_2O 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:

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

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 $p_{ij} \sim N\left(0, \sigma_p^2\right)$; and e_{ijk} are the residual errors with $e_{ijk} \sim N\left(0, \sigma_e^2\right)$. 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:

$$y_{ij} = \mu + b_j + \tau_i + \beta_1 k_{ij} + \beta_{2i} k_{ij} + e_{ij}, \tag{3}$$

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 $e_{ij} \sim N\left(0, k_{ij}^{-1}\,\sigma_e^2\right)$. 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/m^2, 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/m^2, 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/m^2; 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	Day Length	Harvest Maturity *			
	DAS [a]	Date	GDD [b]	Mean Temperature (°C) [c]	(Wh/m^2) [d]	(h) [e]	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 m^2 [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, α = 0.05, mean ± 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 ± 2.1 a	7.77 ± 0.3 a	7.98 ± 1.2 a	10.40 ± 0.7 a
Golden Chia	37.48 ± 2.3 b	2.08 ± 0.2 b	29.42 ± 4.9 b	* 2.76 ± 0.4 b
SALV66	78.60 ± 2.1 a	8.27 ± 0.2 a	15.97 ± 2.4 b	12.26 ± 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 α = 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, α = 0.05).

Factor	Seed Weight (g) Per Single Plant *p*-Value	Number of Seeds Per Single Plant *p*-Value	Yield (kg ha^{-1}) ‡ *p*-Value	TSM (g) ‡ *p*-Value
Variety	ns	ns	**	***
Plants per m^2	-	-	**	ns
Variety × 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].

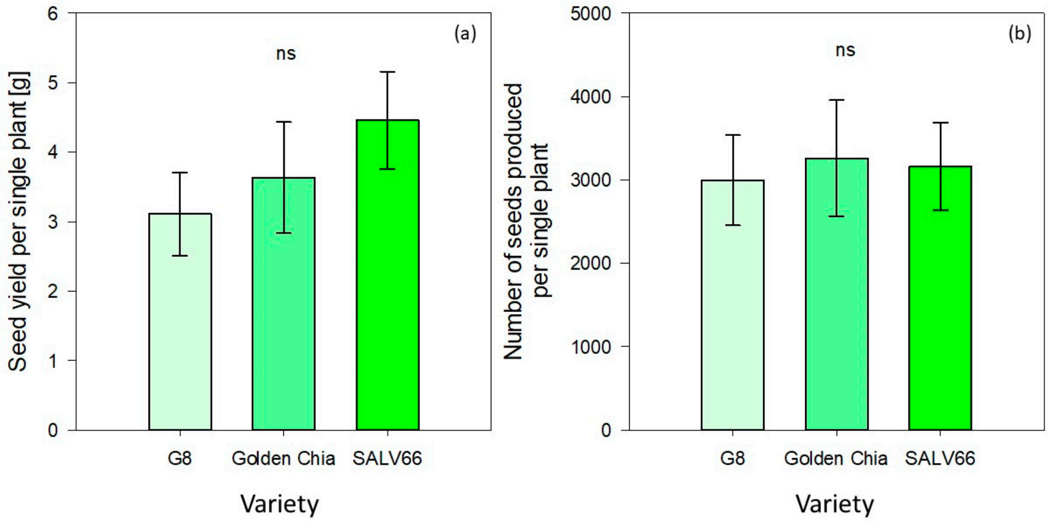

Figure 3. Seed yield (g per plant) (**a**) and number of seeds produced per single plant (**b**) of three chia varieties (G8, Golden Chia, SALV66) cultivated and manually harvested at Ihinger Hof in 2018. Means which share a common lowercase letter are not significant at $\alpha = 0.05$. ($n = 3$, $\alpha = 0.05$, means ± 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 couldbe 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 ± 74.44 kg ha^{-1}) compared to SALV66 (643.99 ± 42.92 kg ha^{-1}), whereas the obtained yield of G8 (491.91 ± 67.45 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].

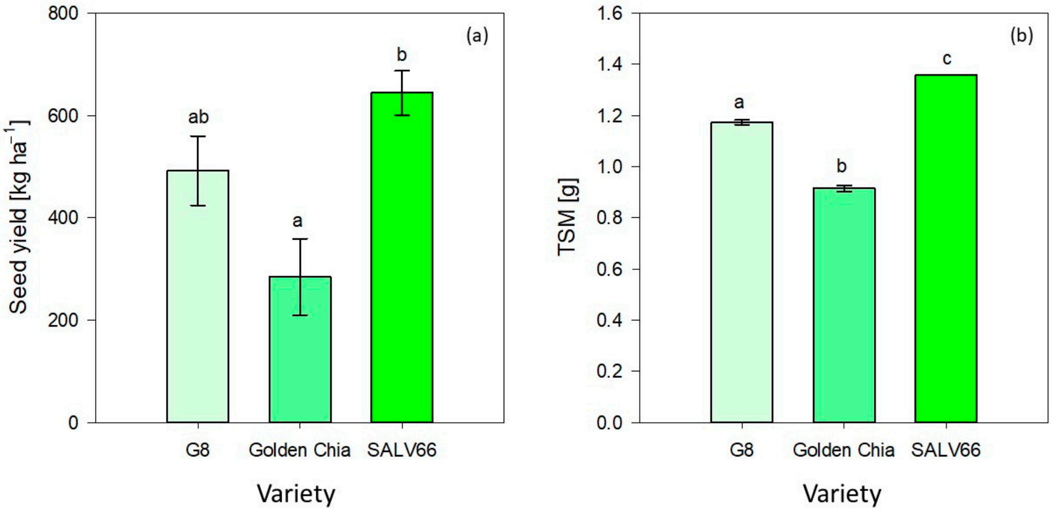

Figure 4. Seed yield (kg ha^{-1}). (**a**) and thousand seed mass (TSM) (g) (**b**) of the three *Salvia* varieties (G8, Golden Chia, and SALV66) cultivated at Ihinger Hof in 2018. Means which share a common lowercase letter are not significant at $\alpha = 0.05$. ($n = 3$, $\alpha = 0.05$, means ± standard 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 (752–1170 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 (0.1–1.3 °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 (3–5.5 °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 ± 0.9% (G8), 22.14 ± 1.1% (Golden Chia), and 24.46 ± 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 ± standard error).

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

Mean ± 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 ± 0.3% (G8), 20.66 ± 0.3% (Golden Chia), and 10.35 ± 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 ± 1.0% (G8) to 28.00 ± 0.6% (Golden Chia) and 31.73 ± 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 ± standard error).

	Palmitic Acid	Stearic Acid	Oleic Acid	Vaccenic Acid	Linoleic Acid ω6	α-Linolenic Acid ω3	PUFA:SFA	ω6: ω3
Varieties								
G8	7.4 ± 0.08 ^{a}	3.8 ± 0.05 ^{a}	7.3 ± 0.17 ^{b}	0.8 ± 0.01 ^{b}	20.2 ± 0.21 ^{a}	60.5 ± 0.49 ^{b}	7.2 ± 0.09 ^{b}	0.3 ± 0.01 ^{a}
Golden Chia	5.7 ± 0.10 ^{b}	3.2 ± 0.06 ^{b}	9.1 ± 0.22 ^{a}	0.9 ± 0.01 ^{a}	15.1 ± 0.27 ^{b}	65.9 ± 0.62 ^{a}	9.0 ± 0.12 ^{a}	0.2 ± 0.01 ^{b}
SALV66	7.7 ± 0.09 ^{a}	3.8 ± 0.06 ^{a}	6.9 ± 0.19 ^{b}	0.9 ± 0.01 ^{a}	20.4 ± 0.24 ^{a}	60.4 ± 0.56 ^{b}	7.1 ± 0.10 ^{b}	0.3 ± 0.01^a
Factor					*p*-values			
Varieties	***	**	***	**	***	**	***	***
Plants per m^2	ns	ns	ns	ns	ns	ns	ns	ns
Varieties × 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 ± SEM, ns = $p > 0.5$, ** $p \leq 0.01$, *** $p \leq 0.001$ Plants per m^2 as a covariate and weighting variable. Means which share a common lowercase letter are not significant at $\alpha = 0.05$.

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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6. General discussion

The main objective of this present study was to select chia species adapted to Central European climatic conditions to establish a local cultivation system for chia obtaining yield and quality traits comparable to its countries of origin. Publication Ⅰ, therefore, examined three early flowering *Salvia hispanica* L. genotypes (G8, Sahi Alba, W13.1) cultivated at two different experimental stations in southwestern Germany in order to observe plant development and determine agronomic and quality traits. Identifying the influence of agronomic practices on chia seed yield and quality traits was the purpose of Publication Ⅱ. The agronomic and qualitative responses to three different row spacings, sowing densities and, N-fertilization rates on agronomic and quality traits of an early flowering *Salvia hispanica* L. genotype (G8) was examined. Publication Ⅲ focused on the comparison of a newly selected *Salvia hispanica* L. (SALV66) and *Salvia columbariae* Benth. (Golden Chia) specie to the early flowering *Salvia hispanica* L. genotype (G8). Phenological, morphological, yield and quality traits were evaluated. The respective results of the individual publications were discussed in detail and therefore will not be repeated in this chapter. The general discussion will provide deeper insight and information beyond the results obtained from the publications. The aim is to highlight the potential and possible limitations of European chia cultivation itself as well as to assess the possible uses and applications of chia and its bioactive compounds.

6.1 Breeding and Selection

Introducing new crop species into proven cultivation systems is usually associated with problems that have to be solved. Regarding chia, the hindrance for its cultivability was its response to day length. It is adapted to tropical (0° - 23.5°) and subtropical (23.5° - 40°) regions, where short-day conditions prevail and corresponding day length (<12 h) and temperature requirements (16 to 26 °C) are met to induce flowering and to complete all development stages (Ayerza & Coates, 2009a; Köppen & Geiger, 1930).

As a first step to prove the cultivability of this short day flowering plant under climatic conditions at a latitude of 48 ° N, a selection of existing plant material was conducted through climate chamber trials in order to examine on a phenotypic level whether the tested strains differed from each other and how much variation within each strain exists. Through field trials conducted at Ihinger Hof and Eckartsweier morphological, agronomic, and quality traits were examined. Currently, underutilized grain crops like, quinoa, amaranth, and chia are not incorporated in high-input farming systems as there is a scarcity of targeted and coordinated breeding efforts aimed at their expansion (Bekkering & Tian, 2019)

To grow chia reliably and safely under local conditions, the following aspects should be further investigated. In the literature, chia is described as highly self-pollinating (Cahill, 2004). This enables the use of different breeding methods to obtain improved plant material as quickly as possible. However, it seems like there is not enough knowledge to select and optimize the optimal breeding method so far. At the State Plant Breeding Institute in Willstätt-Eckartsweier trials are currently conducted to make crosses of different chia origins, which proves to be very difficult as hardly any cross seeds were obtained. When examining the cross-pollination rate, it was observed that chia is not a pure self-pollinator. When white- and blue-flowered origins were closely grown, the progeny showed splits. Therefore, in the future, when developing genotypes, the plants should be bagged. This, however, represents the next obstacle. As bagged plants have a low selfing rate, seed harvest is very poor. It is yet unclear if this is due to the bags. Different materials have been tested in this regard, but none of them worked well. On the other hand, self-incompatibility genes might interfere with the selfing set itself. Palma-Rojas et al. (2017) in this regard observed little genetic variability for the ISSR loci of nine chia provenances analyzed, which they explained by “Selfing and reduced genetic background produced by genetic drift and human selection. This might partially explain the scarce genetic differentiation found among provenances”. Basic genetic information seems to be the key in order to improve and adapt chia to local conditions (Palma-Rojas et al., 2017).

According to Timothy D. Phillips (personal communication, June 19, 2018), mutation breeding attempts to induce random mutations in the hope of changing a single gene to effect a specific change in a specific trait. Most mutations are negative or are not productive for the trait of interest. Hence, it is a tedious process, used only when there is no other source to obtain the desired variation. Gamma irradiation and chemical mutagenesis (ethyl methanesulfonate, EMS) was used to develop the long day length flowering chia line G8. Even though gamma irradiation was found to be twice as effective as EMS in producing the desirable mutants, still less than 1 in 10000 M2 plants led to the desired mutants.

Besides that, Timothy D. Phillips (personal communication, June 19, 2018) mentioned that ploidy could contribute to a genetic advance in chia crop traits. The number of chia chromosomes is doubled (autotetraploidy) from 12 pairs to 24 pairs of chromosomes (diploid chia: $2n=2x=24$ chromosomes, tetraploid chia: $4n=48$) via colchicine treatments. It was assumed that the tetraploid chia lines are highly fertile, develop stouter plants, 30 to 50% larger seeds, and have an altered seed composition compared to the diploid chia lines. The high fertility and the altered seed composition was not observed yet, while first trials in Germany developed stouter plants and to a certain percentage larger seeds, which resulted in a relatively high mucilage content compared to the diploid chia lines (Grimes, Phillips, Claupein, et al., 2018). Regarding the highly selfing characteristic of chia Timothy D. Phillips (personal communication, June 19, 2018), called attention to the fact that inbreeding depression is probably low, which could contribute to a better seed set and seed fertility of diploids and tetraploids crosses.

Throughout the course of this thesis, a selection and evaluation of chia genotypes unknown to Europe has been conducted. One genotype was undergoing a variety testing during the last three years as first results have been promising. Compared to the first released genotype in Europe, namely Oruro (PanamCie, France) maturity begins much earlier (Gravé et al., 2019). Meanwhile, the variety finally got approved. In the future, agricultural practice could benefit from it, since the seeds will be made available to farmers. Further breeding approaches should focus on early and homogeneous ripening in order to incorporate chia more easily into a local crop rotation. Early ripening under prevailing long day conditions represents an extremely important part of the ability to grow chia under local conditions, as it helps to determine harvest maturity. This is essential under the given climate conditions in Central Europe which are increasingly characterized by continental conditions from west to east (Breitenbach et al., 2018). The earlier onset of winter is critical, as chia is highly frost intolerant. At the same time, the hotter summers of the continental climates of Central Europe could lead to faster seed maturation. Nevertheless, in the course of the above, breeding efforts should include the overall frost tolerance with regard to early and late frosts.

Additionally, there should be a very strong focus on the approach of non-shattering genotypes. Seed shattering itself as Dong & Wang (2015) stated, is essential for the propagation of offspring in wild plants while being a main factor causing yield losses in crops. Therefore, the elimination of this seed dispersal trait harbors enormous yield maximizing potential (Lv et al., 2018; Okubo, 2014). Trials comparing hand and machine harvested yields demonstrated that seed losses of *Salvia hispanica* L. genotypes were up to 44.7 % (Coates & Ayerza, 1996). In this regard, Cahill & Provance (2002) reported that there was a complete dominance of open calyx over closed calyx in F1generations of *S. hispanica*. Furthermore, it was stated that several genes must be involved in the closing of the calyxes (Cahill & Provance, 2002). Especially regarding Golden Chia (*Salvia columbariae* Benth.), which proved to be worth cultivating under the given climate conditions, overcoming this obstacle would be highly beneficial (Ayerza & Coates, 2007; Grimes et al., 2020).

In general, however, the breeding and selection of new genotypes should be based on the possible fields of application. In case of a potential application in the packaging industry for example the goal would be the selection of genotypes with high mucilage contents, whereas for an application in the cosmetics industry a selection of higher oil contents could be of more importance.

6.2 The potential of a regional "super food" cultivation: The case of chia

As Arenas-Jal et al. (2020) stated "global concerns are shaping consumer attitudes". The Nielsen Company (2018) in this regard, stated that consumer's "shift towards healthy, plant-based, sustainable, and socially conscious food purchases is enormously influenced by global issues such as climate change, global population aging, child exploitation, food waste, unfair trade or animal abuse and others". Food production on the other hand is especially influenced by global dynamics, culture, socioeconomics, demographics, politics, and environment according to Arenas-Jal et al. (2020). With unprecedented access to information and corresponding consumer power, the food industry is forced to be responsive to the demands and preferences of consumers (Arenas-Jal et al., 2020). With a variety approval in place and further genotypes which, under Central European conditions, go through all necessary development cycles and obtain appropriate yield and quality traits, chia represents a potential new crop for temperate regions enabling a local food chain with its products able to meet consumer demands.

6.2.1 Agronomic management practices

The cultivation of chia in Germany would hold many advantages. One is the fact that existing agronomic management practices can be adopted. Sowing can be done with existing technology, the same applies to weed control and harvesting technology. Referring to harvesting it should be noted, that the combination of a not properly adjusted combine and/or its inappropriate operation, with a highly seed shattering genotype could nevertheless lead to severe seed loss (Coates & Ayerza, 1996). More diversified farming systems and wider crop rotations harbor great potentials as they can contribute to reduced risks from climate-change challenges and environmental degradation while simultaneously ensuring stable yields and therefore food for a steadily increasing population (Bowles et al., 2020; Klein, 2018). It might not give the maximum yield, but in return, the plants perform reliably (Klein, 2018). The classic three-field crop rotation involves soil-improving crops such as clover grass or field beans for example, highly nutrient demanding crops such as wheat or corn, and less demanding, ablative crop such as triticale, rye, oats, spring barley (Klein, 2018). With its low N demand chia could be cultivated after a demanding crop in a conventional crop rotation, and would contribute to the widening of existent crop rotations as in recent years a sharp decline in crop diversity, and the dominance of few insect-unfriendly species (wheat, barley, and corn) can be observed (Butz, n.d.). It as well offers great potential for organic cultivation as its agricultural and environmental requirements are generally low (Jamboonsri et al., 2012). Chia cultivation in general might therefore lead to the increase in overall agricultural productivity, and to the safety of the food base whiles adapting to the effects of climatic change (Graeff-Hönninger & Khajehei, 2019).

Another factor advocating the cultivation of *Salvia* species, which could be observed, is the fact that its blossoms exerted a highly attractive effect on insects of all kinds. Chia provides food for pollinators at times when almost nothing else is blooming on the field besides white mustard (*Sinapis alba* L.), oil radish (*Raphanus sativus* var. *oleiformis* Pers.), Phacelia (*Phacelia tanacetifolia* Benth.), or buckwheat (*Fagopyrum esculentum* Moench). This is of particular interest in terms of the worldwide discussion about biodiversity loss as food production and consumption has been identified to be among the major drivers in this regard (Chaudhary & Kastner, 2016; Crenna et al., 2019; Hallmann et al., 2017; Notarnicola et al., 2017). Corresponding biodiversity action plans are implemented globally. The EU Commission implemented a biodiversity Strategy for 2030 (European Commission, 2020). While the federal German government for example intends to comprehensively combat the decline in insect population through the Insect Conservation Action Program and thus actively contribute to the preservation of biodiversity (Bundesministerium für Umwelt Naturschutz und nukleare Sicherheit, 2018). One of the goals is to achieve a binding insect protection law including changes in plant protection-, nature conservation-, water- and fertilizer laws, the protection and restoration of insect habitats in all landscapes, and distinct specifications for environmentally and ecologically compatible pesticide applications (Bundesministerium für Umwelt Naturschutz und nukleare Sicherheit, 2018).

Nevertheless, the meaningfulness of cultivating new plant cultures is regularly discussed and questioned. In view of *Salvia hispanica* L. becoming a potentially invasive species this discussion is certainly not to be neglected but should be considered with a conscience as risk assessments conducted did neglect environmental limitations like (in)capacity to produce seeds and frost tolerance. (Z. Kaplan et al., 2018; Šilc et al., 2020; Verloove & Aymerich, 2020). Besides that risk of invasiveness, it is, especially in regard to chia, often argued that there are native species like flax (*Linum usitatissimum* L.) with a similar nutrient profile. (Saphier et al., 2017) in this regard concluded that chia seeds are richer in polyphenols than flax seeds by approximately 42%. Additionally, chia seeds pose one major advantage. Its seeds are neutral in taste, which makes them versatile (Ho et al., 2013). Flax seeds on the other hand have a pronounced nutty intrinsic flavor and must be ground to release their nutrients, but chia seeds do not (Nitrayová et al., 2014). All in all, each additional crop offers a contribution to more diversity in today's cereal and rapeseed-dominated agriculture (Jäkel, 2016). However, as in any other industry, demand determines supply. For this reason, the next chapters deal with ecological potentials, socioeconomic issues, and potential applications of chia.

6.2.2 Chia and its ecological potential versus socioeconomic issues

Adapting agriculture to the negative impacts of global climate changes today and in the future poses a tremendous challenge (Klocker et al., 2018). The occurrence of extreme weather events in connection with rising temperatures (heat waves, droughts) and increased precipitation (storms and floods) is on the rise (Agovino et al., 2019). In connection with the dependency on a shrinking list of crops, such as wheat, rice, and maize it has major consequences for human nutrition and global food security (Khoury et al., 2014).

Under European, more narrowly focused German conditions the frequent occurrence of dry periods with high temperatures increased during the last decades (Ministry of the Environment Climate Protection and the Energy Sector, 2012). In this regard, trials with pseudocereals like quinoa (*Chenopodium quinoa* Willd.), amaranth (*Amaranthus* sp.), and chia (*Salvia hispanica* L.) being highly adaptable to different pedoclimatic conditions were studied in the last decade (Gimplinger & Kaul, 2009; Grimes et al., 2020; Herman et al., 2016; Präger et al., 2018; Roitner-Schobesberger & Kaul, 2013). Chia represents an alternative crop in terms of food security and climate change (Herman et al., 2016). In addition to the previously mentioned general health aspects associated with chia consumption and nutritional trends, consumers increasingly value local and regional food products and buy them in high proportions (Fernández-Ferrína et al., 2018). In the context of fulfilling the increasing demand, and working towards a sustainable superfood industry local cultivation of chia under the given environmental conditions of Germany could lead to a decrease of GHG emissions and better CO_2 footprint related to the omission of transportation and packaging (Mordor Intelligence, 2019; Vermeulen et al., 2012). Further, food safety issues like pesticide residues and contamination (toxins, microorganisms) would no longer play any role for importing countries, among others Germany as the largest chia seed consuming and importing country in 2016 worldwide (Centre for the Promotion of Imports from developing countries, 2021b, 2021a; Melo et al., 2019; Mordor

Intelligence, 2019; Vermeulen et al., 2012). However, a local cultivation outside its countries of origin is a double-edged sword.

The shift from traditional cultivation practices to intensive agricultural production practices to meet the growing demand is leading to implications for food security, local livelihoods, and the environment in the native growing countries (Campbell et al., 2018; Magrach & Sanz, 2020). Overall, the demand of predominately outside of its centers of origin consumed "superfoods" has different environmental (water depletion, soil degradation decrease in biodiversity, and increasing land conversion within natural habitats) and social effects (plunge in prices, neglecting the local production of food for the population) in the countries of origin as a large area of agricultural land is used for the cultivation of chia purely for export (Jacobsen, 2011; Magrach & Sanz, 2020). Short-term gains of large corporations will lead to boom-and-bust cycles eroding local food systems which commonly were healthy, sustainable, and socially just (Gonzalez, 2011; Magrach & Sanz, 2020).

Due to the intensive monoculture cultivation of chia, a decrease in productivity and genetic variability might potentially lead to a loss of their ascribed characteristics (Magrach & Ghazoul, 2015; Magrach & Sanz, 2020). In terms of environmental and social impacts of superfood production and consumption in general, questions arise whether foods branded as "superfoods" reinforce concepts of cultural diversity and promote paradigms of worldwide social inequality (Loyer, 2014; Magrach & Sanz, 2020). In this regard, attention should be drawn to the increased consumer awareness considering the environmental impacts of their food choices coupled with stronger regulations on marketing and distribution. This could lead, among other things, to the fulfillment of the diverse nutritional needs of a steadily growing population while maintaining biodiversity (Magrach & Sanz, 2020). An overall sustainable food production in the countries of origins will only be achieved if active changes in production practices, consumer diets and governance will take place (Charles et al., 2014; Magrach & Sanz, 2020).

6.2.3 Chia and its economic potential

The global market demand for so-called super foods which are linked to health-promoting properties is highly dynamic (Salanță, 2020). According to statistics, the global superfood market will register a CAGR of almost 17% by 2023 and 22.3% by 2025 (Grand View Research, 2019; Technavio, 2019). In 2018, the largest market for chia was Europe with Germany holding its most significant market share (Grand View Research, 2019; Mordor Intelligence, 2020). Prices for chia seeds stabilized (conventional: 1.8 - 2.2 euros/kg, organic: 2.5 - 3.0 euros/kg after the "chia boom" in 2013, which led to prices of up to 6 - 9 euros per kilo (Centre for the Promotion of Imports from developing countries, 2021a). In this regard, it should be pointed out that chia seed retail margins are enormous. Depending on the distribution channel and product, chia seeds are sold for 4.5 - 6 euros/kg (bulk packages/online shops) and up to 20 euros/kg (organic brands/specialized stores) (Centre for the Promotion of Imports from developing countries, 2021a).

The application of chia seeds in the food industry is plenty fold. Several studies reported that chia seeds, their flour, oil, and mucilage can be used as substitutes for hydrocolloids, eggs, wheat flour, and as emulsifiers and stabilizers in a large variety of bakery products including pasta, bread, and cake as well as in ice cream, and sausages (Campos et al., 2016; Gallo et al., 2020; Menga et al., 2017; Sayed-Ahmad et al., 2018; Švec et al., 2016). The incorporation of chia in foods potentially improves the nutritional, bio-intelligent values regarding general health and well-being, nutrition, and consumption, which could contribute to the prevention of civilization-related-diseases, like diabetes, blood pressure, and asthma (Graeff-Hönninger & Khajehei, 2019; Grand View Research, 2019). Consumer awareness linking physical health with healthy diets possibly represents the most prominent factor driving the product demand and therefore food market growth (Figure 3) (Grand View Research, 2019; Statista GmbH, 2016).

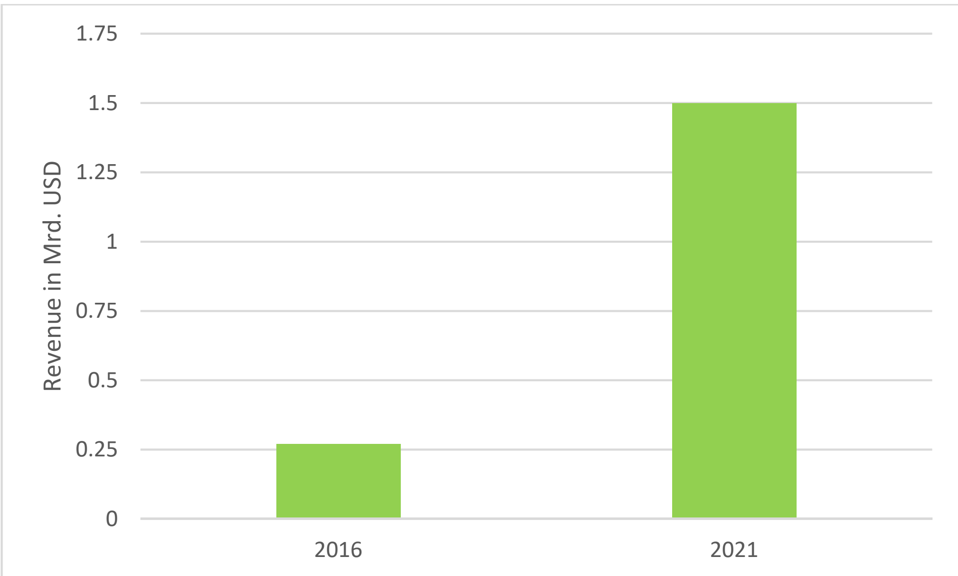

Figure 3 Forecast of global revenue from chia (*Salvia hispanica* L.) seeds by 2021 amended according to Statista GmbH (2016).

Inspiring food technologies in connection with suitable raw materials could potentially influence, lead and form the food sector of the future (Graeff-Hönninger & Khajehei, 2019). Especially regarding sports nutrition, and healthy, convenient, ready-made products, there is a huge economic potential which is already discovered and implemented by major brand companies like Nestlé, Danone, and Kellogg's (Centre for the Promotion of Imports from developing countries, 2021b).

Therefore, a possible processing flow for chia seeds is illustrated in Figure 4. Different sequential extraction methods could lead to a holistic, diverse, and economically sound product manufacturing of reduced-, fat-free -, and high protein chia flour, chia protein, chia oil, and fiber. The fiber could be used to produce packaging material as stable as cardboard by fiber casting, representing one of the possible multipurpose uses of chia (BIO-LUTIONS International AG, 2019).

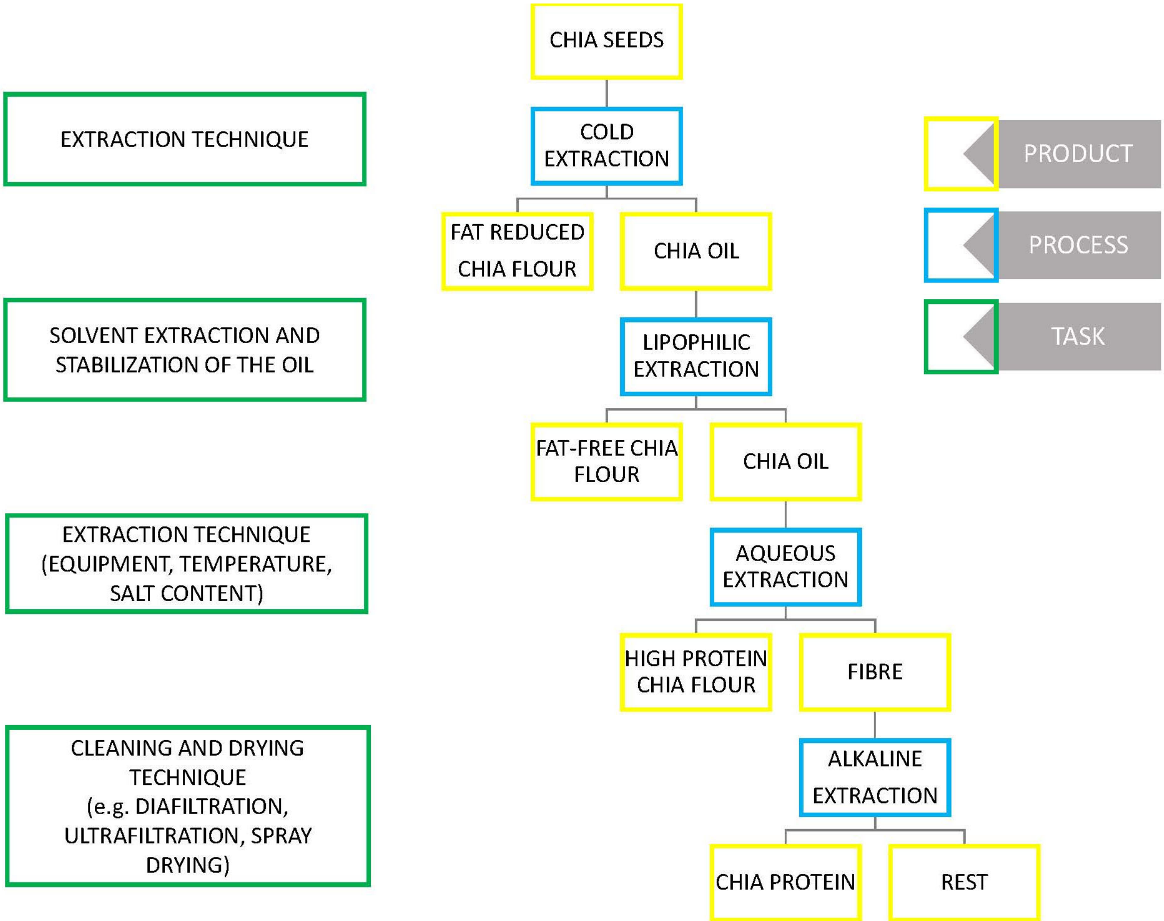

Figure 4 Possible processing flow for chia (*Salvia hispanica* L.) seeds amended according to © AcanChia UG & Co. KG, Kümmersbruck, Germany (personal communication, February 2, 2017).

The points mentioned in the previous paragraphs led to the impulse to establish a regional, low input production of chia, as a possible health-promoting so-called "superfood" under Central European climate conditions. From "superfood" to "local food" offers the opportunity to meet consumers ecological, regional, and health consciousness while potentially strengthening regional market growth as it harbors increasing economic and business opportunities along the value chain (Figure 5) (Aschemann-Witzel et al., 2020; Centre for the Promotion of Imports from developing countries, 2021a; Grand View Research, 2019).

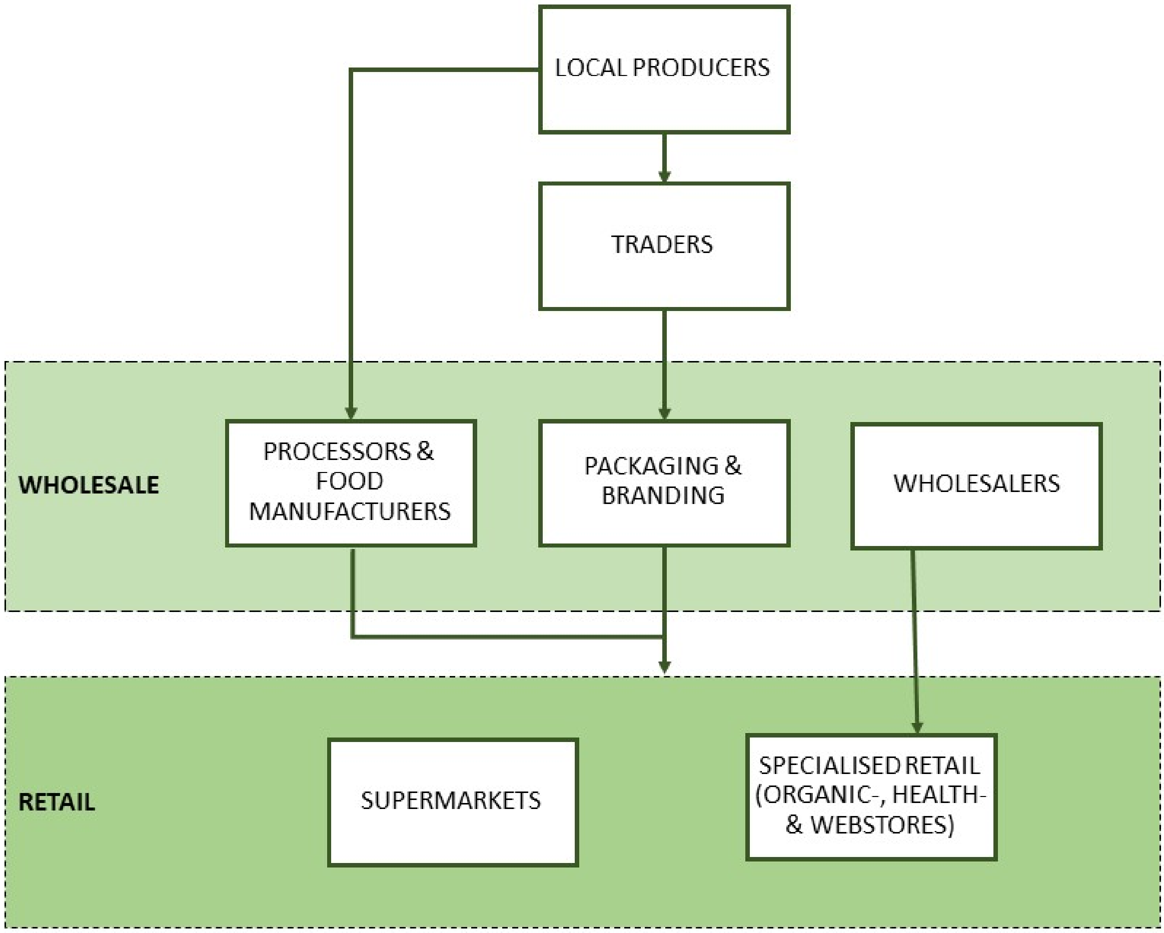

Figure 5 Market channels for chia (*Salvia hispanica* L.) seeds amended according to the Centre for the Promotion of Imports from developing countries (2021a).

6.3 Potential usage and applications of chia: Present and future

Consumers worldwide are adopting to a more and more plant-based diet as it is perceived as healthy, ethical, and environmentally friendly (Angus & Westbrook, 2018; Aschemann-Witzel et al., 2020). This lifestyle trend shows the necessity to rethink and transform food use and distribution as consumers are highly aware of the environmental and climatic impacts due to intensive agricultural production, associated with livestock farming and animal-based foods (Aschemann-Witzel et al., 2020; Keating et al., 2014; Reisch et al., 2013; Röös et al., 2017).

The entire chia plant including its seeds represents a valuable raw material as its technological and health-promoting properties have excellent future prospects in food, feed, pharmaceutical, medical, and neutraceutical industries (Kaur & Bains, 2019; Kulczyński et al., 2019; Ullah et al., 2015).

6.3.1 Chia protein

Angus & Westbrook (2019) and Aschemann-Witzel et al. (2020) reported consumers' food choices for plant-based foods, plant-based alternative proteins as well as food market innovations, pointing into this consumer trend, led to a double-digit percentage growth from 2017 to 2018 in dairy and meat substitutes alone. Poore & Nemecek (2018) in this regard stated that animal food production and the production of animal products effects on greenhouse gas emissions, water use, and land use "despite providing only 37% of our protein and 18% of our calories". Apparently, the demand for plant-based protein products being free-from and being produced from the point of sustainability and animal welfare are unique selling points leading to dominant sales globally (Schmidt, 2014). Protein is the preferred food, which has become increasingly attractive to consumers over the last ten years (Arenas-Jal et al., 2020; Bergström et al., 2020; Schmidt, 2014).

Besides proteins being a nutrient component of food they are also fulfilling physiochemical functions which promote health as peptide sequences that are encrypted in the parent protein lead to the physiological activities of proteins (Banan-Mwine Daliri et al., 2017; Rizzello et al., 2016).

Grancieri et al. (2019) evaluated the composition of chia seeds as well as the effects of their proteins, and peptides on the human body and concluded that "chia seeds (*Salvia hispanica* L.) and their peptide sequences have auspicious biological potentials […], however the mechanisms of action contributing to the observed health benefits need to be further investigated". Several studies indicated for example that antioxidant capacity, more precisely, the in vitro inhibition of the NF-κB transcription factor, reduced the inflammatory and carcinogenic processes (Aggarwal & Shishodia, 2006; Ellulu, 2017; Rahman et al., 2006). This, according to Hou et al. (2003), Marcinek & Krejpcio (2017), and Parejo et al. (2002), could protect the organism from pathologies like neurological diseases, Alzheimer's and Parkinson's diseases, ischemic heart disease, immunodeficiency, strokes, inflammation, and cancer. Furthermore, it has been demonstrated that chia proteins and their bioactive peptides are capable to inhibit cholesterol homeostasis (Coelho et al., 2018). While Hernández-Pérez et al. (2020) showed that 1) globulins represent the major protein fraction followed by albumins, glutelins, and prolamins and 2) peptides from globulin and albumin fractions harbor the greatest potential against angiotensin-converting enzymes. Hence, the mentioned characteristics validate the possible use of peptides generated from chia as potentially health-improving nutraceuticals for pharmaceutical and food applications (Meisel, 1997; Muñoz et al., 2013).

Besides the above mentioned possible health-promoting features for the human body the antimicrobial properties of the bioactive peptides derived from chia seed proteins via microwave, leading to improved bioactivity and functionality, and enzymatic hydrolysis accommodate potential in the prevention of food spoilage and food-borne illnesses and could therefore be possibly used as antimicrobial agents in the food industry and various therapeutic applications (Aguilar-Toalá et al., 2020; Aguilar-Toalá & Liceaga, 2020; Urbizo-Reyes et al., 2019).

Moreover, chia proteins could also be a valuable ingredient for the cosmetic industry. A study conducted by Aguilar-Toalá & Liceaga (2020) implied that ”chia seed peptides possess amino acids that participate during the enzymatic inhibitory, which could improve skin health as aging-related enzymes such as elastase might contribute to the degradation of the protein matrix of the skin”. Additionally, data obtained by Bilalis et al. (2016) indicated that chia could be used as a feasible forage crop for lactating ruminants due to high acid and neutral detergent fiber contents as its milk fat content positively relates with their percentages.

6.3.2 Chia mucilage

According to Brütsch et al. (2019) and Coorey et al. (2014), chia seed mucilage is functionally and nutritionally an extremely auspicious ingredient regarding the pharmaceutical and food industry as it bears great potential in the development of food products as emulsifier, thickener, and stabilizer while being non-toxic, biodegradable, and digestible. Studies specifically examining the rheological properties of chia mucilage came to the same conclusion (Campos et al., 2016; Capitani et al., 2015; Cuomo, Iacovino, Cinelli, et al., 2020; Cuomo, Iacovino, Messia, et al., 2020).

It was shown that the viscosity of chia mucilage emulsified with lemon grass essential oil remained constant as the anti-oxidative properties of the essential oil prevented short-term polymer degradation of the chia mucilage mixture leading to enhanced colloidal stability (Cuomo, Iacovino, Cinelli, et al., 2020; Cuomo, Iacovino, Messia, et al., 2020). Chia mucilage could therefore be used as a base for a series of innovative plant-based foods like meat and dairy alternatives as they harbor enormous economic potential in the long term (Aschemann-Witzel et al., 2020; Campos et al., 2016; Pintado, 2019).

The tremendous adhesion of the mucilage layer to the seed, which is produced when in contact with water, represents an enormous disadvantage as it necessitates complex extraction methods (Brütsch et al., 2019). Its mucilage needs to be separated by e.g. hydration, freeze-drying, rubbing off the gel, ultrasonic removal, and purification (Capitani et al., 2013, 2015; Castejón et al., 2017; Goh et al., 2016; Urbizo-Reyes et al., 2019). Establishing a method that makes the extraction economically viable would bring a great advantage to the food business sector (Aschemann-Witzel et al., 2020).

Regarding the most effective and productive extraction method, the temperature, the extraction duration, and the water: seed ratio, among others, must be taken into account. Here further research approaches arise which should be investigated in the future. Especially with regard to the use of chia mucilage as a plastic and packaging material, this is of enormous interest.

Plastic and packaging materials have become indispensable components of everyday life increasingly replacing paper, board, or cardboard packaging, therefore, becoming the largest use of plastic in Europe (Figure 6) (Chidambarampadmavathy et al., 2017; NABU, 2017; PlasticsEurope Deutschland e.V., 2019). The impact of non-biodegradable packaging materials on the environment and the consequences resulting from it are of alarming concern (Chidambarampadmavathy et al., 2017).

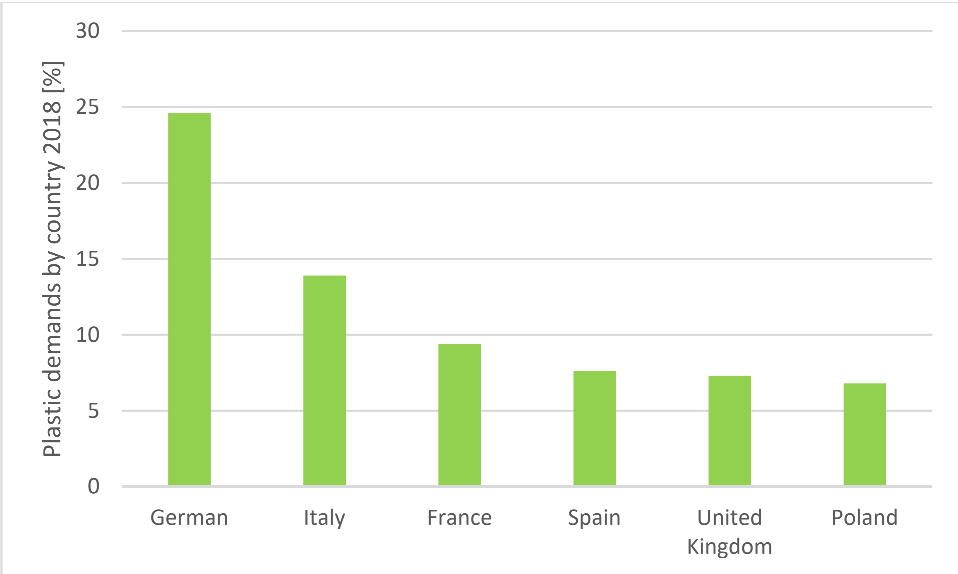

Figure 6 The six largest European countries (in terms of population) and the Benelux cover almost 80% of the European demand. Amended according to PlasticsEurope Deutschland e.V. (2019).

The demands for solutions leave politics, trade, and research no other choice, but to develop alternative packaging materials or coating options. Bioplastics made of organic material such as sugar, starch, or cellulose seemed to be the solution to the plastics and packaging problem (Haas, 2017). This resulted in a highly competitive market with double-digit growth rates around the world (Haas, 2017). Bioplastics sales were growing rapidly, but so far in Germany, as almost everywhere else in the world, they are still only at just under 2 percent (Haas, 2017). However, as the term is not clearly defined, much of this turned out to be greenwashing, as the production of these bioplastics consumes a lot of chemicals and water, and the natural materials could only be processed with plasticizers (Haas, 2017). Bioplastics can either be bio-based, biodegradable, or both. The methods of production are independent of the definition. Hence, one of the new research approaches resulting amongst others from an increased consumer's environmental consciousness and sensibility towards the global environmental hazards (climate change, micro plastic problems) of fossil-based and non-biodegradable plastics increased during the last decades, led to the exploration of chia proteins and mucilage usage as biodegradable films (Angus & Westbrook, 2019; Asgher et al., 2020; Capitani et al., 2015; Evans et al., 2020; Salazar Vega et al., 2020; Vasara et al., 2019; White & Lockyer, 2020).

Studies affirmed that chia seed mucilage could be used as a polymer blend for edible and functional films and coating, as wall material for nano-encapsulation, and as biodegradable/compostable alternatives to conventional plastics like cups, bottles, trays, carrier bags, etc (Campos et al., 2016; Cazón et al., 2017; I. S. V. da Silva et al., 2018). In this regard, Venkatesh et al., (2018) pointed to the fact that in food packaging barrier coating serves primarily the purpose of shelf life extension of the food contained within the packaging, color, odor, taste, and quality preservation, in an attempt to reduce food waste.

Plastic is everywhere. Therefore, further areas of applications seem possible, for example in cosmetic and technology industries (Asgher et al., 2020; Capitani et al., 2016; de Campo et al., 2017; Hrnčič et al., 2020; Ullah et al., 2015). Chia hence holds enormous potential in this application range and the resulting evolving market meeting consumers' ever-increasing demands for plastic alternatives (Spierling et al., 2018).

The selection of genotypes with high mucilage contents can be defined as a first goal. Besides, a process has to be developed to produce a packaging or coating material, based on the mucilage. In this respect, special attention must be paid to the extraction possibilities of the mucilage. Our experiments showed, that the extraction of unprocessed seeds was more advantageous for the processing than the extraction of ground seeds, which, however, had a higher mucilage content (surface enlargement). When talking about a natural, biological, and environmentally friendly packaging product, the solvents used for extraction should also be tested for compatibility or new approaches, and new products should be tested and evaluated for these components. Also, the evaluation of possible mixing partners meeting those requirements could possibly contribute to the improvement of the final product. The compatibility with food is indispensable. Nothing is more undesirable than the migration of components of the packaging material/packaging to the product (AlFiPa GmbH & Co. KG, n.d.). Depending on the application of the new packaging or coating material, the mechanical, thermal, optical, and chemical requirements need to be tested (AlFiPa GmbH & Co. KG, n.d.). The economic potential/added value resulting from the worldwide demand for environmentally friendly manufactured and degradable plastic alternatives (industry/consumers) must be clearly emphasized.

A further application of chia mucilage is related to soil improvement. Di Marsico et al. (2018) came to the conclusion that “mucilage extracted from chia seeds, exerts a dose-dependent significant increase of soil aggregate stability, which persists in time after 30 days from application” via 13C– Cross Polarization/Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy and Scanning Electron Microscope images of the diaspora soil surface and of mucilage enriched soil. Results of Di Marsico et al. (2018) further indicated that chia seed exudates could lead to an improvement of soil structure and an enhancement of herbicides sorption. The reduction of their mobility - herbicides and non-desired pollutants – could therefore contribute to an overall decrease in environmental pollution (Di Marisco et al., 2018). It is however explicitly pointed out that the application of organic materials to soils needs to be thoroughly tested as mass balance and efficiency of amendments is strongly dependent on soil quality and type (Di Marisco et al., 2018).

Moreover, the structural polysaccharide xyloglucan, which can be isolated from chia seed mucilage, was examined regarding its promising applications in pharmaceuticals and biotechnology as it might be used as a base for hydrogel wound dressings (Brun-Graeppi et al., 2010; De Souza et al., 2013). Xyloglucan is able to form gels, biocompatible, adhesive, non-carcinogenic, non-irritating, non-toxic, non-mutagenic, and compatible with blood (Arruda et al., 2015; Mahajan & Mahajan, 2016; Martínez-Ibarra et al., 2018, 2019; Simi & Abraham, 2010; Yan et al., 2016). Respective results indicated that the incorporation of plant-derived polymers such as xyloglucan enhance the chitosan properties (non-toxicity, biodegradability, bioadhensivity, hemostasis, and antigenic capacity) while leaving its antimicrobial capacity unaffected (Martínez-Ibarra et al., 2018, 2019). Thus, biomaterials and mixtures of chitosan and xyloglucan represent propitious applicability for the creation of hydrogel for wound dressings as they offer a considerable level of dehydration to improve its re-epithelialize ability in addition to bestowing infection-causing antimicrobial capacity of the majority of pathogenic microorganisms for medical applications (Martínez-Ibarra et al., 2018, 2019). Additionally, it could be confirmed that the xyloglucan generally improves mechanical durability and liquid absorption properties of hydrogels obtained by chitosan (Martínez-Ibarra et al., 2018).

Another study recently tested the suppression effect of chia mucilage (*Salvia hispanica* L.) regarding iron ore particulate matter transported by railways (de Almeida Gama et al., 2020). Its ability to bind fine dust particles, which represents a promising application in the mining sector and thus could help to prevent diseases caused by these particles, was demonstrated (de Almeida Gama et al., 2020). The areas of application of chia mucilage are manifold and diverse. However, further research needs to be conducted in view of the mentioned topics.

6.3.3 Chia oil

According to Taga et al. (1984), chia seeds have an oil content of 25% to 35%. The high level of polyunsaturated fatty acids present in chia seeds, mainly α-linolenic acid (ω-3 fatty acid), is due to eight proteins which are specially related to the production of the plant lipids (Amato et al., 2015; Bochicchio, Phillips, et al., 2015; Cruz-Tirado et al., 2021; Grancieri et al., 2019). Particularly the high proportion of α-linolenic acid (>60 %) in addition to high proportions of fiber and polyphenolic compounds, which have been found to impact human health positively, led to chia being considered a functional food (Ullah et al., 2015). Among others, ω-3 fatty acids can lead to a decrease of glycerides and cholesterol levels, it acts cardioprotective, antidiabetic, anti-inflammatory, and hepatoprotective (Ali et al., 2012; de Falco et al., 2018; Grancieri et al., 2019; Ullah et al., 2015). Also, it is assumed that it protects the human body against arthritis, autoimmune disease, and cancer (Ali et al., 2012; de Falco et al., 2018; Grancieri et al., 2019; Ullah et al., 2015). While, ω-6 fatty acids also act anti-hypertensive, anti-thrombotic, anti-inflammatory, and anticancerogen (Ali et al., 2012; de Falco et al., 2018; Grancieri et al., 2019; Ullah et al., 2015). According to Hrnčič et al. (2020), chia oil is one of the most valuable oils on the market nowadays. Because of the high levels of PUFAs, it is of extraordinary interest as a food ingredient for the food industry, especially in view of the sports foods and nutrition trends (Arenas-Jal et al., 2020; Imran et al., 2016). Therefore, to protect the essential fatty acids of extracted chia oil, Imran et al. (2016) stated that innovative technologies like antioxidants, adequate preparation, refining, or packaging are needed. In this regard, Bordón et al. (2019) drew attention to vegetable oil blending, as a common practice in the edible oil industry. Edible oil blends improve oxidative stability, enhance the presence of fatty acids and therefore the nutritional properties and sensory characteristics while leading to cost savings. It was concluded that chia oil seed blends present an innovative option for the food industry, currently interested in obtaining non-conventional oils and providing novel food products to potentially enhance health and human nutrition (Bordón et al., 2019). As different extraction methods and solvents are causing variations in extractable chia seed oil yields, quality, content of fatty acids, dietary fibers, and antioxidant content, a state of the art overview of these quality traits of produced chia oils, along with extraction methods was given (Hrnčič et al., 2020; Ishak, Ghani, & Nasri, 2020). Demonstrating chia oilseed yield varied from 7.2 % (Subcritical fluid extraction with CO_2 as a solvent) to 90.3 % (Subcritical fluid extraction, with Ethanol as a solvent), while ω-6 fatty acids ranged from 2.97 % (Screw pressing and Soxhlet extraction with n-hexane as a solvent) to 35.2 % (Subcritical fluid extraction with CO_2 as a solvent) and ω-3 fatty acids varied between 3.5 % (Screw pressing with n-hexane as a solvent) and 69.3 %

(Soxhlet extraction with n-hexane as a solvent and pressing) (Ixtaina et al., 2010; Ixtaina, Martínez, et al., 2011; Ixtaina, Mattea, et al., 2011; Uribe et al., 2011; Villanueva-Bermejo et al., 2019). Results of a recent study conducted by Ishak, Ghani, & Yuen (2020) found that chia seed oil extracted by acetone increase carotenoid and total phenolic contents in addition to antioxidant activity as the antioxidant compounds strongly react to the strong polarity of acetone.

Regarding their biological properties (antimicrobial, anti-alzheimer, hypotensive, antioxidant, anti-cancer, anti-hyperglycemia, anti-hyperlipidemia, and skin curative agents) *Salvia* plants are of great interest for pharmaceutical applications (Sharifi-Rad et al., 2018). It was reported that there is a diverse phytochemical richness in the genus of *Salvia*, which could lead to the discovery of new biologically active compounds (Sharifi-Rad et al., 2018). Especially *Salvia* plant essential oils show a wide range of pharmacological activities and, therefore have great potential to be used as a naturally derived food preservative (Sharifi-Rad et al., 2018). According to literature, *Salvia* species essential oil yield ranges from 0.07 to 6% (Karousou et al., 2000; Rajabi et al., 2014; Sharopov et al., 2015). Terpenes and terpenoids are organic compounds, which occur as a main component of essential oils (Yannick Stephane & Jean Jules, 2020).

Luis et al. (1994) identified the following diterpenoids in the aerial parts of *Salvia columbariae* Benth.: 11,12-di-O-methylcarnosol, 11,12-di-O-methylrosmanol, salvicanol, carnosol, rosmanol, isorosmanol, rosmadial, epirosmanol, and carnosic acid while Adams Jr. et al. (2005) identified cryptotanshinone, miltionone II, and tanshinone IIA diterpenoids in plant extracts of *Salvia columbariae* Benth. roots. Additionally, β-sitosterol lithospermic acid, a compound similar to salvianolic acid B, was detected (Adams Jr. et al., 2005). These are assumed to enhance cerebral blood flow, block platelet aggregation, act anti-inflammatory, and have neuro- and cardioprotective properties, therefore, being of potential interest in stroke and heart attack treatment (Adams Jr. et al., 2005; Fan et al., 2019).

Tanshinones are also discussed as potential therapeutics in cancer research as well as in the treatment of hypertrophic scar tissue and psoriasis (Dai et al., 2020; Li et al., 2016; Zhang et al., 2018; Zhou et al., 2020). The Münster research project demonstrated that lipophilic drug extracts - and in particular the isolated cryptotanshinone - showed unexpectedly promising effects on the differentiation of human skin cells (keratinocytes) (Hensel et al., 2018).

Through antimicrobial tests, Elshafie et al. (2018) argued that the essential oil (Sesquiterpenes main constitute), extracted from the aerial parts of chia (stem and leaves) represents a promising natural alternative to synthetic chemical treatments which negatively affect the environment and human health (Elshafie et al., 2018). Additionally, the problem of pest resistance to insecticides led to an increased interest in naturally derived compounds for insect control (Le Goff & Giraudo, 2019; C. M. Oliveira et al., 2014). The exact mode of action for chia essential oil still needs to be examined but the growth of some phytopathogenic fungi (*A. fumigatus*, *P. expansum, M. laxa,* and *M. fructigena*) and Gram-positive bacterial strains could be inhibited significantly (Elshafie et al., 2018). Contents of terpenes and terpenoids (Secondary metabolites), like germacren-B, β- caryophyllene, α-humoleno, globulol, γ-muroleno, widdrol, and β-pinene, present in the leaf oil composition of *Salvia hispanica* L. are further believed to have strong repellent characteristics to a wide range of insects (Ahmed et al., 1994; Bochicchio, Phillips, et al., 2015; Dambolena et al., 2016; Hrnčič et al., 2020; Ullah et al., 2015). Besides the essential oils present in the leaves of chia hold great aromatization, flavoring, and fragrance potential (Ahmed et al., 1994). The extracts and oils can be used as emollient, moisturizing substances for skincare (Bährle-Rapp, 2020). First results of Jeong et al. (2010) additionally indicated chia seed oils great potential to be used as an effective topical for pruritus and xerosis treatment.

6.3.4 *Salvia hispanica* L. and its multipurpose potential

The potential applications of chia were discussed in detail in the previous chapters. Clearly emphasizing its multipurpose potential as the aerial parts, roots, and seeds, including their plant constituents, can be implemented to various uses and processes leading to a diverse range of products and the corresponding enormous economic potentials. The prospect of being able to use chia holistically serves as a continuous incentive for future research. The following Figure 7 demonstrates those present and future possibilities in condensed form.

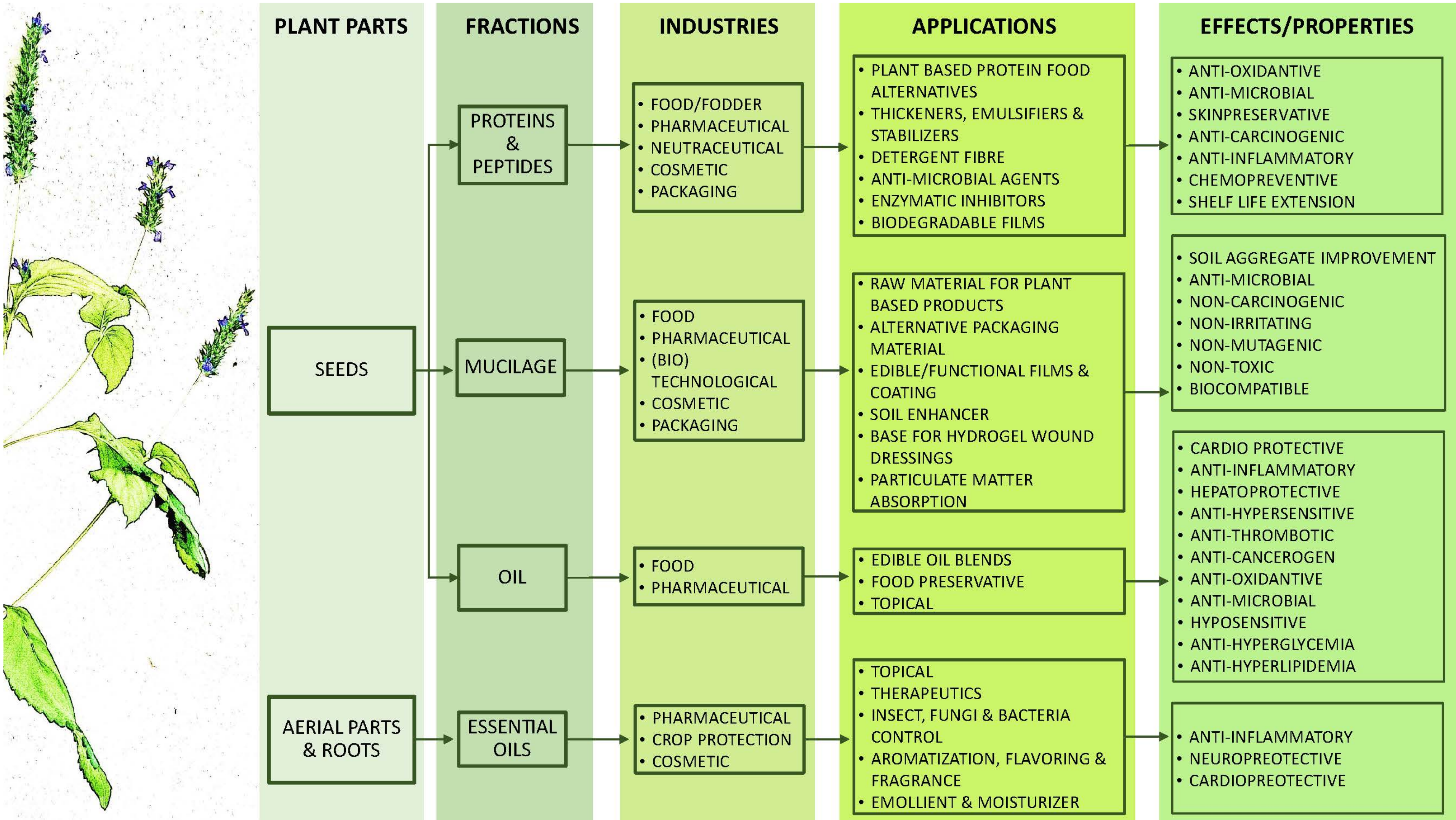

Figure 7 Overview of chia (*Salvia hispanica* L. & *Salvia columbariae* Benth.) plant parts and their potential applications, effects, and uses in different industries (own photo).

7. Summary

Nowadays, lifestyle and health consciousness are leading to an ever-increasing consumer demand for so-called "superfoods", which are believed to provide multiple simultaneous health benefits beyond their nutritional value. In parallel, there is greater awareness regarding food origin, social and environmental impacts of consumer's food choices. To meet those consumer demands, a regional, sustainable production of chia as the most well-accepted "superfood" among German consumers, presents a highly promising opportunity in different industries (packaging, cosmetic, medical, pharmaceutical, neutraceutical, technological, and crop protection) and fields of applications. The potential usage and application of chia is plenty fold and live up to the status of a multi-purpose crop. However, as chia is a short-day plant with a threshold of 12–13 h, cultivation was geographically limited to the tropics and subtropics. Yet cultivation expansion of chia to temperate regions (40°-60°) within the cool temperate climate as it prevails for example in Germany however, required an adaptation of genotypes regarding day length sensitivity as cultivation is only feasible during the summer months as the temperature conditions are only fulfilled in these months. Due to the efforts of breeders the photoperiodic sensitivity was overcome and new long-day flowering species, able to induce flower formation under day lengths of up to > 15 hours, were developed.

The breakthrough in overcoming the photoperiodic sensitivity enabled the cultivation of chia for its seed production in Europe and made the development of a holistic cultivation system for chia under the given climatic conditions essential. However, abroad from day length additional climate requirements need to be fulfilled and agronomic management practices need to be developed to finally meet chis seed yield and quality goals.

Therefore, within the current thesis the following objectives were evaluated to select new chia genotypes and establish agronomic management practices improving chia yield and quality traits under European conditions:

- to investigate the cultivability of different chia genotypes under Central European climate conditions
- to determine and specify agronomic and quality traits of different chia genotypes cultivated under Central European climate conditions
- to optimize and establish an applicable cultivation system for chia under the climatic conditions given in Central Europe
- to select new chia genotypes that can be cultivated under Central European climatic conditions

In this respect, climate chamber and field experiments at the University of Hohenheim and its experimental stations 'Ihinger Hof' and 'Eckartsweier' were conducted between 2015 to 2018. Findings of the first publication showed that three cultivated chia genotypes either day length insensitive (Sahi Alba 914) or adapted to day lengths greater than 12 h surpassed the yields obtained by their countries of origin, and obtained oil, protein, and mucilage contents in line with current literature and the Novel Food-Regulation (EU) 2015/2283, thus possibly representing an attractive alternative to local farmers in Germany. Considering the results, it became apparent that Germany could be seen as a seminal opportunity to expand the cultivation area of chia to latitudes up to 48° N. In times of climate change the broadening of the food base with respective crops could be crucial in the future.

Results presented within the second publication confirmed that the given environmental conditions significantly influenced seed yield and nutrient composition of chia, while the applied agronomic management practices (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}) showed a limited influence. Nevertheless, under the prevailing conditions in Germany, 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} could be recommended. It became obvious that more field trials were necessary to provide information regarding the influence of agronomic management practices on overall chia performance and to adapt management practices to maximize chia seed yields and their associated seed quality traits under the given conditions.

Within the third publication it could be concluded that within the given biodiversity of *Salvia* species, there are existing *Salvia hispanica* L. (SALV66) and *Salvia columbariae* Benth. (Golden Chia) varieties adapted to day lengths greater than 12 h showing seed yields almost in line with those of their countries of origin. Both selected chia varieties SALV66 and Golden Chia represented very promising raw materials from a nutritional point of view. The significant seed shattering level of Golden Chia, however, is a huge drawback regarding its commercial production. Therefore, especially concerning Golden Chia breeding efforts and scientific studies on agronomic management practices are of enormous importance.

The establishment of chia cultivation under local conditions could be considered as the first step towards a local and sustainable chia value chain in Europe. Chia cultivation in Europe including Germany would on the one hand fulfil the steadily increasing demand for healthy, environmentally sustainable, and regionally produced food and non-food products. While on the other hand, it would simultaneously represent a profitable economic source of income for local farmers, potentially leading to an economically and environmentally profitable way of

broadening the food chain and food base. Improved basic understanding about chia cultivation, the effect of plant constituents, and processing possibilities could help to translate research into new technological advances.

8. Zusammenfassung

Ein zunehmendes Lifestyle- und Gesundheitsbewusstsein der Verbraucher führte in jüngster Zeit zu einer gesteigerten Nachfrage nach so genannten "Superfoods", welchen gesundheitsfördernde Eigenschaften zugeschrieben werden. Parallel dazu werden Lebensmittelkaufentscheidungen immer stärker durch das erhöhte Bewusstsein der Verbraucher hinsichtlich der sozialen und ökologischen Auswirkungen in den Herkunftsländern beeinflusst. Eine regionale, nachhaltige Produktion von Chia in Mitteleuropa, kann dieser Nachfrage und dem entsprechenden Verbraucherbewusstsein entgegenkommen. Das breite Spektrum an Einsatz- und Anwendungsmöglichkeiten in diversen Industrien (Verpackung, Kosmetik, Medizin, Pharmazie und Nutrazeutika) abseits der Nahrungsmittelindustrie spricht darüber hinaus auch aus wirtschaftlicher Sicht für den regionalen Anbau.

Da Chia jedoch eine Kurztagpflanze ist, welche Tageslängen von 12-13 Stunden für die Blütenbildung benötigt, war der Anbau bislang geographisch auf die Tropen und Subtropen beschränkt. Eine Ausweitung des Anbaus von Chia in gemäßigte Regionen (40°-60°) innerhalb des kühl-gemäßigten Klimas, wie es z.B. in Deutschland vorherrscht, erforderte jedoch eine Anpassung der Genotypen hinsichtlich der Tageslängenempfindlichkeit. Der Anbau wäre bis dato nur in den Sommermonaten möglich, da nur in diesen Monaten die Temperaturbedingungen erfüllt wurden, dies allerdings dem Tageslängenanspruch einer Kurztagpflanze entgegensteht. Durch züchterische Bemühungen wurde die photoperiodische Sensibilität überwunden und neue Sorten entwickelt, die in der Lage sind, die Blütenbildung unter Tageslängen von bis zu > 15 Stunden zu induzieren.

Durch die Überwindung der photoperiodischen Sensibilität wurde der Anbau von Chia für die Samenproduktion in Mitteleuropa ermöglicht. Dies erfordert jedoch die Entwicklung eines ganzheitlichen Anbausystems für Chia, um letztendlich die geforderten Ertrags- und Qualitätsziele für Chiasamen unter den gegebenen klimatischen Bedingungen zu erreichen.

Daher wurden im Rahmen der vorliegenden Arbeit folgende Ziele evaluiert, um neue Chia-Genotypen zu selektieren und agronomische Managementpraktiken zu etablieren, die zur Verbesserung des Ertrags und der Qualitätsmerkmale von Chia unter mitteleuropäischen Bedingungen beitragen könnten:

- Untersuchung der Anbaufähigkeit verschiedener Chia-Genotypen unter mitteleuropäischen Klimabedingungen

- Bestimmung und Spezifizierung von agronomischen und qualitativen Merkmalen verschiedener Chia-Genotypen, die unter mitteleuropäischen Klimabedingungen angebaut werden
- Optimierung und Etablierung eines Anbausystems für Chia unter den in Mitteleuropa gegebenen klimatischen Bedingungen
- Selektion neuer, weiterer Chia-Sorten, die unter mitteleuropäischen Klimabedingungen anbaufähig sind

Hierzu wurden in den Jahren zwischen 2015 und 2018 Klimakammer- und Freilandversuche an der Universität Hohenheim und den Versuchsstationen 'Ihinger Hof' und 'Eckartsweier' durchgeführt.

Die Ergebnisse der ersten Publikation zeigten, dass drei kultivierte Chia-Genotypen, die entweder tageslängenunempfindlich (Sahi Alba 914) oder an Tageslängen von mehr als 12 Stunden angepasst waren, Erträge erreichten, die über den Erträgen der Herkunftsländer lagen. Des Weiteren wurden Öl-, Protein- und Schleimstoffgehalte erzielt, die der aktuellen Literatur und der Novel-Food-Verordnung (EU) 2015/2283 entsprachen und somit stellt Chia möglicherweise eine attraktive, alternative Feldfrucht für hiesige Landwirte in Deutschland dar, da in Anbetracht der Ergebnisse deutlich wurde, dass der Anbau von Chia auf Breitengrade bis 48° N ausgedehnt werden könnte. In Zeiten des Klimawandels könnte die Erweiterung der Nahrungsmittelbasis mit entsprechenden Nutzpflanzen essentiell sein.

Die in der zweiten Publikation vorgestellten Ergebnisse bestätigten, dass die gegebenen Umweltbedingungen einen signifikanten Einfluss auf den Samenertrag und die Nährstoffzusammensetzung von Chiasamen hatten, während die angewandten agronomischen Managementpraktiken (i) Reihenabstand (35, 50 und 75 cm), (ii) Aussaatstärke (1, 1,5 und 2 kg ha^{-1}) und (iii) N-Düngungsrate (0, 20 und 40 kg N ha^{-1}) einen begrenzten Einfluss aufwiesen. Dennoch konnte unter den in Deutschland vorherrschenden Bedingungen ein Reihenabstand von 50 cm, eine Aussaatdichte von 1,5 kg ha^{-1} und eine N-Düngermenge von 20 bis 40 kg N ha^{-1} empfohlen werden. Es wurde deutlich, dass weitere Feldversuche notwendig sind, um Informationen über den Einfluss der agronomischen Managementpraktiken auf die allgemeine Pflanzenentwicklung zu erhalten und final den Saatgutertrag und die entsprechenden Qualitätsmerkmale von Chia unter den gegebenen Bedingungen zu maximieren.

Im Rahmen der dritten Publikation konnte festgestellt werden, dass innerhalb der gegebenen Diversität der *Salvia*-Arten, bereits *Salvia hispanica* L. (SALV66) und *Salvia columbariae* Benth. (Golden Chia) Sorten verfügbar waren, die an Tageslängen von mehr als 12 Stunden angepasst waren und deren Samenerträge annähernd der der Herkunftsländer entsprachen.

Beide ausgewählten Chia-Sorten SALV66 und Golden Chia stellen aus ernährungsphysiologischer Sicht vielversprechende Rohstoffe dar. Der signifikante Samenausfall von Golden Chia ist jedoch ein großer Nachteil für die kommerzielle Produktion. Daher sind insbesondere hinsichtlich der Golden Chia Züchtungsbemühungen und wissenschaftliche Studien zu agronomischen Managementpraktiken von enormer Bedeutung.

Die Etablierung des Chia-Anbaus unter lokalen Bedingungen könnte als erster Schritt zu einer lokalen und nachhaltigen Chia-Wertschöpfungskette in Mitteleuropa angesehen werden. Der Chia-Anbau in Europa, einschließlich Deutschland würde einerseits die steigende Nachfrage nach gesunden, nachhaltigen und regional erzeugten Lebensmitteln und Non-Food-Produkten erfüllen. Andererseits würde er gleichzeitig eine lukrative wirtschaftliche Einnahmequelle für lokale Landwirte darstellen. Dies könnte zu einer ökonomisch und ökologisch gewinnbringenden Erweiterung der Nahrungskette und Nahrungsmittelbasis führen. Ein verbessertes Grundverständnis hinsichtlich des Chia-Anbaus, der Inhaltsstoffe und Verarbeitungsmöglichkeiten könnte helfen, Forschung in neue technologische Entwicklungen umzusetzen.

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Annex 3

Declaration in lieu of an oath on independent work

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

Screening and cultivation of chia (*Salvia hispanica* L.) under Central European conditions: The potential of a re-emerged multipurpose crop

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Stuttgart-Hohenheim, 19th of March 2021 ____________________________________

Place, Date Signature

Curriculum vitae

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Publications

Conference proceedings	Grimes, S. J., Phillips, T. D., Claupein, W., & Graeff-Hönninger, S. (2018). Chia^4: Is a new tetraploid chia (Salvia hispanica L.) genotype able to revolutionize European agriculture? *The Food System Approach: New Challenges for Education, Research and Industry*, 79.

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Stuttgart-Hohenheim, 19th of March 2021

Place, Date Signature