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## *Developing cropping systems for the ancient grain chia (*Salvia hispanica* L.) in two contrasting environments in Egypt and Germany*

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## Table of Contents

<b>LIST OF FIGURES AND TABLES .....</b>	<b>II</b>
<b>LIST OF ABBREVIATIONS AND ACRONYMS.....</b>	<b>III</b>
<b>1 INTRODUCTION .....</b>	<b>1</b>
1.1 GLOBAL CHALLENGES IN AGRICULTURAL CROPPING SYSTEMS.....	1
1.2 CHIA ( <i>SALVIA HISPANICA</i> L.).....	3
1.3 CULTIVATION OF CHIA – AGRONOMIC ASPECTS .....	6
1.4 CROP MODELING - CHIA GROWTH AND YIELD SIMULATION .....	9
1.5 PROJECT BACKGROUND AND FRAMEWORK .....	10
1.6 OUTLINE AND OBJECTIVES.....	11
<b>2 PUBLICATIONS.....</b>	<b>13</b>
<b>3 SOWING DATE IN EGYPT AFFECTS CHIA SEED YIELD AND QUALITY .....</b>	<b>15</b>
<b>4 NONDESTRUCTIVE LEAF AREA ESTIMATION FOR CHIA.....</b>	<b>30</b>
<b>5 ADAPTING THE CROPGRO MODEL TO SIMULATE CHIA GROWTH AND YIELD.....</b>	<b>43</b>
<b>6 GENERAL DISCUSSION .....</b>	<b>65</b>
6.1 FUTURE CHALLENGES FOR AGRICULTURAL CROPPING SYSTEMS .....	65
6.2 CROP MODELING - CROPGRO .....	66
6.3 APPLICATION OF THE CROPGRO-CHIA MODEL TO OTHER REGIONS.....	68
6.4 MODEL IMPROVEMENTS AND FUTURE RESEARCH .....	70
6.5 EXPANSION OF CHIA CROPPING TO OTHER REGIONS - CHIA AS A CROP ON THE WORLD.....	71
6.6 CROP ROTATION OR HOW CHIA CAN BE INTEGRATED INTO CROP SEQUENCE .....	74
6.7 FUTURE POTENTIALS.....	76
<b>7 SUMMARY .....</b>	<b>77</b>
<b>8 ZUSAMMENFASSUNG.....</b>	<b>80</b>
<b>9 REFERENCES .....</b>	<b>84</b>
<b>APPENDIX .....</b>	<b>95</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>101</b>

## List of Figures and Tables

<b>Figure 1.</b> Botanical habitat of chia (A). Chia field at flowering stage in Germany (B).	4
<b>Figure 2.</b> Charcoal chia seeds.	5
<b>Figure 3.</b> Chia experiments in Germany at early vegetative stage (A); flowering stage (B); ripening stage (C).	7-8
<b>Figure 4:</b> Chia cultivation and dispersal of chia crop around the world (Bohicchio et al. 2015b; Sosa et al. 2016; Modified).	72
<b>Figure 5:</b> Worldwide potential distribution of <i>Salvia hispanica</i> cultivation (A, rainfed; B, irrigated) based on the Maxent model.	74
<b>Figure 6.</b> Cropping calendar for integrating chia into existing crop rotation in Egypt.	76
<b>Figure A 7.</b> Mechanical harvesting of chia in Germany.	95
<b>Figure A 8.</b> Traditional field drying of chia in Egypt.	95
<b>Figure A 9.</b> Traditional and manual sieving of field dried chia seeds in Egypt.	96
<b>Figure A 10.</b> Monitoring of cultivated chia plants at the SEKEM experimental station (Adeja farm) in Egypt.	98
<b>Table 1.</b> Taxonomy of chia.	3
<b>Table 2.</b> Global growing regions and seasons of chia.	73
<b>Table 3.</b> Integrating chia into existing crop rotation in Egypt.	75

## List of Abbreviations and Acronyms

°C	degree Celcius
€	Euro
AC	anno Christi
ALA	alpha-linolenic acid
a.s.l.	above sea level
b	regression or shape coefficient
BC	before Christ
C	carbon
CAP	common agricultural policy
CC	climate change
CR	crop rotation
CSDL	critical short daylength below which reproductive development progresses as rapidly as possible with no daylength effect
CSM	Cropping System Model
CUL	Cultivar
d	days
DSS	decision support systems
DSSAT	Decision Support System for Agrotechnology Transfer
e.g.	exempli gratia (for example)
ECO	Ecotype
et al.	et alii, and others
EU	European Union
FA	fatty acid
FAME	fatty acids methyl esters
FID	flame ionization detector
GC	gas chromatography
GDP	gross domestic product
GDD	growing degree days
ha	hectare
HI	harvest index
IBSNAT	International Benchmark Sites Network for Agrotechnology Transfer
LA	leaf area

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List of Abbreviations and Acronyms

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LAI	leaf area index
LAISH	shaded LAI
LAISL	sunlit LAI
LC	lack of correlation
LL	leaf length
LR	likelihood ratio
LW	leaf width
MSD	mean squared deviation
N	nitrogen
NH <sub>4</sub> <sup>+</sup>	ammonium
NO <sub>3</sub> <sup>-</sup>	nitrate
NSTRES	nitrogen stress
NU	non-unity-slope
OLA	observed leaf area
P	phosphorus
PD	photothermal days
PLA	predicted leaf area
PRO	protein content
PP-SEN	slope of the relative response of development vs. photoperiod
PUFA	polyunsaturated fatty acids
r	Pearson's correlation coefficient
REML	restricted maximum likelihood
RMSE	root mean square error
SB	squared bias
SFA	saturated fatty acids
SLA	specific leaf area
SPE	Species
SWD	sowing date
SY	seed yield
SYP	seed yield per plant
Tb	base temperature
TD	thermal days
TDF	total dietary fiber
TKW	thousand kernel weight

## List of Abbreviations and Acronyms

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Tmax	maximum temperature
Tmin	minimum temperature
TNC	total non-structural carbohydrate concentration
Topt	optimum temperature
U.S.	the United States
WPA	Water People Agriculture
WTPSD	maximum weight per seed under non-limiting substrate (g / seed)
yr	years
XLAI	leaf area (one side) per unit of ground area

# 1 Introduction

## 1.1 Global challenges in agricultural cropping systems

Worldwide, the biggest problems and key challenges under climate change (CC) in arid and semiarid regions are water and food security (Misra 2014). The most obvious impacts are e.g. growing deserts and an increase in the extent of floods and droughts. The effects of CC on crop yields have already been reported in experimental data (Campbell et al. 2016). In arid and semi-arid regions, an enormous drop in crop yields has led to food shortages and a rise in food inflation (Misra 2014). In the future, CC and water scarcity will significantly threaten agriculture and sustainable development (UNFCCC 2016; Zeibisch et al. 2005). Thus, the demand for water will become one of the tenacious resource problems of the 21<sup>st</sup> Century (Abdin and Gaafar 2009). The challenge for water use in many parts of the world is linked with access, equity, and the response to growing requirements. Water analysts predict an augmented competition among water users in satisfying the growing demand. They predict that the competition will globally increase among the three largest water users, agriculture, industry, and municipal and domestic uses. Despite increasing urbanization in Africa, Asia and Latin America, agriculture is the dominant water user in these regions, accounting for approximately 85% of all water used (Abdin and Gaafar 2009; FAO 2016). In more developed regions of Europe and North America, industry is the major water consumer. Rainfall and other sources of freshwater are unevenly distributed around the world and not only located where human water demand arises. Countries are considered to be water stressed when the annual renewable water supply drops below 1700 m<sup>3</sup> per capita, and water is scarce when it drops below 1000 m<sup>3</sup> (Soliman et al. 2019). Egypt has been classified as a water-scarce state. Eckstein (2009) stated that the challenge of water scarcity linked with the effects of CC could produce significant security problems for nations around the world, particularly where adequate fresh water resources are missing (Eckstein 2009). So, investments are required for improving future food security by developing and adopting climate resilient varieties, improving irrigation infrastructure, and reforming international food trade (Hanjra and Qureshi 2010).

### **The two contrasting environments, Egypt and Germany**

Water resources in Egypt are becoming scarce. Egypt's demand for fresh water has increased dramatically in recent years. Increased population pressure and upstream agricultural development, mainly in irrigation projects, will have effects on the volume of water in the Nile reaching Egypt and therefore on food security. Hot dry summers from May to October and mild winters from November to April characterize Egypt's climate. Rainfall is very low, irregular, and unpredictable. Annual rainfall ranges between a maximum of about 200 mm in the northern coastal region to a minimum of nearly zero in the south, with an annual average of 51 mm (FAO 2016). Egypt is classified as a lower middle-income country. The agricultural sector is important for the national economy. Agriculture

contributes about 11% to the gross domestic product (GDP), while its share of employment amounts to 26% of total employment. The main food crops grown in the country are wheat, maize, rice, sugarcane, vegetables, and fruits. About 11% of total export earnings (as averaged for the period from 2012 to 2016) is provided by the agricultural sector (FAO 2016). The Egyptian government indicated that by 2050 CC could deteriorate productive agricultural areas in Egypt by reducing country's production of barley, wheat, maize, sorghum, and soybean between 18% and 27% (Eckstein 2009). Egypt grows an enormous quantity of crops requiring irrigation (e.g. wheat and cotton). Policies are aimed at an increased efficiency in irrigation and the use of water efficient crops that earn high revenues in international markets (El-Sadek 2010). A positive argument for chia is its drought tolerance (Ayerza and Coates 2009; Peiretti and Gai 2009), thus, able to contribute to saving the scarce source, water, in Egypt. Further, it offers the chance to export the high value chia seeds, generating foreign exchange for reimporting e.g. wheat characterized by a higher water demand (Kijne 2006). However, information on chia cropping systems and management practices for Egypt being outside of the center of origin are still missing.

In Germany, 53% of the area is used for agriculture. The main crops are wheat for bread making, barley for fodder and industrial use, as well as other fodder crops like clover, lupine etc. (Zebisch et al. 2005). Agricultural yields in Germany have improved progressively within the last fifty years, particularly due to technological progress (development of new seeds, improvements in plant protection, new and improved sowing, cultivation and harvest techniques, and enhanced fertilization) (Zebisch et al. 2005). However, the German agricultural sector faces problems like the reduction of market supporting measures (e.g. subsidies), increasing globalization, eastward enlargement of the European Union (EU) and the liberalization of prices that represent economic risks to farmers and a destabilization of incomes in agriculture (Ortlof 1998, in: Zebisch et al. 2005). Further, agriculture is vulnerable to CC. Thus, a reduced wheat yield by 14% by 2055 is assumed, because of decreased precipitation and negative impacts of temperature increase in summer (Zebisch et al. 2005).

### **The increasing demand of chia seeds**

Chia seeds are becoming increasingly widespread and present in new food products in Europe due to their nutritional and health properties. The production of chia offers an attractive alternative because of high revenues in international markets. The retail prices for chia seeds are in the range of 8.95 €kg<sup>-1</sup> (NATURACEREAL Chia Samen) and 22 €kg<sup>-1</sup> (Alnatura Chia Samen (organic)) (CBI 2017). In 2016, around 16,182 tons (€31 million) of chia seeds were imported to Europe. This was an average annual increase of 27% (in volume) since 2012. With a share of 40%, Germany was the largest European importer of chia seeds in 2016 (CBI 2017). Chia seeds are attractive to the European market due to the classification of “free from” products meaning food products that do not contain allergens or other ingredients such as gluten, dairy, sugar or eggs that consumers avoid. Chia is interesting

especially for vegan and vegetarian diets. Germany has the most vegetarians in Europe and the vegetarian population is estimated to grow. Being gluten-free, chia seeds also generate attention among consumers suffering from gluten-intolerance (celiac disease) or those who prevent gluten intake (lifestyle choice). This expected increase indicates growth opportunities for chia seeds. Further, the production of chia seeds augmented considerably within the last years. However, there are no official data, it is expected that the global market for chia seeds totals around 40 to 60 thousand tons. Keeping in mind the above-mentioned facts, the increasing demand of chia seeds could also be interesting for both contrasting environments, Germany and Egypt.

## 1.2 Chia (*Salvia hispanica* L.)

### Botanical description

Chia (*Salvia hispanica* L.) is an annual herb of the Lamiaceae or Labiateae and it belongs to the mint family (Jamboonsri et al. 2012; Cahill 2003; Mohd Ali et al. 2012). It is a diploid with only 12 chromosomes ( $n = 6$ ) (Bushway et al. 1981). The taxonomy (Table 1) can be described as follows (Muñoz et al. 2013):

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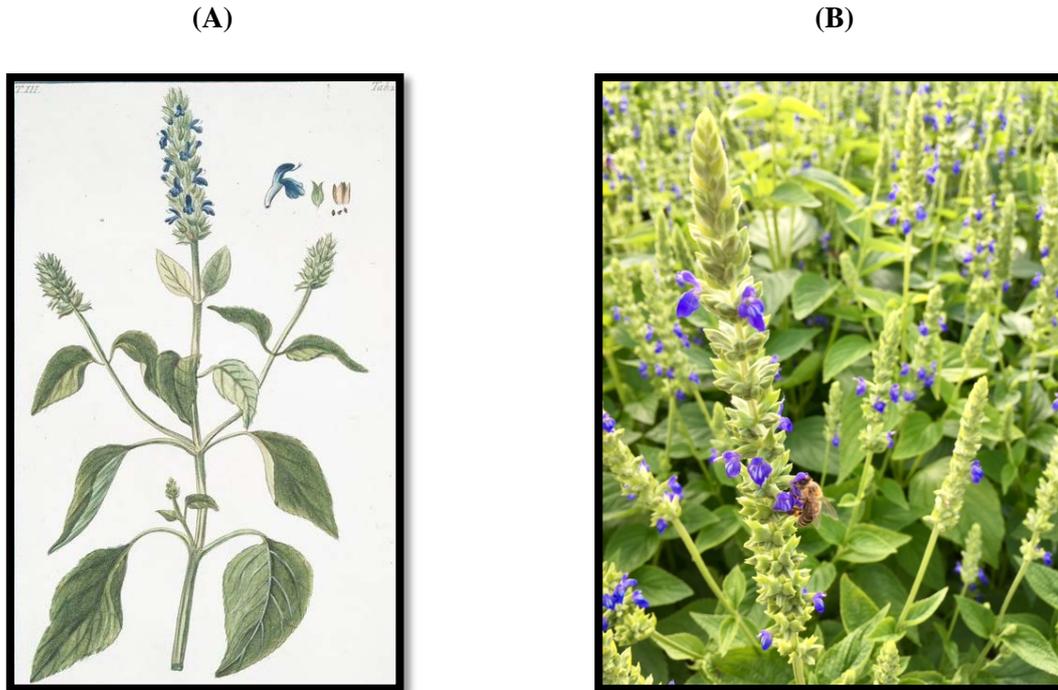
**Table 1. Taxonomy of chia.**

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Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genera	Salvia
Specie	hispanica

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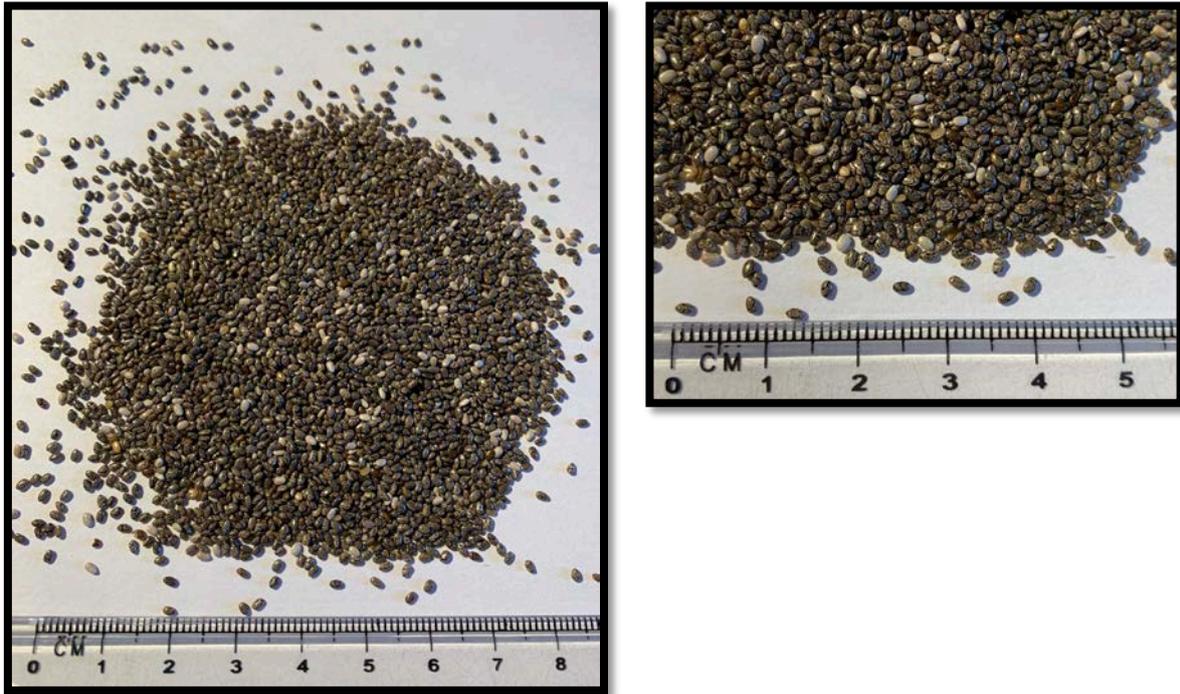
Chia (Figure 1 A-B) is a native plant from the regions of west-central Mexico to northern Guatemala (Cahill 2003; Muñoz et al. 2013). It can grow in height about 1-2 m having opposite arranged, petiolate, and serrated leaves that are 4-8 cm long and 3-5 cm wide (Jamboonsri 2010; Mohd Ali et al. 2012; Muñoz et al. 2013). Stems are obtusely quadrangular, ribbed, and hairy. The small hermaphrodite flowers (3-4 mm) with small corollas are shaped in terminal and axillary fourcornered spikes protected by small bracts with long sharp points. The fused flower parts result in a high self-pollination rate (Jamboonsri 2010; Mohd Ali et al. 2012). Each chia flower produces up to four fruits (called nutlets) in the calyx “capsule” (Figure 1 A). Commercially, each of these fruitlets is named “seed” (Capitani et al. 2013; Kochhar 2016). The seed color varies from black, gray, and black spotted (charcoal) to white (Figure 2). The seeds are oval shaped having a size ranging from 1 to 2 mm (Mohd Ali et al. 2012). Mean seed mass of domesticated chia is 15 mg 100 seeds<sup>-1</sup> (Jamboonsri 2010).



**Figure 1. Botanical habitat of chia (A).** Source: available online on <https://www.vegetarianproteinlist.com/chia-seeds.html> (left). **Chia field at flowering stage in Germany (B).** © Picture (1 B) by Laura Mack.

### **Nutritional value**

The cultivation of chia has recently regained attention. Chia seeds are getting increasingly popular as a functional superfood in Western diet due to their beneficial nutritional health-related properties, especially their high concentration of  $\alpha$ -linolenic acid (Ayerza and Coates 2011; Mohd Ali et al. 2012). Chia seeds (Figure 2) contain protein (15-25%), fats (30-33%), carbohydrates (26-41%), high dietary fiber (18-30%), ash (4-5%), minerals, vitamins, and dry matter (90-93%). Chia may be the best source of healthful soluble fiber known. It also contains a high amount of antioxidants (Ayerza and Coates 2005).



**Figure 2. Charcoal chia seeds.** © Picture by Laura Mack.

The chia seed contains as much as protein compared to flaxseed and even more protein in relation to any other grain, exceeding that of traditional cereals such as wheat, corn, rice, oats, and barley and other seeds such as amaranth and quinoa (Muñoz et al. 2013). Further, chia does not contain gluten which makes it interesting for vegetarian diets (Mohd Ali et al. 2012). Lipid of chia seed contains the highest known percentage of  $\alpha$ -linolenic (omega-3) fatty acid (up to 68%) of any plant source (Ayerza, 1995), compared to 36%, 53%, and 57% for camelina (*Camelina sativa* L.), perilla (*Perilla frutescens* L.), and flax (*Linum usitatissimum* L.), respectively (Ayerza and Coates 2011). The oil also contains linoleic acid (omega-6). Both are essential fatty acids, required by the human body for good health, and they cannot be synthesized (Mohd Ali et al. 2012). There are some studies that reported the benefits of chia on human health. Diets supplemented with chia have been documented to reduce risks from cardiovascular diseases, cancers, and diabetes (Mohd Ali et al. 2012; Muñoz et al. 2013; Jin et al. 2012).

### **Historical aspects**

Chia seeds in human consumption started around 3500 BC. In pre-Columbian Mesoamerica, chia was widely used for medicinal, culinary, artistic, and religious purposes. It was one of the main staple crops of pre-Columbian civilizations between 1500 and 900 BC (Bushway et al. 1981; Jamboonsri 2010; Muñoz et al. 2013). After the Spanish conquest, many of the traditions of the pre-Columbians and agricultural production were devastated due to beliefs and religious conflicts. That was why, the

cultivation of chia was drastically reduced and substituted by foreign crops such as wheat, barley, and carrots, which were demanded by the conquerors (Mohd Ali et al. 2012; Muñoz et al. 2013). In 1753, the Swedish botanist Carl Linnaeus classified chia and gave it the name *Salvia* (save or cure) *hispanica* (Spanish) that in Latin means Spanish plant to save. However, it is evident that this name was incorrectly set because this crop is not native from Spain. Cristobal Colón carried it from Mexico to Spain. The origin of the word “chia” is a Spanish adaption of the Nahuatl (language of the Aztecs) word *chian* that means “oily” (Muñoz et al. 2013). The Aztecs used the word “chia” to refer to all spices of *Salvia* genera with high oil content (Sosa et al. 2016).

Today, chia seeds are used for different purposes in various countries, such as Mexico, Argentina, Chile, New Zealand, Japan, the United States, Canada, and Australia. Chia and chia oil are used as human food (as an ingredient in cereal bars, breakfast cereals, cookie, fruit juices, cake biscuits, pasta, bread, snacks, and yogurt), animal feed, drying oil in paints, and ingredients in cosmetics (Jamboonsri 2010; Muñoz et al. 2013). Chia leaf oil may be useful in fragrances and as a natural pesticide (Muñoz et al. 2013). In 2000, the U.S. Dietary Guidelines recommended that chia seed could be consumed as a primary food not exceeding 48 g day<sup>-1</sup>. The EU Novel Food Regulation also limits the use of chia seeds in industrial applications. In 2009, the European Parliament and Council of Europe approved it as a “novel food”. Thus, chia seeds are intended to be used under the European Commission Implementing Decision 2013/50/EU authorizing an extension of use of chia seed (*Salvia hispanica* L.) as a “novel food” ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27<sup>th</sup> January 1997 (EFSA 2009). This regulation defines a “novel food” as a food, which has no significant history of human consumption within the European Community before 1997. Chia seeds have historically been consumed in South America, but have not previously been used for human consumption to a significant degree within the European Community. Thus approval, as a “novel food” ingredient is required and chia seeds in bread products should not exceed at a maximum of 5% (Armstrong 2004; EFSA 2009; Official Journal of the European Union 2014). If legislation changes, it would mean a meaningful market growth for chia seeds. Thus, it is estimated that the market could grow by as much as 10 times within the next five years after legislative changes occur (CBI 2017).

### **1.3 Cultivation of chia – Agronomic aspects**

Chia is seen as a future crop because it has a short crop cycle and it is considered to require less water to grow than cereals or other oil seeds (Peiretti and Gai 2009; Segura-Campos et al. 2014; Muñoz et al. 2013). Hence, it was explored as a future crop for more diversity in regions like Argentina and the U.S. (Ayerza and Coates 2005; Coates and Ayerza 1996). It has also been recommended as a highly economic substitute for common field crops supporting diversification and stabilization of the local economy (Coates and Ayerza 1996). Especially in arid and semiarid regions, where water availability

is the main limitation to crop production (Ayerza and Coates 2009; Peiretti and Gai 2009), the cultivation of chia may be attractive. Not only in arid regions, such as Egypt, CC and water scarcity will be major concerns in the future. In Egypt, agriculture mainly relies on irrigation. Therefore, policies aim at the use of water efficient crops with high revenues in international markets (Abdin and Gaafar 2009) to substitute water-intensive crops such as wheat or cotton. Presently, chia is grown for its seeds in Mexico, Bolivia, Argentina, Colombia, Ecuador, Australia, and Guatemala (Mohd Ali et al. 2012) where latitude ranges from 20°55'N to 25°05'S (Ayerza and Coates 2005; Jamboonsri 2010; Muñoz et al. 2013). It grows naturally in tropical and subtropical environments with an elevation ranging from 400 to 2500 m a.s.l. and a temperature range of 11-36 °C (Baginsky et al. 2016). Chia is a short-day plant and anthesis is naturally induced by short days (Jamboonsri et al. 2012). At higher latitudes such as Choele-Choele (39°11'S) in Argentina, the plant is destroyed by frost before flowering and thus cannot produce seeds (Ayerza and Coates 2005). However, new cultivars have been bred that are insensitive to daylength or adapted to regions with daylength greater than 12 h (Hildebrand et al. 2013). Figure 3 A-C shows the cultivation of chia in Germany at different developmental stages.

(A)



(B)



(C)



**Figure 3. Chia experiments in Germany at early vegetative stage (A); flowering stage (B); ripening stage (C).**  
© Pictures by Laura Mack.

The crop cycle varies between 90 and 150 days depending on the latitude of cultivation area. The sowing rate of chia is 5-8 kg ha<sup>-1</sup> with a row spacing between 0.50 (Yeboah et al. 2014) and 0.80 m (Bochicchio et al. 2015a; Jamboonsri 2010). Chia is semi-tolerant to acid soils (from slightly less than 7.5 to less than 5) (Ramírez-Jaramillo and Lozano-Contreras 2015) and drought, so it develops in light to medium clay and sandy soils, and even in arid soils that have good drainage but it does not tolerate flooded soils (Jamboonsri 2010). Chia is sensitive to water deficit, but attempts to make osmotic

adjustments to maintain the leaf water balance resulting in a greater tolerance to water deficit and no significant yield loss (de Falco et al. 2018; Silva et al. 2016).

Jamboonsri (2010) and Coates (2001) reported that low nitrogen (N) fertility reduced yield. In other studies, N fertilization varied from 21 to 115 kg ha<sup>-1</sup> (Bochicchio et al. 2015a; Coates and Ayerza 1996). Based on the experiments of Sosa-Baldivia and Ruiz Ibarra (2018) nitrogen fertilization could improve seed yield (SY) by 63%. Chia is often harvested mechanically (Figure A 7). The main problem is the unequal ripening of the central inflorescence compared to the side shoots that stay green. However, waiting until all seeds are mature rises the risk of seed loss due to shattering problems, damage from birds, and abiotic factors like rain and wind (Jamboonsri 2010). Based on Coates and Ayerza (1996) and Coates (2011), commercial seed yields of chia can vary between 500 and 600 kg ha<sup>-1</sup>. Depending on location, seed yields varied between 175-1355 kg ha<sup>-1</sup> in Argentina (Coates and Ayerza 1996), and could reach even 2500 kg ha<sup>-1</sup> when irrigation and nitrogen fertilizer were applied (Muñoz et al. 2013), or 446-1753 kg ha<sup>-1</sup> in Ecuador (Ayerza and Coates 2009), and 70 to 2500 kg ha<sup>-1</sup> in Chile (Baginsky et al. 2016). SY reached almost 3000 kg ha<sup>-1</sup> in Mexico, when chia was established at an optimal sowing date in November or December (Sosa-Baldivia and Ruiz Ibarra 2018). Climate changes, differences in the location, nutrient availability, year of cultivation, or soil conditions are essential factors influencing SY (Ayerza 2009; Ayerza and Coates 2009) and also seed quality like protein and oil content (Mohd Ali et al. 2012). Although, chia seed has been commercialized for a long time in Argentina, the production there is still small-scale (Figure A 8). Thus, the current production of chia seeds is not able to fully meet the world market demand (Coates and Ayerza 1996; Mohd Ali et al. 2012). Sosa-Baldivia and Ruiz Ibarra (2018) reported that low productivity on chia is because agronomic management has not been modernized (Figure A 9). Further, it still is mainly cultivated in the rainy season, and the use of fertilizers, pesticides, and improved varieties is rare.

#### **1.4 Crop Modeling - Chia growth and yield simulation**

Since chia is highly reliant on the environment to evolve its maximum agronomic potential, it is necessary to define the factors that influence yield of chia (de Freitas et al. 2016). There is no broad experience available on the cultivation of chia out of its centers of origin. Thus, the most appropriate geographic regions and cropping systems for the production of chia should be identified. Field experiments are laborious, expensive, and time consuming. In this context, crop models may be useful instruments to assess different management options for cultivation of chia in other regions than its origin. Process-oriented crop growth simulation models are advantageous for adapting and improving management systems (Boote et al. 1996). In the last few years, crop models have become increasingly important as research tools for management and decision making in cropping systems in diverse locations where soils and climate are different (Jones et al. 2003; Naab et al. 2015). Since 1989,

researchers worldwide have used the Decision Support System for Agrotechnology Transfer (DSSAT). The origin of the DSSAT system goes back to the International Benchmark Sites Network for Agrotechnology Transfer (IBSNAT) Project that was supported by the U.S. Agency for International Development from 1982 to 1993 (Thorp et al. 2014). DSSAT integrates models of about 46 different crops with software that enables the assessment and application of the crop models for different reasons. The DSSAT cropping system model design has a modular structure to simplify more efficient integration of new scientific advances, applications, documentation, and maintenance (Hoogenboom et al. 2017). The CROPGRO model is embedded in the DSSAT (Jones et al. 2003). An advantageous feature of CROPGRO is that a new crop can be introduced via the crop template approach which allows model developers to modify values in a species crop template file without changing any of the FORTRAN code (Boote et al. 1996; Jones et al. 2003). CROPGRO was developed as a generic approach for modeling crops with one joint source code initially developed for legume crops (Boote et al. 1998b; Wilkerson et al. 1983). It can predict the growth of several crops, such as soybean, peanut, dry bean, chickpea, cowpea, faba bean, tomato, macuna, cotton, and safflower based on weather, plant, soil, and management inputs (Boote et al. 1998a; Hoogenboom et al. 2017). The CROPGRO model uses external data files that specify species, ecotype, and cultivar traits (Hoogenboom et al. 2017; Jones et al. 2003). For example, in the CROPGRO species file the base temperatures ( $T_b$ ) and optimum temperatures ( $T_{opt}$ ) are defined for developmental processes (e.g. rate of emergence and leaf appearance), and growth processes (e.g. photosynthesis, seed growth, and N mobilization). Phenology is an essential part of the CROPGRO crop template approach. Life cycle progresses depending on a physiological day accumulator as a function of temperature and daylength (Jones et al. 2003). Chia is sensitive to daylength, whereas other crops such as peanut are not. So far, no chia model exists. Enhancing the production of chia and adapting it to new regions demands the comprehensive understanding of processes linked to growth, development, and yield formation.

## **1.5 Project background and framework**

The present doctoral thesis was based on a project embedded in the graduate school Water-People-Agriculture (WPA) at the University of Hohenheim funded by the Anton-&Petra-Ehrmann foundation. This research training group focuses on key water issues and water related challenges of today's society on five main topics such as “Water as a resource”, “Water and climate”, “Water productivity”, “Water and health”, and “Water as a societal challenge”. This thesis is to settle in the versatile area of water productivity meaning efficiency of agricultural and industrial production systems, “more crop per drop”, improving irrigation systems, managing green water resources in rainfed systems, dry farming, water saving, water and rain use efficiency, affecting water pathways by management, adapt systems to climate variability or change, industrial water use efficiency,

sustainable intensification, sustainable water use, and salinity. Young scientists are encouraged to focus on water related issues of ecologic, societal, and economic dimensions such as water and food security, sustainable water use and productivity, water and ecosystem functions, and water governance and trade, or water pollution. The most important resource for life is fresh water that is the only resource having no substitute. Its accessibility enriches the quality of life and the economy of a community. In the light of the above, it is concluded that the future agricultural systems should use the restricted and valuable source, water, in an appropriate way and therefore implement more suitable water sound systems for e.g. novel emerged crops. Agriculture is facing global challenges like securing the world diet, supply of raw material and climate and environmental protection. The world agrarian report comes to an unequivocal judgment: The world will live ecologically or not at all. A promising attempt for a structural change to a more sustainable production that can make an economic prosperity possible with an ecological and social compatibility at the same time needs to strengthen the knowledge-based support of research and innovation (Kijne 2006). The topic about water scarcity in Egypt matches with the WPA research areas by transferring and developing cropping systems for the grain “chia” in developing countries such as Egypt, where agriculture consumes the largest amount of the total water demand (Sallam 2014). Thus, policies are aimed at an increased efficiency in irrigation and the use of water efficient crops while ensuring at the same time high revenues in international markets (e.g. less wheat and cotton) (El-Sadek 2010). Being drought tolerant, chia may offer an alternative to increase farm income under arid conditions by diversifying products, hedging risks, expanding markets, increasing exports, decreasing imports, improving human and livestock diets, and creating new industries based on agricultural resources. This project was also linked with ongoing chia projects in Germany to explicitly compare two contrasting environments and to test not only Egypt but also Germany for cultivation of chia.

## **1.6 Outline and objectives**

This doctoral thesis focused on the transfer and development of cultivation practices for the ancient grain chia in other regions than its origin. The main aim was to expand the knowledge on cultivation practices for new environments (e.g. Egypt and Germany) to increase seed production and seed quality. Further, a CROPGRO-chia model was preliminarily developed and adapted to simulate chia crop growth and yield. Based on this developed model, researchers can use this model to extend the knowledge on the eco-physiology of chia and to improve its production and adaption to other regions.

The specific objectives of this thesis were

- to evaluate the effect of six different sowing dates on: (i) agronomic traits and (ii) seed quality with the main aim to determine the optimum sowing date (SWD) for chia cultivation in Egypt,

- to develop a leaf area (LA) estimation model for chia using leaf width (LW) and length (LL) measurements,
- to determine whether population and nitrogen fertilizer level have an effect on the accuracy of a LA estimation model for chia,
- to adapt the CROPGRO model for simulating chia growth and yield under European climate conditions.

For the fulfillment of the above mentioned objectives, field experiments were conducted at the experimental station 'Ihinger Hof' of the University of Hohenheim in southwestern Germany from 2015 to 2017 and in Egypt during the cropping season 2015 to 2016 at the SEKEM experimental station located 50 km Northeast of Cairo. The field experiments in Germany comprised eight varieties of black- and white-seeded chia (South American short-day genotypes: 07015 ARG, 06815 BOL, 06915 ARG, photoperiod insensitive new cultivars: G8, G7, G3, W13.1, and Sahi Alba 914) and three nitrogen (N) fertilizer levels (0, 20, and 40 kg N ha<sup>-1</sup>). The experiment in Egypt included six different SWDs with a commercial short-day flowering genotype with charcoal seeds available in the local supermarket. The experimental design and the outcomes are presented in three scientific publications, which form the main body of the present thesis.

**Publication 1** deals with the effect of six different sowing dates on yield attributes and seed quality of chia (*Salvia hispanica* L.) to determine the suitable SWD for chia in Egypt. Further, the relationships between thermal time at onset of flowering and the corresponding prevailing daylength, and seed yield, protein content, and oil content were investigated. In **Publication 2**, a nondestructive LA estimation model for chia was developed that can take into account differences in leaf shape, population, and N-fertilization. Therefore, a meta-regression was used to integrate the data accounting for heterogeneity between experiments, populations, and N-levels. The LA is an important parameter for modeling. With the final goal of developing cropping systems for chia for other regions, a modeling approach was developed. For this purpose, a LA model is needed. **Publication 3** used the derived field data and LA estimation model developed in Publication 2 to finally adapt the plant growth simulation model DSSAT CROPGRO for chia cultivated within a temperate climate zone.

Publications 1-3 present the results that have already been published in peer-reviewed journals. The details of each publication are given in the following chapter 'Publications'. Additional contributions and presentations in the context of the dissertation are listed in the appendix.

## 2 Publications

The present cumulative thesis contains three articles, which have been published in peer-reviewed, international high standard referenced journals. For citation of the three articles, which correspond to the chapters 3-5 of the present thesis, please use the references given below.

### **Publication I**

Mack, L., Munz, S., Capezzone, F., Hofmann, A., Piepho, H.-P., Claupein, W., & Graeff-Hönninger, S. (2018). Sowing Date in Egypt Affects Chia Seed Yield and Quality. *Agronomy Journal*, 110(6), 2310–2321. doi:10.2134/agronj2018.05.0324.

### **Publication II**

Mack, L., Capezzone, F., Munz, S., Piepho, H.-P., Claupein, W., Phillips, T., & Graeff-Hönninger, S. (2017). Nondestructive Leaf Area Estimation for Chia. *Agronomy Journal*, 109(5), 1960–1969. doi:10.2134/agronj2017.03.0149.

### **Publication III**

Mack, L., Boote, K.J., Munz, S., Phillips, T., & Graeff-Hönninger, S. (2020). Adapting the CROPGRO Model to Simulate Chia Growth and Yield. *Agronomy Journal*, 1–19. doi.org/10.1002/agj2.20305.



### 3 Sowing Date in Egypt Affects Chia Seed Yield and Quality

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#### Publication I

**Mack, L., Munz, S., Capezzone, F., Hofmann, A., Piepho, H.-P., Claupein, W., & Graeff-Hönninger, S. (2018). Sowing Date in Egypt Affects Chia Seed Yield and Quality. *Agronomy Journal*, 110(6), 2310–2321. doi:10.2134/agronj2018.05.0324.**

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*Chia seeds have become increasingly popular as a functional superfood due to their health related benefits. It has been highly suggested as a substitute crop for the field crop industry, because it can grow in arid environments. However, knowledge on cultivation practices in arid regions is rare, as many studies have focused on seed characteristics. More information is needed for improvement of its cultivation and adaption to other regions with the aim to diversify and stabilize local agricultural economy and to meet the growing demand. To establish chia successfully in Egypt, the understanding of the influence of daylength on yield and relevant quality traits is required. From a nutritional perspective, it is essential that the quality of chia sold in the market does not vary significantly, since the benefits attained by consumption depend on its seed composition. Early studies reported a wide range in oil content and fatty acid composition of seeds grown under different climatic conditions, and in diverse geographical regions. The following article provides preliminary results from a field experiment in an arid region. It evaluates the effect of six different sowing dates on agronomic and quality traits to determine the optimal sowing date for chia in Egypt. Further, a correlation analysis by bivariate linear mixed models was performed in the following article that accounts for both the treatment structure and the randomization layout, something that is often neglected in correlation analysis.*

# Sowing Date in Egypt Affects Chia Seed Yield and Quality

Laura Mack,\* Sebastian Munz, Filippo Capezzone, Angela Hofmann, Hans-Peter Piepho, Wilhelm Claupein, and Simone Graeff-Hönninger

## ABSTRACT

Chia (*Salvia hispanica* L.) has recently been rediscovered as functional “superfood” for human nutrition. Chia is a short-day plant and it naturally grows in tropical and subtropical environments. It can cope with water stress and thus could also be cultivated in arid regions. The aim of this study was to determine the suitable sowing date (SWD) for chia in Egypt. Therefore, the effect of six different sowing dates (August to October) on agronomic traits like seed yield (SY), plant height, seed yield per plant (SYP), harvest index (HI) and quality traits such as protein, oil, mucilage content, and fatty acid profile was evaluated. The last SWD resulted in a significantly lower SY (125.91 kg ha<sup>-1</sup>), HI (0.11), oil content (27.08%), content of polyunsaturated fatty acids (PUFA) (81.46%), and ratio of PUFA to saturated fatty acids (7.24), but higher thousand kernel weight (TKW) (1.51 g), protein content (26.03%), and higher content of saturated fatty acids (SFA) (8.21%) compared with the other SWDs. The maximum observed SY (664.94 kg ha<sup>-1</sup>) was recorded for SWD 4 (3 Oct. 2015). In this study, the thermal time at onset of flowering and the corresponding prevailing daylength showed a strong positive relationship for daylengths higher than 10.4 h that corresponded to about 600°C d (between SWD 5 and 6). Considering the obtained results and the possible risk of high temperature stress for very early sowings (SWD 1 and 2), sowing dates between middle and end of September are recommended to achieve a marketable seed quality and higher yields.

## Core Ideas

- Bivariate linear mixed models should be used to estimate correlations between traits in designed experiments.
- The fourth sowing date resulted in highest seed yield and harvest index with high PUFA/SFA ratio and content of PUFA and protein.
- Under later sowing, protein content and saturated fatty acids increased whereas oil content and PUFA decreased.
- Sowing chia in Egypt is recommended between the middle and end of September to achieve higher yields and good quality.

CHIA (*SALVIA hispanica* L.) is an annual herb of the Lamiaceae family originating from the regions of west-central Mexico to northern Guatemala (Cahill, 2003; Muñoz et al., 2013). In pre-Columbian times, it was one of the basic foods of several Central American civilizations (Ayerza, 2016). Chia is a short-day plant (Jamboonsri et al., 2012) and grows naturally in tropical and subtropical environments from 400 to 2500 m a.s.l. with an optimum temperature range of 16 to 26°C (Bochicchio et al., 2015a). Chia can grow in light to medium, clay, and sandy soils, and even in arid soils that have a good drainage (Mohd Ali et al., 2012; Muñoz et al., 2013). However, chia is sensitive to water deficit, but attempts to make osmotic adjustments to maintain the leaf water balance resulting in a greater tolerance to water deficit and no significant yield loss (Silva et al., 2016; de Falco et al., 2018). Today, chia is commonly cultivated in Mexico, Bolivia, Argentina, Colombia, Ecuador, Australia, and Guatemala (Jamboonsri et al., 2012; Segura-Campos et al., 2014; Baginsky et al., 2016). Chia is rich in polyunsaturated fatty acids (PUFA) and has been recommended as a highly economic substitute for common field crops supporting diversification and stabilization of the local economy (Coates and Ayerza, 1996). Especially in arid and semiarid regions, where water availability is the main limitation to crop production (Peiretti and Gai, 2009; Ayerza and Coates, 2009) the cultivation of chia may be attractive. Agriculture in Egypt mainly relies on irrigation, wherefore policies aim at the use of water efficient crops with high revenues in international markets (Abdin and Gaafar, 2009) to substitute, such as with water intensive crops like wheat and cotton. To introduce chia to Egypt, experiments studying different sowing dates and their influence on yield and relevant quality traits under Egyptian conditions are required. Sowing is one of the main management practices used to adjust the timing of occurrence of crop phenological phases and therefore determines the environmental conditions under which crops grow (Hirich et al., 2014) and the potential seed yield and seed quality (Ayerza, 2010; Ayerza and Coates, 2011). Due to the sensitivity to short photoperiods,

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**Abbreviations:** FA, fatty acids; FAME, fatty acids methyl esters; GDD, growing degree days; HI, harvest index; PUFA, polyunsaturated fatty acids; SWD, sowing date; SFA, saturated fatty acids; SY, seed yield; SYP, seed yield per plant; TKW, thousand kernel weight.

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chia can produce seeds only in a limited range of latitudes (Jamboonsri et al., 2012). As the market of chia expands, new regions for cultivation need to be investigated. Under Egyptian conditions, sowing dates in July or August experience very high temperatures in the beginning while later sowing dates are characterized by cooler temperatures and shorter daylengths throughout the entire growing period. Chia is a short-day plant (Jamboonsri et al., 2012), thus sowing date will strongly influence the timing of flowering and therefore the potential seed yield and seed quality (Ayerza, 2010; Ayerza and Coates, 2011). Hence, late sowing dates will lead to very early floral initiation which shortens the vegetative period. In general, a longer growing period (i.e., early sowing dates) favors plant growth and thus the formation of generative parts which results in higher yields (Baginsky et al., 2016). Oil is the major carbon storage form in the oilseeds, which provides energy during germination and is stored during later developmental stages at the costs of starch and protein (De la Vega and Hall, 2002; Vigeolas et al., 2004; Pal et al., 2017). Further, temperature affects oil quality (Flagella et al., 2002). Cool climatic conditions postpone the maturity of the seeds and thus offer a longer period for oil and fatty acid synthesis (Mirshekari et al., 2012) and colder temperatures generally increase the level of unsaturated fatty acids (Ayerza and Coates, 2009). Chia seeds contain mucilage, which is part of the soluble dietary fiber (Capitani et al., 2013; Coelho and de las Mercedes Salas-Mellado, 2014). The mucus layer prevents the plant and seeds from water loss, facilitates germination by forming a favorable environment, and providing a reserve of carbohydrates (Uschapovsky et al., 2015). The food industry is interested in high contents of seed mucilage as a stabilizer or a thickener similar to guar (*Cyamopsis tetragonoloba* L. Taub.) gum (Oomah et al., 1995). Further, the total dietary fiber has become important for the daily diet because total dietary fiber has health beneficial effects like reduction of cholesterolaemia, or adjustment of the glycemic and insulinaemic responses (Reyes-Caudillo et al., 2008). Most of the previous studies were conducted in countries of the origin of chia and focused on seed characteristics. To the best of our knowledge, no previous studies have reported on chia cultivation in Egypt. Therefore, the objectives of this study were to evaluate the effect of six different sowing dates on: (i) agronomic traits, such as seed yield (SY), yield components, harvest index (HI), and (ii) seed quality traits like contents of protein, oil, mucilage, and fatty acid profile with the aim to determine the optimum sowing date for chia cultivation in Egypt.

## MATERIAL AND METHODS

### Site Characteristics

A field experiment was conducted in Egypt during the cropping season 2015 to 2016 (22 Aug. 2015 to 30 Mar. 2016) at the SEKEM experimental station located 50 km Northeast of Cairo (30°25' N 31°38' E, 35 m a.s.l.). During the growing season, the average temperature was 19.2°C and the photoperiod ranged between 10.2 and 13.0 h (Fig. 1). Precipitation was limited between end of September and middle of October with a total amount of 14.4 mm. The soil was classified according to USDA soil taxonomy as Aridisol, which is a loamy sand soil (FAO, 2006) with a texture in the topsoil (0 to 0.3 m) consisting of 67.9% sand, 15.6% silt, and 16.5% clay (determined by the handbook of soil analysis following the DIN ISO 11277).

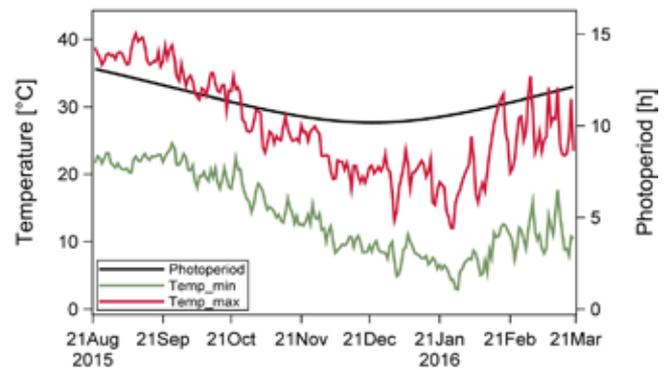


Fig. 1. Maximum and minimum temperature and photoperiod between sowing and harvest at the experimental station (30°25' N, 31°38' E). Data provided by NOAA (National Oceanic and Atmospheric Administration, <https://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html>) and NASA Prediction of Worldwide Energy Resource (POWER, <https://power.larc.nasa.gov/cgi-bin/agro.cgi?email=agroclim@larc.nasa.gov>).

### Experimental Design

The experiment comprised six different sowing dates (SWD) which varied for 2 wk between 22 Aug. and 31 Oct. 2015 leading to differences in temperature, growing period and daylengths (Table 1).

For seedbed preparation, the soil was plowed followed by manually forming of raised seed beds of 10.0-m length, 1.0-m width, and 0.3-m height. The trial was laid out as a randomized complete block design. Each plot consisted of a raised bed with two rows of chia with 0.3 m between rows and plants within a row. Sowing was done by hand with a sowing depth of 2.0 cm and a sowing density of 100 seeds m<sup>-2</sup>. Twenty days after sowing plants were thinned out to 30 plants m<sup>-2</sup>. Weeds were controlled manually by hoeing. A commercial short-day flowering genotype with charcoal seeds available in the local supermarket was used for the experiment. The trial was irrigated every day with 6 mm via drip irrigation to ensure a constant water supply. The amount of water was based on average evapotranspiration and an assumed crop transpiration coefficient of 0.95. Plants were fertilized with a total of 157 kg N ha<sup>-1</sup> by fertigation using vinasse. The harvest was performed at physiological maturity on 18 Jan. 2016 for SWD 1 to 3, on 30 Jan. 2016 for SWD 4, on 14 Feb. 2016 for SWD 5, and for the last SWD on 24 Mar. 2016 (Table 1). Physiological maturity was defined to occur when over 75% of the plants were completely senescent. The six sowing dates resulted in vegetation periods ranging between 120 and 150 d and growing degree days (GDD) between 821 and 1805°C d. The daily GDD (GDD<sub>*i*</sub>) were calculated after (Edey, 1977) as:

$$GDD_i = \frac{X_{i,max} + X_{i,min}}{2} - X_{base} \quad \text{if } GDD_i < 0, \text{ then } GDD_i = 0 \quad [1]$$

where  $X_{i,max}$  and  $X_{i,min}$  are the daily maximum and minimum temperatures on day  $i$  and  $X_{base}$  is the base temperature which was set to 10°C for chia (Baginsky et al., 2016) as for many tropical crops. The GDD of the vegetation period were the sum of the single GDD<sub>*i*</sub> from sowing to harvest.

### Agronomic Traits

Ten plants (always the same) were evaluated twice per week to determine the dates of emergence, initiation of branching,

Table 1. Characteristics of the six sowing dates including date of sowing and harvest, growing period (d), temperature (°C), growing degree days (°C d) and day length (h).

Characteristic	Sowing date					
	1	2	3	4	5	6
Date of sowing	22 Aug. 2015	5 Sept. 2015	19 Sept. 2015	3 Oct. 2015	17 Oct. 2015	31 Oct. 2015
Date of harvest	18 Jan. 2016	18 Jan. 2016	18 Jan. 2016	30 Jan. 2016	14 Feb. 2016	20 Mar. 2016
Growing period, d	150	136	122	120	121	142
Temperature, °C						
Max.†	28.4	27.5	26.2	24.0	22.6	22.8
Min.†	15.7	15.1	14.3	12.4	10.9	10.2
Mean‡	22.1	21.3	20.3	18.2	16.8	16.5
Growing degree days, °C d	1805	1530	1249	988	821	931
Day length, h						
Max.†	13.1	12.7	12.3	11.8	11.4	11.0
Min.†	10.2	10.2	10.2	10.2	10.2	10.2
Mean‡	11.2	11.0	10.8	10.7	10.6	10.8

† Absolute maximum or minimum value during growing period.

‡ Average of each sowing date.

beginning of anthesis and physiological maturity. For this purpose, a description of the developmental stages for chia was developed (Supplemental Table S1). At harvest, plant height, number of side shoots and inflorescences of three randomly chosen plants per plot were measured. For the determination of the harvest index, biomass, and seed yield of those plants were recorded. Then, all remaining plants from 10 m<sup>2</sup> were cut at the soil surface, sun-dried until the seed material was completely dry to be threshed out by hand. Absolute seed yield (SY) was determined by drying the sun-dried seeds for additionally 24 h at 40°C in a drying oven until constant weight. Since differences in plant density per plot were detected at harvest, these differences were later accounted for in the statistical analysis. Seed weight was determined by measuring the thousand kernel weight (TKW) from two replications per plot.

### Chemical Analysis of Seeds

#### Determination of Protein Content

The nitrogen content was determined by the Dumas method (Müller, 2017) using a Macro Analyzer Vario Macro Cube CHNS (Elementar Analysensysteme GmbH, Hanau, Germany). Then, the protein content was calculated with the conversion factor of 5.71 (Ayerza and Coates, 2011; Müller, 2017).

#### Oil Extraction and Determination

Seed samples of 20 g were ground for 30 s using an analytical mill (Type A 10; IKA, Staufen, Germany). Oil content was determined using a Soxhlet apparatus following the Commission Regulation (EC) No. 152/2009 procedure B (European Union, 2009). The method was modified as follows: An amount of 2.5 g of the sample was heated up with 100 mL of hydrochloric acid (3 mol L<sup>-1</sup>) for an hour. The residue was washed with cold water until a neutral filtrate was obtained, dried for 1 h in an oven at 95°C and submitted to an extraction (Soxhlet apparatus) for 6 h with light petroleum (100 mL, boiling range 30–50°C). The solvent was distilled off and the residue was dried for 1 h in a drying oven at 103°C. The content of crude fat was expressed as weight percentage on dry basis (% d.b.).

#### Determination of Fatty Acids

**Sample, chemicals, and equipment.** The oil sample was received by Soxhlet extraction of ground chia seeds as described above. Boron-trifluoride-methanol-complex solution (FLUKA 61626, CAS: 2802-68-8) was used. The two following methyl ester standard mixtures were used in which the identity and concentration of several major peaks are known: Marine Oil FAME Mix, 35066 (100 mg mL<sup>-1</sup>), including 20 FAMES (C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 (oleat.), C18:1 (vacc.), C18:2, C18:3, C20:0, C20:1, C20:2, C20:4, C20:3, C20:5, C22:0, C22:1, C24:0, C22:6, C24:1) and Marine Oil FAME Mix, 35021 (20 mg mL<sup>-1</sup>), containing 5 FAMES (C13:0, C15:0, C17:0, C19:0, C21:0). These two marine source fatty acid methyl esters were purchased from Restek (Restek Corporation, Bellefonte, PA). An internal standard of C19:0 (H<sub>38</sub>O<sub>2</sub>, MW 298.5; Sigma–Aldrich, Zwijndrecht, the Netherlands) was used. The analysis was performed by a gas chromatograph (GC-2010 Plus, Shimadzu, Nakagyo-ku, Kyōto, Japan) equipped with an auto sampler (AOC-201, Shimadzu, Nakagyo-ku, Kyōto, Japan) with an injection volume of 1.0 mL.

**Sample preparation.** The FAMES were determined according to the AOCS official method (Firestone, 1997). In brief, the fatty acids were split off by saponification with methanolic sodium hydroxide, and then methylated with boron trifluoride–methanol reagent (Jalali-Heravi and Vosough, 2004). The corresponding FAMES were extracted with hexane by adding salt solution for complete recovery (Eder, 1995). For determination of percentage of each component in the real sample, the prepared sample was directly injected in the GC-2010 column. The method was adapted as follows: 400 µg of extracted oil was treated with 0.5 mL of methanolic KOH (0.5 M) for 5 min at 80°C. After cooling, 1 mL of methanolic BF (FLUKA 61626, CAS: 2802-68-8) was added and heated for another 5 min at 80°C. Then, the reaction vials were cooled in an ice bath (10 min), 2 mL of saturated sodium chloride solution and 2 mL of *n*-hexane were added, and the organic phase including the FAMES was separated and 1 mL hexane solution of fames subjected to gas chromatographic analysis (Thurnhofer and Vetter, 2005).

**Gas chromatographic analysis.** In the gas chromatographic system, a FAMEWAX (polyethylene glycol) column (30 m by 0.25-mm inner diameter, 0.25-µm film thickness; Restek

Corporation, Bellefonte, PA) was used. The column temperature program was as follows: The initial column temperature was set to 180°C and programmed to increase in increments of 5°C min<sup>-1</sup> until 220°C was reached. This temperature (220°C) was held for 1 min followed by an increase of 5°C min<sup>-1</sup> until 240°C was reached. This temperature was held for 8 min then increased to 250°C and held for 4 min. The total run time was 26 min. A flame ionization detector at 250°C was used. Helium was taken as carrier with a total flow of 25.7 mL min<sup>-1</sup>. The results were expressed as the relative percentage of each individual fatty acid (FA) present in the sample.

### Mucilage Extraction and Determination

The method of mucilage extraction performed in previous studies (Muñoz et al., 2011; Campos et al., 2016) was adjusted as follows: Samples of 5 g of whole seeds were placed in a 250-mL beaker with distilled water in a seed/water ratio of 1:40. The pH was recorded by a pH meter (Mettler Toledo EL 20, Gießen), pH was adjusted and maintained at 8.0 using 0.2 M NaOH. The temperature of the water bath was maintained at 80 ± 1.5°C. The mixtures were stirred with a magnetic stirrer (Relax top, Heidolph, Schwabach) for better hydration. After 2 h, the aqueous suspension was spread on a drying tray and exposed to a temperature of 50°C for 10 h. The dried mucilage was separated from the seed by rubbing over a 0.5-mm mesh screen, and the weight was recorded. The difference to the weight at the beginning represents the weight of the mucilage.

### Statistical Analysis

For statistical analysis, different models were fitted accounting for the data collected either from individual plants or from one sampling area within each plot, respectively. Three plants per plot were measured for determining HI, plant height, and number of side shoots and inflorescences. Accordingly, the following model (Eq. [2]) was fitted:

$$y_{ijk} = \mu + b_i + \tau_j + p_{ij} + e_{ijk} \quad [2]$$

where  $y_{ijk}$  is the measurement on the  $k$ th plant of the  $j$ th sowing date in the  $i$ th block,  $\mu$  is the overall intercept,  $b_i$  is the effect of the  $i$ th complete block,  $\tau_j$  is the effect of the  $j$ th sowing date,  $p_{ij}$  is the random effect of the plot with the  $j$ th sowing date in the  $i$ th block with  $p_{ij} \sim N(0, \sigma_p^2)$ , and  $e_{ijk}$  is the residual error associated with  $y_{ijk}$  with  $e_{ijk} \sim N(0, \sigma_e^2)$ .

A linear mixed model with fixed effects for block and sowing date, and a random plot effect was fitted using the MIXED procedure of the statistical program SAS 9.4. The random plot effect was integrated to account for the fact that plants measured on the same plot constitute pseudo-replicates. The residual error corresponds to the effect of plants within plots (Piepho, 1997). Variance components were estimated by restricted maximum likelihood (REML). Model assumptions (i.e., normal distribution and homogeneity of variance) were graphically assessed based on plots of 'studentized' residuals. The normality assumption was assessed by QQ-plots, and histograms. Homogeneity of variance was evaluated by plots of residuals vs. predicted values. If necessary, the response variables were transformed to achieve the fulfillment of assumptions. The effect of the qualitative factor 'sowing date' was studied by Wald-type  $F$ -tests at a significance

level of 5%. In case of a significant Wald-type  $F$ -test, the levels of sowing date were compared by pairwise  $t$ -test. The multiple  $t$ -test controls the comparison-wise Type I error rate, but not the family-wise Type I error rate, which is controlled by other procedures such as the Tukey test. For a discussion of these issues and reasons favoring the  $t$ -test over alternatives see Saville (2015).

The other traits, such as SY, SYP, TKW, seeds per plant, seeds per inflorescence, protein, oil, and mucilage content, and FA, were assessed for all remaining plants together on each plot. However, the final plant density per plot varied. Thus, estimations were different in precision (Piepho, 1997). The number of plants per plot ( $k$ ) was known and was integrated into the model to improve the analysis. By using  $k$  as weighting factor for the observations (using the WEIGHT statement) the statistical package can differentiate between plot variance ( $\sigma_p^2$ ) and error variance ( $\sigma_e^2$ ), leading to more accurate estimates and tests (Piepho, 2018). In addition, number of plants per plot ( $k$ ) was integrated as covariate into the fixed part of the following model (Eq. [3]):

$$\bar{y}_{ij\bullet} = \mu + b_i + \beta k_{ij} + \tau_j + \beta_j k_{ij} + p_{ij} + \bar{e}_{ij\bullet} \quad [3]$$

where  $\bar{y}_{ij\bullet}$  is the average measurement of the  $j$ th sowing date in the  $i$ th block,  $\mu$  is the overall intercept,  $b_i$  is the effect of the  $i$ th complete block,  $\beta$  is the slope in a regression on the number of plants per plot,  $k_{ij}$  is the number of plants on the  $ij$ th plot,  $\tau_j$  is the effect of the  $j$ th sowing date,  $\beta_j$  is a sowing date specific deviation from the common slope,  $p_{ij}$  is the random effect of the plot with the  $j$ th sowing date in the  $i$ th block with  $p_{ij} \sim N(0, \sigma_p^2)$ , and  $\bar{e}_{ij\bullet}$  is the residual error associated with  $\bar{y}_{ij\bullet}$  with  $\bar{e}_{ij\bullet} \sim N(0, k_{ij}^{-1}\sigma_e^2)$ .

This model (Eq. [3]) was saturated with respect to the fixed model part and different variance-covariance structures were compared: a model without any weighting and plot effect; a model with weighting but no plot effect and a model with plot effect and weighting. The most appropriate variance covariance structure was selected based on the lowest AIC. The model (Eq. [3]) without weighting analysis of plants was also fitted to the duration of developmental stages (emergence, vegetative period, bud formation, flowering and ripening measured in GDD).

Finally, the relationships of seed yield per plant (SYP) and protein content, as well as protein content and oil content were explored to see how these traits were correlated with each other. Pearson correlation coefficients computed for two or more traits could possibly give insight about the association of traits, but they are suboptimal if data come from a designed experiment with blocking and treatment factors. Qualitative factors can interfere with correlations and can cause misleading conclusions. Piepho (2018) suggested the use of bivariate linear mixed models. Such models account for the treatment and blocking structure and correlation can be partitioned according to the various levels of the design. The following bivariate model (Eq. [4]) was used to explore the relationship of seed yield per plant and protein content:

$$\begin{pmatrix} y_{ij1} \\ y_{ij2} \end{pmatrix} = \begin{pmatrix} \mu_1 \\ \mu_2 \end{pmatrix} + \begin{pmatrix} b_{j1} \\ b_{j2} \end{pmatrix} + \begin{pmatrix} \tau_{i1} \\ \tau_{i2} \end{pmatrix} + \begin{pmatrix} e_{ij1} \\ e_{ij2} \end{pmatrix} \quad [4]$$

where  $y_{ij1}$  and  $y_{ij2}$  are the observations of the two traits on the same plot. Otherwise, the nomenclature follows that of models in Eq. [2] and [3]. Effect of blocks ( $b_{j1}, b_{j2}$ ), sowing dates ( $\tau_{i1}, \tau_{i2}$ ), and errors ( $e_{ij1}, e_{ij2}$ ) were assumed to follow a bivariate normal distribution and allowed to be correlated.

Several hypotheses about correlations in the bivariate model were tested using likelihood ratio tests. The tests were performed using the COVTEST statement in the GLIMMIX procedure. Correlations were tested individually for significance (tests with one degree of freedom) at the levels of block, sowing date and residual error. A test statistic ( $T$ ) was computed as the difference between the  $-2 \log$  likelihood of a model with and without correlation ( $\rho_{b(1,2)} = 0$ ,  $\rho_{\tau(1,2)} = 0$ ,  $\rho_{\epsilon(1,2)} = 0$ ). The  $p$ -value was computed from a  $\chi^2$  distribution with degree of freedom equal to the difference in number of parameters (Piepho, 2018). In addition, all correlations were simultaneously tested (test with three degrees of freedom) and homogeneity of correlations was tested by the computation of contrasts between pairs of correlations. If correlations of different effects within one model were found to be homogeneous, the marginal correlation was computed from the sum of all variances and covariances which were estimated in a model. If heterogeneity of correlations was found between the different effects, individual correlations on the level of each effect were interpreted, separately.

The relationship between onset of flowering and day-length at flowering was analyzed by a horizontal-linear-plus-quadratic model (similar to the quadratic-plus-plateau model; see Bullock and Bullock, 1994). The model was derived according to Sosa-Baldivia et al. (2017) and is given by  $y_i = \eta(d_i) + e_i$ , where  $y_i$  is the thermal time at onset of flowering for the  $i$ th sowing date,  $d_i$  is the prevailing day-length at onset of flowering for the  $i$ th sowing date,  $e_i$  is a residual error, and  $\eta(d)$  is defined as

$$\eta(d) = \begin{cases} \eta_1 = \alpha_1 & \text{for } d < \theta \\ \eta_2(d) = \alpha_2 + \beta_1 d + \beta_2 d^2 & \text{for } d \geq \theta \end{cases} \quad [5]$$

We impose two smoothness conditions for the function at the change point  $\theta$ :  $\eta_1 = \eta_2(\theta)$  and  $\eta_2'(\theta) = 0$ , where the prime denotes the first derivative. We can use the first condition  $\eta_1 = \eta_2(\theta)$  to replace  $\alpha_2$  by  $\alpha_2 = \alpha_1 - \beta_1 \theta - \beta_2 \theta^2$ . The second condition  $\eta_2'(\theta) = 0$  yields  $\beta_1 = -2\beta_2 \theta$ . These relations allow fitting the model by nonlinear least squares for parameters  $\alpha_1$ ,  $\beta_2$ , and  $\theta$ . Parameters were estimated using the NLIN procedure in SAS.

## RESULTS

### Plant Growth

Seed yield per plant (SYP) and per hectare (SY), harvest index (HI), and other yield components like TKW, numbers of inflorescences and side shoots per plants were analyzed according to the previously described models. At harvest, plant density varied across sowing dates (Table 2). The lowest plant density with 10 and 14 plants  $m^{-2}$  was recorded for SWD 1 and 2 and the highest plant density was observed for SWD 4 with 26 plants  $m^{-2}$ . When the covariate 'plants  $m^{-2}$ ' was not significant, it was excluded from the model (Table 2).

In general, TKW increased from early (SWD 1) to late sowing date (SWD 6), whereas SYP and seeds per plant decreased (Table 2). Seed yield and HI increased until SWD 4 and then decreased. Seed yield varied across sowing dates between 126 and 665  $kg\ ha^{-1}$ . The highest SY was achieved under SWD 4 and the lowest under the latest sowing (SWD 6), the latter being significantly lower than all other sowing

Table 2. Seed yield, thousand kernel weight, harvest index, number of side shoots, inflorescences and seeds per plant, and number of seeds per inflorescence, followed by the 95% confidence intervals (in parenthesis) for the six different sowing dates (SWD).

Sowing date	Plant density No. $m^{-2}$	Seed yield† kg $ha^{-1}$	Seed yield‡ plant $^{-1}$	1000 kernel weight† g	Harvest index†	Plant height‡ cm	Side shoots‡	Inflorescences†		Seeds per inflorescence†	
								No. plants $^{-1}$	No. plants $^{-1}$	No. plants $^{-1}$	No. plants $^{-1}$
SWD 1	10.7	378.29 (190.72;750.32) a§	36.93 (15.99;57.88)	1.26 (1.19; 1.33) c	0.24 (0.19;0.29) a	97.44 (87.35; 107.53) b	14.11 (12.07; 16.16)	36.33 (24.84;47.83)	3008.35 (1328.00;4688.6)	81.98 (33.52; 130.43)	
SWD 2	13.7	342.97 (187.86;626.15) a	28.12 (9.72;46.53)	1.25 (1.19; 1.31) c	0.24 (0.19;0.29) a	99.89 (89.80; 109.98) b	12.78 (10.73; 14.82)	34.44 (22.95;45.94)	2217.32 (740.39;3694.26)	66.60 (24.01; 109.19)	
SWD 3	17.3	414.24 (240.73;712.83) a	28.33 (11.72;44.93)	1.33 (1.27; 1.39) bc	0.28 (0.23;0.33) a	93.33 (83.24; 103.42) b	10.78 (8.73; 12.82)	27.89 (16.40;39.38)	2129.72 (797.90;3461.5)	78.35 (39.94; 116.75)	
SWD 4	25.7	664.94 (428.49; 1031.89) a	26.37 (12.93;39.81)	1.36 (1.31; 1.41) b	0.28 (0.23;0.33) a	102.44 (92.35; 112.53) b	13.22 (11.18; 15.27)	38.11 (26.92;49.60)	1939.55 (861.31; 3017.80)	50.90 (19.80;82.00)	
SWD 5	17.0	333.84 (194.26;573.72) a	20.63 (4.07; 37.19)	1.40 (1.34; 1.45) b	0.16 (0.10;0.21) b	118.00 (107.91; 128.09) a	12.78 (10.73; 14.82)	35.89 (24.40;47.38)	1477.70 (149.09;2806.3)	42.26 (3.95;80.58)	
SWD 6	22.0	125.91 (77.91; 203.49) b	4.53 (-10.14;19.21)	1.51 (1.46; 1.56) a	0.11 (0.06;0.16) b	97.67 (87.58; 107.76) b	13.11 (11.07; 15.16)	28.22 (16.73;39.71)	284.31 (-893.56;1462.1)	9.59 (-24.38;43.55)	
p-value of plant density		0.2659	0.0829	0.4182	—	—	—	—	0.0953	0.0360	
p-value of SWD		0.0053**	0.1275	0.0003***	0.0018**	0.0389*	0.2893	0.6258	0.1196	0.0897	

\* Significant at the 0.05 probability level based on the F-test.

\*\* Significant at the 0.01 probability level based on the F-test.

\*\*\* Significant at the 0.001 probability level based on the F-test.

† Means based on Eq. [3].

‡ Means based on Eq. [2].

§ Means in the same columns that share the same letter do not differ significantly at  $\alpha = 5\%$  by pairwise t test.

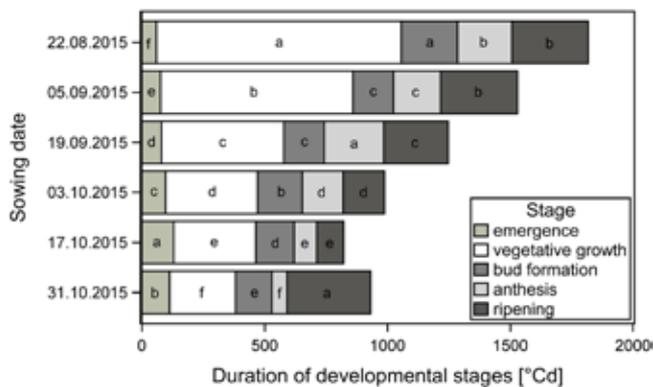


Fig. 2. Duration of developmental stages in growing degree days ( $^{\circ}\text{C d}$ ) of chia: emergence, vegetative growth, bud formation, anthesis and ripening for the six different sowing dates. Means of the duration of one developmental stage (computed by Eq. [3]) that share the same letter do not differ significantly at  $\alpha = 5\%$  by pairwise *t*-test.

dates ( $F = 6.77; p = 0.0053$ ). The highest SYP was recorded for SWD 1 ( $37 \text{ g plant}^{-1}$ ) and the lowest seed yield for the last sowing date ( $5 \text{ g plant}^{-1}$ ). Although this effect was not significant ( $F = 2.26; p = 0.1275$ ), there was a decreasing trend across sowing dates. The last sowing date (SWD 6) had a significantly higher TKW ( $1.51 \text{ g}$ ), whereas the early sowing dates (SWD 1 and 2) had the significantly lowest TKWs with  $1.26$  and  $1.25 \text{ g}$ , respectively ( $F = 14.02; p = 0.0003$ ) (Table 2). Earlier sowing (SWD 1 to 4) showed a significantly higher HI of  $0.24$  to  $0.28$  than later sowings (SWD 5 to 6) of  $0.11$  to  $0.16$ . Plant height was significantly higher for SWD 5 ( $118.0 \text{ cm}$ ) compared with the other dates ranging between  $93.3$  and  $102.4 \text{ cm}$  (Table 2). There was no significant effect of SWD on side shoots per plant ( $F = 1.45; p = 0.2893$ ) with an average of  $10$  to  $14$  across all sowing dates. Further, SWD did not significantly influence the number of inflorescences per plant ( $F = 0.72; p = 0.6258$ ) nor seeds per plant ( $F = 2.33; p = 0.1196$ ), nor the number of seeds per inflorescence ( $F = 2.57; p = 0.0897$ ). However, there was a tendency that with more seeds per plant for example under SWD 1 ( $3008.00$  seeds), consequently, more seeds per inflorescence ( $82.00$  seeds) were produced compared with SWD 4 ( $1939.55$  and  $50.90$  seeds, respectively). The number of plants per square meter was significant for seeds per inflorescence. Further, if more seeds were produced ( $3008$  seeds for SWD 1), seeds tended to be smaller as TKW was lower ( $1.26 \text{ g}$ ) compared with the later sowings.

### Plant Development

Sowing date significantly influenced the duration of all stages of development such as time to emergence ( $F = 806.33; p < 0.0001$ ), vegetative growth ( $F = 20763.1; p < 0.0001$ ), bud formation ( $F = 342.98; p < 0.0001$ ), anthesis ( $F = 3015.69; p < 0.0001$ ) and ripening ( $F = 5890.79; p < 0.0001$ ) (Fig. 2). Generally, the growing period became shorter from SWD 1 to 5. This effect was most prominent for the vegetative growth period. Chia seedlings needed about  $60$  to  $100^{\circ}\text{C d}$  for emergence. The duration of vegetative growth was longest for SWD 1 ( $996^{\circ}\text{C d}$ ) and shortest for SWD 6 ( $268^{\circ}\text{C d}$ ). The shortest duration of bud formation was recorded for SWD 6 ( $149^{\circ}\text{C d}$ ), whereas SWD 1 had the longest bud formation period ( $230^{\circ}\text{C d}$ ). Anthesis was shortest for SWD 6 ( $62^{\circ}\text{C d}$ ) and early sowing dates (SWD 1 to 3) showed the longest duration between  $193$  and  $242^{\circ}\text{C d}$ .

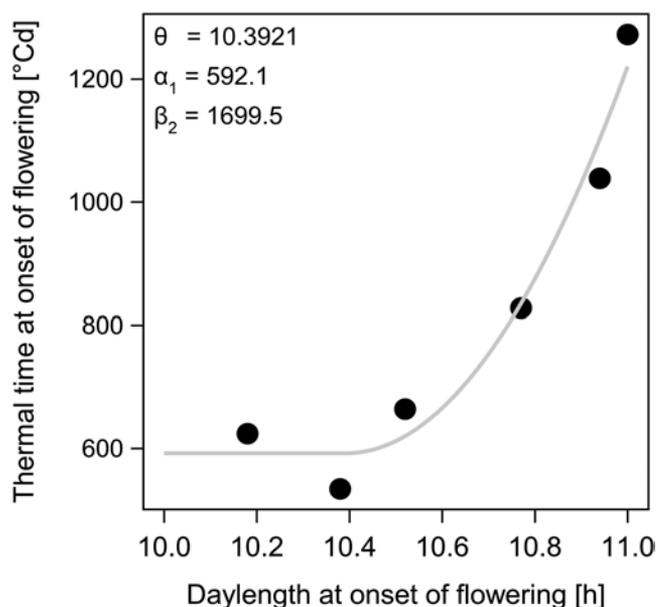


Fig. 3. Relationship between thermal time [ $^{\circ}\text{C d}$ ] and daylength [h] at the onset of flowering across six different sowing dates using Eq. [5].

Likewise, the duration of ripening decreased from SWD 1 and 2 toward SWD 5. However, SWD 6 showed the longest duration of ripening which was associated with several plants still being green and not fully ripe. Hence, the last stage of SWD 6 was prolonged.

The onset of flowering in GDD and the corresponding prevailing daylength showed a strong positive relationship for daylengths higher than  $10.4 \text{ h}$  (Fig. 3). Further, this model differentiates a phase of daylength where thermal time decreased with declining daylength. In this phase, the relationship of thermal time at onset of flowering and daylength was described by a quadratic slope ( $\beta_2$ ). In the second phase a further reduction of daylength did not reduce thermal time to initiate flowering. The daylength, which is the demarcation of these two phases ( $\theta$ ), was estimated to  $10.4 \text{ h}$  daylength with a thermal time of  $592^{\circ}\text{C d}$ .

### Chemical Composition of Seeds

Earlier sowing dates (SWD 1 to 4) generally resulted in lower protein contents than later sowing dates (SWD 5 and 6), whereas concerning the oil content, later sowing dates (SWD 5 and 6) showed lower oil contents than earlier sowing dates (SWD 1 to 4) (Fig. 4a-b). Sowing date showed a significant effect on protein content ( $F = 19.30; p = 0.0001$ ; Eq. [3]). The last sowing date showed the highest ( $26.03\%$ ) and SWD 1 the lowest protein content ( $18.04\%$ ) (Fig. 4a). Sowing date had also a significant effect on oil content ( $F = 6.56; p = 0.0077$ ). The lowest oil content ( $27.08\%$ ) was recorded under SWD 6 while higher oil contents ( $30.68$  to  $32.94\%$ ) were observed for the other sowing dates (Fig. 4b). There was no significant effect of SWD on mucilage content ( $F = 1.21; p = 0.3731$ ). The mucilage content was in the range between  $9.66$  and  $11.3\%$  across all dates (Fig. 4c).

In the bivariate analysis untransformed response variables were used, as residual plots did not show any departure from normality or homoscedasticity. The relationship of SYP and protein content was investigated using the model in Eq. [4]. The variance components at the levels of blocks were estimated to

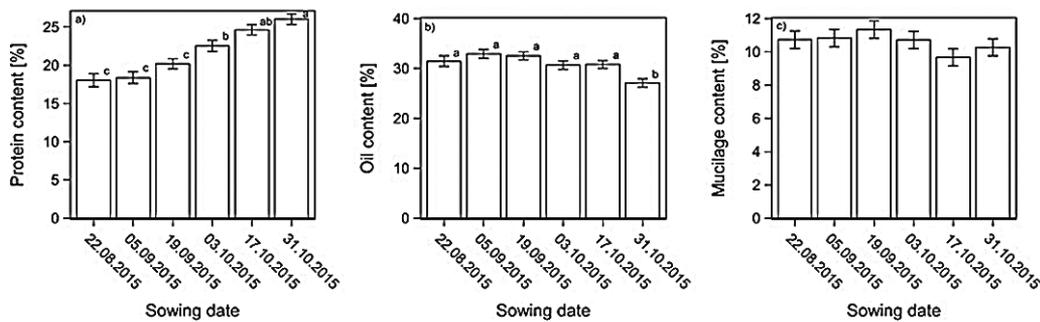


Fig. 4. Mean estimated contents of protein (a), oil (b), and mucilage (c) for the six different sowing dates (SWD) based on Eq. [3]. The bars represent the standard error. Means that share the same letter do not differ significantly at  $\alpha = 5\%$  by pairwise *t* test.

Table 3. Variance components for a bivariate mixed model fitted to seed yield per plant (SYP) and protein content (PRO), standard errors of estimates (SE), and likelihood ratio (LR) tests for correlations being equal to one.

Effect	Estimate†	SE	LR test‡
Block-specific variances ( $\sigma_{b(1)}^2, \sigma_{b(2)}^2, \rho_{b(1,2)}$ )	0	–	
Variance for PRO in SWD ( $\sigma_{\tau(1)}^2$ )	90.2683	69.3879	
Variance for SYP in SWD ( $\sigma_{\tau(2)}^2$ )	7.2385	4.1709	
Correlation of PRO and SYP in SWD ( $\rho_{\tau(1,2)}$ )	–1.000§	–	$T = 5.64, p = 0.0176$
Variance for PRO in residual ( $\sigma_{\epsilon(1)}^2$ )	2525.27	832.82	
Variance for SYP in residual ( $\sigma_{\epsilon(2)}^2$ )	30.3007	10.7859	
Correlation of PRO and SYP in residual ( $\rho_{\epsilon(1,2)}$ )	0.8098	0.09052	$T = 13.83, p = 0.0002$

† Covariance-parameter-estimates based on Eq. [4].

‡ *T* is the test statistic of a likelihood ratio test with the null hypotheses that  $\rho_{\tau(1,2)}$  and  $\rho_{\epsilon(1,2)}$  are equal to zero. *T* is computed from the difference of the  $-2 \log$  likelihood of two mixed models. In addition, a test for  $\rho_{\tau(1,2)}$  and  $\rho_{\epsilon(1,2)}$  being simultaneously zero was significant:  $T = 19.45, p < 0.0001$ . A test for homogeneous correlations for  $\rho_{\tau(1,2)}$  and  $\rho_{\epsilon(1,2)}$  was significant:  $T = 13.71, p = 0.0002$ .

§ Correlation converged toward the upper bound during REML iterations.

zero and therefore excluded from the model, as well as the correlation at the level of blocks. Table 3 shows the variance components estimated by Eq. [4] and *p*-value of the likelihood ratio tests. The correlations at the levels of SWD and residuals were significantly different ( $T = 13.71, p = 0.0002$ ) and were therefore interpreted separately. The correlation at the level of SWD was significantly different from zero ( $T = 5.64, p = 0.0176$ ) and negative ( $\rho_{\tau} = -1.000$ ). This negative relationship between protein content and SYP at the level of sowing dates implied a trade-off between protein content and SYP: (i) the higher the SYP the lower the protein content and (ii) early sowing dates resulted in higher SYP but lower protein content (Fig. 5). At the level of residual errors, the correlation between the two traits was positive ( $\rho_{\epsilon} = 0.8098$ ) (Table 3). Hence, at the levels of plots, the positive correlation between traits indicated that more fertile plots reached both higher protein contents and higher SYP.

Second, the bivariate model (Eq. [4]) was used to determine the correlation of oil and protein content. Block variances were estimated as zero and thus dropped from the model. Variance component estimates, as well as the marginal correlation, which is computed from the sum of effect variances and covariances, are shown in Table 4. Correlations indicated a negative relationship of protein and oil content ( $\rho_y = -0.4752$ ) (Table 4). Figure 6 presents the scatter plot of oil and protein content with a negative marginal correlation.

Concerning the FA profile, palmitic acid, stearic acid, and oleic acid generally increased across sowing dates, whereas alpha-linolenic acid and linoleic acid decreased. Hence, SFA content was highest (11.3%) for SWD 6, whereas SWD 1 to 5 showed lower contents (9.5–10.0%) (Table 5). For PUFA,

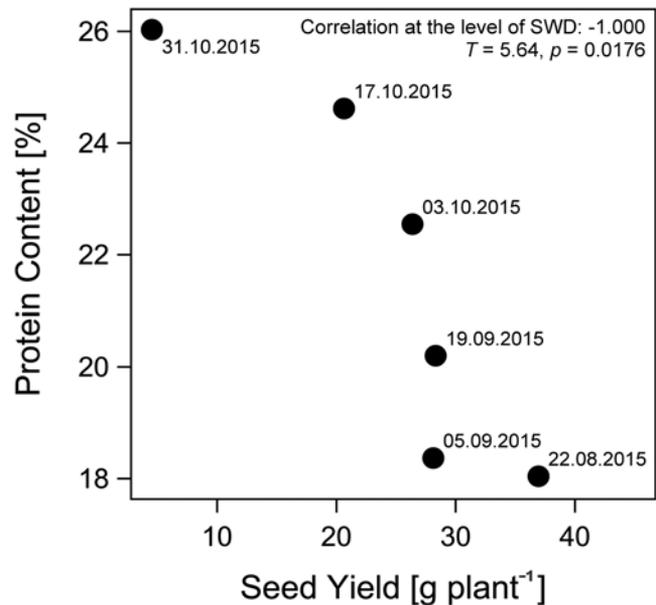


Fig. 5. Correlation between seed yield per plant ( $\text{g plant}^{-1}$ ) and protein content (%) across six different sowing dates. Means were estimated from Eq. [3] (see also Table 2; Fig. 4a). The correlation was estimated from Eq. [4] and tested for significance by a likelihood ratio test.

highest contents ( $\sim 84.0\%$ ) were recorded for sowing dates SWD 1 to 5 and the lowest content (81.5%) was observed for SWD 6. The PUFA/SFA ratio was higher for SWD 1 to 5 because the contents of PUFA were higher and SFA lower (Table 5). Palmitic acid ( $F = 3.54; p = 0.0421$ ), stearic acid ( $F = 14.91; p = 0.0002$ ), oleic acid including vaccinate acid ( $F = 5.45; p =$

Table 4. Variance components for a bivariate mixed model fitted to oil content (OIL) and protein content (PRO), standard errors of estimates (SE), and likelihood ratio (LR) tests for six different sowing dates (SWD).

Effect	Estimate†	SE	LR test‡
Block-specific variances and correlation ( $\sigma_{\delta(1)}^2, \sigma_{\delta(2)}^2, \rho_{\delta(1,2)}$ )	0	–	
Variance for OIL in SWD ( $\sigma_{\tau(1)}^2$ )	2.3095	1.8874	
Variance for PRO in SWD ( $\sigma_{\tau(2)}^2$ )	6.6199	4.6852	
Correlation of PRO and OIL in SWD ( $\rho_{\tau(1,2)}$ )	–0.8541	0.2127	$T = 3.60, p = 0.0578$
Variance for OIL content in residual ( $\sigma_{\epsilon(1)}^2$ )	65.9201	26.6681	
Variance for PRO in residual ( $\sigma_{\epsilon(2)}^2$ )	62.1343	25.4149	
Correlation of PRO and OIL in residual ( $\rho_{\epsilon(1,2)}$ )	–0.4564	0.2300	$T = 2.70, p = 0.1004$
Marginal variance for OIL ( $\sigma_y^2(1)$ )	68.2293		
Marginal variance for PRO ( $\sigma_y^2(2)$ )	68.7545		
Marginal correlation ( $\rho_y(1,2)$ )	–0.4752		

† Covariance-parameter-estimates based on Eq. [4].

‡  $T$  is the test statistic of a likelihood ratio test with the null hypotheses that  $\rho_{\delta(1,2)}$ ,  $\rho_{\tau(1,2)}$  and  $\rho_{\epsilon(1,2)}$  are equal to zero.  $T$  is computed from the difference of the  $-2 \log$  likelihood of two mixed models. In addition, a test for  $\rho_{\tau(1,2)}$  and  $\rho_{\epsilon(1,2)}$  being simultaneously zero was significant:  $T = 7.17, p = 0.0277$ . A test for  $\rho_{\tau(1,2)}$  and  $\rho_{\epsilon(1,2)}$  being equal was not significant:  $T = 0.98, p = 0.3222$ . Correlations were homogeneous and the marginal correlation ( $\rho_y = -0.4752$ ) could be computed which is largely dominated by the residual correlation.

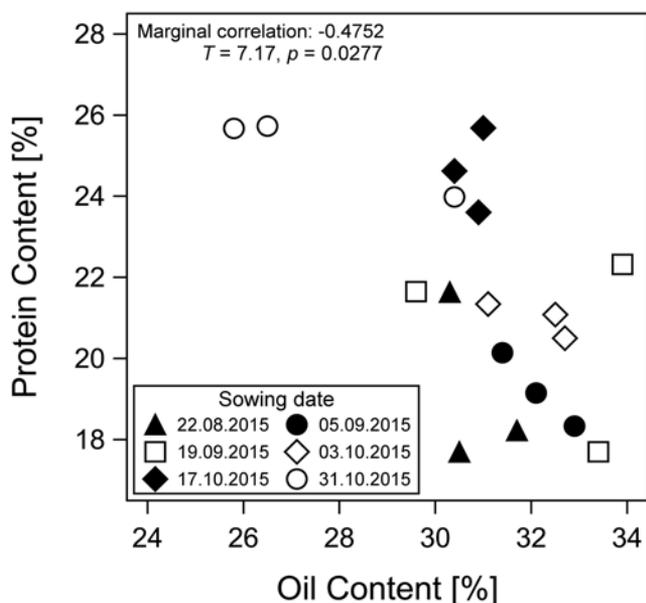


Fig. 6. Marginal correlation between oil content (%) and protein content (%) across six different sowing dates. Correlation was estimated from Eq. [4]. The correlation was tested for significance by a likelihood ratio test.

0.0112), linoleic acid ( $F = 10.42; p = 0.0010$ ), SFA ( $F = 6.95; p = 0.0048$ ), PUFA ( $F = 6.63; p = 0.0057$ ), and the PUFA/SFA ratio ( $F = 7.07; p = 0.0045$ ) were significantly influenced by SWD. Alpha-linolenic acid ( $F = 0.3; p = 0.6834$ ) and the ratio of alpha-linolenic to linoleic acid ( $F = 2.93; p = 0.0698$ ) did not show any significant effect.

## DISCUSSION

Chia is sensitive to daylength, and anthesis is normally induced by short days (Jamboonsri et al., 2012). Consequently, the growing period of chia is determined by the latitude where it is cultivated. In our study, the growing period in Egypt at  $30^{\circ}25' N$  varied between 120 and 150 d depending on SWD. Earlier sowings had longer growing periods with the most pronounced differences in the duration of the vegetative growth stage. In comparison, chia was harvested after 90 d in Columbia

( $04^{\circ}15' N$ ), whereas, sown in Argentina ( $23-28^{\circ}20' S$ ), a growing period of 160 d was similar to SWD 1 (Coates, 2011). At higher latitudes (i.e., Choele-Choele in Argentina [ $39^{\circ}11' S$ ] or Tucson, AZ, USA [ $32^{\circ}14' N$ ]), chia did not produce seeds, because frost killed the crop before the seed matured (Coates, 2011). Thus, grain production of chia seeds was originally restricted to latitudes of  $22^{\circ}55' N$  to  $25^{\circ}05' S$  (Baginsky et al., 2016). However, breeding efforts have generated long-day flowering genotypes to extend the cultivation of this crop to other temperate areas where chia is killed by frost before seed-set (Jamboonsri et al., 2012; de Falco et al., 2018).

The observed maximum SY was recorded for SWD 4 with  $664.94 \text{ kg ha}^{-1}$ , which is at a medium level compared to yields reported in the literature. Based on Coates (2011), commercial seed yields of chia can vary between 500 and  $600 \text{ kg ha}^{-1}$  in the countries of its origin. In an experimental study conducted in Ecuador, seed yields of  $446 \text{ kg ha}^{-1}$  ( $00^{\circ}03'26'' S$ , 2200 m elevation, 135 d growing period),  $1075 \text{ kg ha}^{-1}$  ( $00^{\circ}29'47'' N$ , 1621 m elevation, 130 d growing period), and  $1753 \text{ kg ha}^{-1}$  ( $01^{\circ}18'50'' S$ , 2042 m elevation, 125 d growing period) were achieved depending on location (Ayerza and Coates, 2009). In Argentina, seed yields between  $175 \text{ kg ha}^{-1}$  ( $27^{\circ}36' S$ , 248 m elevation, 170 d growing period) and  $1355 \text{ kg ha}^{-1}$  ( $25^{\circ}30' S$ , 858 m elevation, 173 d growing period) were recorded (Coates and Ayerza, 1996). Baginsky et al. (2016) reported seed yields of  $70 \text{ kg ha}^{-1}$  ( $33^{\circ}30' S$ , 12 m elevation, 160 d growing period) to  $2500 \text{ kg ha}^{-1}$  ( $18^{\circ}30' S$ , 230 m elevation, 135 d growing period) in Chile. Variations were due to different genotypes and site conditions implying an environment-genotype interaction (Ayerza, 2009). Further, seed yield also depends on plant density (Yeboah et al., 2014; Bochicchio et al., 2015b). For instance, Bochicchio et al. (2015b) reported seed yields of  $133.7 \text{ kg ha}^{-1}$  (low plant density of  $4 \text{ plants m}^{-2}$ ) and  $518 \text{ kg ha}^{-1}$  (high plant density of  $125 \text{ plants m}^{-2}$ ) in Italy. In this study, the final plant densities across sowing dates were comparatively low. Even though, plant density had no significant effect neither on SY nor SYP, SWD 4 had the highest plant density and highest SY compared to SWD 1 with the lowest plant density (Table 2). The compensation growth of SWD 1 in response to a low plant density shown by a larger SYP was not sufficient. This indicates that a higher plant density

might increase yields. Sowing dates in late October resulted in lower seed yields probably due to lower HI, seeds per plant and seeds per inflorescences. The SY of SWD 6 (125.91 kg ha<sup>-1</sup>) was dramatically reduced. This could be due to a short growing cycle and temperatures not being in the optimum range for chia during growth, flowering, and grain-filling stage. It is not unexpected that the lower temperatures affected growth and yield of chia, keeping in mind that the species is adapted to temperatures between 11 and 36°C with an optimum range between 16 and 26°C (Bochicchio et al., 2015a). Within this context, the very high temperatures during August and September might explain why the longest vegetative growth periods of the earliest sowing dates did not result in the highest SY by limiting plant growth or even resulting in plant death indicated by the lower plant densities for early sowings. Other authors reported that during harvest a great problem is the scalarity of flowering and maturation, where the central flower bud ripens while inflorescences of side shoots are still green (Bochicchio et al., 2015a). Waiting until all seeds are dry can increase the risk of seed losses due to rain, wind, or birds (Jamboonsri et al., 2012). These might be reasons for the high variability of SY, as well. Further, genetic variability of the seed material used can also increase variability of yield (Ayerza and Coates, 2009).

The relationship of thermal time and daylength at onset of flowering was described by the model in Eq. [5] (Fig. 3). In this study, we observed a threshold of 10.4 h daylength with a thermal time of 592°C d. A further reduction of daylength will not decrease the thermal time lower than 592°C d within the observed range. Under longer daylength conditions in Egypt, flowering of chia will occur considerably later when a definite quantity of GDDs was reached (Fig. 3). Another study conducted in Chile reported that photoperiods of less than 11.8 h defined the lower thermal requirement of chia plants to start flowering which corresponded to about 500°C d, while longer daylength increased the thermal time (600 and 700°C d) necessary to initiate anthesis. The quantitative differences are most probably related to genotypic differences (Baginsky et al., 2016).

The productivity of a crop is determined, not only by its photosynthetic efficiency, but also by the effective translocation of assimilates to the seeds. Several authors reported that HI of other crops was also affected by SWD (Cirilo and Andrade, 1994; Hirich et al., 2014; Bonelli et al., 2016). In this study, the highest HI values were recorded when chia was sown in August to September (0.24 and 0.28, respectively) and lowest values were obtained when chia was sown late in October (0.11). This might be due to the fact that less assimilates have been used for grain production resulting in lower HI which is in accordance with former findings (Baginsky et al., 2016; Hirich et al., 2014).

The economic return will finally be determined by seed yield and seed composition. Several factors may cause variations in seed composition, for instance, differences in the environment, nutrient availability, or cultivar selection. These factors influence basic metabolic processes of plants and thus affect seed protein, and oil quantity and quality (Ayerza, 2009). Crude protein contents of chia in this study were in the range of 18 to 26%, which is in line with other authors reporting contents between 20 and 23% (Ayerza, 2009; Ayerza and Coates, 2011; Mohd Ali et al., 2012). A longer growing period reduced seed crude protein, which is in accordance with former literature

Table 5. Fatty acid profile including saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) followed by the 95% confidence intervals (in parenthesis) for the six different sowing dates (SWD); †

Sowing date	C16:0	C18:0	C18:1	C18:2	C18:3	SFA†	PUFA†	ALA/LA ratio‡	PUFA/SFA ratio
SWD 1	7.51 (7.04; 7.98) b†	2.57 (2.32; 2.84) b	6.37 (5.77; 6.96) b	21.31 (20.56; 22.06) a	62.17 (60.29; 64.06)	10.14 (9.30; 10.98) b	83.49 (82.10; 84.88) a	2.92 (2.73; 3.11)	8.28 (7.54; 9.02) a
SWD 2	7.16 (6.68; 7.63) b	2.35 (2.14; 2.58) b	6.14 (5.61; 6.66) b	21.17 (20.51; 21.83) a	63.19 (61.53; 64.85)	9.51 (8.78; 10.25) b	84.35 (83.13; 85.57) a	2.99 (2.82; 3.16)	8.86 (8.22; 9.53) a
SWD 3	7.13 (6.66; 7.61) b	2.40 (2.20; 2.60) b	6.01 (5.54; 6.48) b	21.16 (20.57; 21.76) a	63.37 (61.88; 64.86)	9.46 (8.79; 10.12) b	84.54 (83.43; 85.63) a	3.00 (2.85; 3.15)	8.92 (8.33; 9.51) a
SWD 4	7.26 (6.79; 7.73) b	2.36 (2.20; 2.52) b	6.04 (5.66; 6.43) b	21.19 (20.71; 21.67) a	63.17 (61.96; 64.38)	9.61 (9.07; 10.15) b	84.35 (83.46; 85.24) a	2.98 (2.86; 3.10)	8.78 (8.30; 9.25) a
SWD 5	7.37 (6.90; 7.85) b	2.41 (2.21; 2.61) b	6.46 (5.99; 6.94) b	20.60 (20.00; 21.18) a	63.20 (61.70; 64.69)	9.75 (9.09; 10.41) b	83.79 (82.69; 84.89) a	3.07 (2.92; 3.22)	8.59 (8.00; 9.17) a
SWD 6	8.21 (7.73; 8.68) a	3.21 (3.01; 3.42) a	7.20 (6.78; 7.62) a	19.25 (18.72; 19.77) b	62.22 (60.89; 63.54)	11.34 (10.75; 11.93) a	81.46 (80.49; 82.44) b	3.23 (3.10; 3.37)	7.24 (6.72; 7.75) b
P-value	0.0421*	0.0002***	0.0112*	0.0010**	0.6834	0.0048**	0.0057**	0.6098	0.0045**

\* Significant at the 0.05 probability level based on the F-test.

\*\* Significant at the 0.01 probability level based on the F-test.

\*\*\* Significant at the 0.001 probability level based on the F-test.

† Data are given as weight percentage of total fatty acid methyl esters. Results are averages of duplicate analyses.

‡ SFA consists of palmitic acid and stearic acid, PUFA consists of linoleic and linolenic acid.

§ Ratio of alpha-linolenic acids to linoleic acids.

¶ Means in the same columns that share the same letter do not differ significantly at  $\alpha = 5\%$  by a pairwise t test.

(Bhargava et al., 2007) and matches with the negative relationship of SYP and protein content that was found in this study (Fig. 5). In general, increases in protein content are at the expense of yield. This can be explained physiologically by differences in source-sink relations for starch deposition and protein deposition during the grain filling stage (Jenner et al., 1991). As stated in a study of Fertilizer et al. (2012), assimilated assimilates will be transported to more sinks at higher yields and are thus less available for the formation of grain proteins. In this study, under early sowing more inflorescences and more seeds per plant were produced compared to later sowings (SWD 5 and 6), even though these differences were not statistically significant. In the literature for chia and for other crops, it is documented that a reduction of the protein content is related to the temperature during seed development. For example, as altitude decreased, temperature increased and the protein content of soybeans, sorghum and chia tended to increase (Ayerza, 2009, 2010). Further, there is a trade-off between oil content and crude protein in oilseeds. A shorter duration of vegetative growth (i.e., late sowing dates) leads to less carbon partitioning to oil and higher stores of protein within the seed (Pritchard et al., 2000). The oil contents in this study (27–33%) are in accordance with previous studies reporting values of 29 and 33% (Ayerza, 2009; Ixtaina et al., 2011; Mohd Ali et al., 2012). Other authors showed that later sowing dates had a negative effect on oil content due to less assimilates accumulated in the leaves (source) that can be transported to the seeds (sink) (De la Vega and Hall, 2002; Mirshekari et al., 2012; Gallardo et al., 2014). The oil content of chia seed contains one of the highest percentages of alpha-linolenic acid (n-3 PUFA; 62–64%) known in plants and linoleic acid (n-6 PUFA, 20%) (Ayerza and Coates, 2011; Mohd Ali et al., 2012). The ratio of alpha-linolenic to linoleic acid is likely to be around 15 to 20 in the present Western diet (Ansorena and Astiasarán, 2004). However, it should fall to below 5 or 4 (Wood et al., 2004) to avoid health problems induced by a high level of n-6 PUFA (Craig, 2004). Chia contains high amounts of n-3 FA and a favorable ratio of alpha-linolenic to linoleic acid (Ixtaina et al., 2011) which makes it very attractive as a functional food (Coelho and de las Mercedes Salas-Mellado, 2014). Comparing the results of chia seed oil composition in this study with those of Ayerza and Coates (2011), similar values for palmitic acid (7.13–8.21%) and stearic acid (2.35–3.21%), higher values for alpha-linolenic acid (62.17–63.37%), linoleic acid (19.25–21.31%), and PUFA (81.46–84.54%) and a lower value for oleic acid (6.01–7.20%) were achieved. Similar to our results, Ixtaina et al. (2011) categorized the FA in the following order of frequency: linolenic acid (C18:3) > linoleic acid (C18:2) > oleic acid (C18:1) > palmitic acid (C16:0) > stearic acid (C18:0). The relationship between elevation, FA composition and oil saturation known for chia is related to a temperature–elevation interaction since elevation showed a strong negative relationship with temperature. Our results are in accordance with previous studies (Flagella et al., 2002; Zheljzkov et al., 2009) which recorded lower PUFA but higher SFA contents for SWD 5 and SWD 6 due to an increase in oleic acid and a consistent decrease in linoleic acid. A reason is the synthesis or activation of oleate desaturase at low temperature and the reversible inhibition of this enzyme at high temperature (Flagella et al., 2002). In this study, linoleic acid tended to decrease as the level of alpha-linolenic acid increased

thus PUFA/SFA increased. This is in agreement with Ayerza (2009) who reported a significant negative relationship of alpha-linolenic contents with the more saturated 18-C fatty acids, oleic and linoleic. Chia seeds contain mucilage, which is part of the soluble dietary fiber (Capitani et al., 2013). Chia gum consists of polysaccharide composing of b-D-xylopyranosyl, a-D-glucopyranosyl, and 4-O-methyl-a-D-glucopyranosyluronic (Muñoz et al., 2011). The highest observed mucilage content in this study (11.33%) was lower than reported by Marin et al. (2008) (15.10%), higher than recorded by Muñoz et al. (2011) (6.97%) or Reyes-Caudillo et al. (2008) (6.00%) but similar to that of Segura-Campos et al. (2014) (10.90%). Our results showed values twice as high as compared to Reyes-Caudillo et al. (2008). Muñoz et al. (2011) reported that varied levels of yields usually depend on extraction and hydration methods. In this study the mucilage extraction was repeated thrice because the seeds could be soaked into water up to three times. Segura-Campos et al. (2014) used a centrifuge for gum extraction and Marin et al. (2008) developed a patent for exact mucilage determination. Diederichsen et al. (2006) reported a higher influence on mucilage content by genotype than by environment. Environmental influences on mucilage were observed by Dorrell and Daun (1980), who described that extreme humidity during the harvest period caused significantly lower mucilage content. None of the six sowing dates in the present study faced such unfavorable weather conditions during or immediately before harvest. The effects of different sowing dates influencing this trait could not be identified from our experiment. The differences found between the sowing dates were not significant.

An increasing population in Egypt demands not only an increase in food grain production, but also a shift toward environmentally sound sustainable agriculture. Chia could help to diversify and stabilize the local agricultural economy in semiarid environments. Results indicated that desert environments in the North of Cairo provided conditions for growth of chia. It was shown that seed yield and quality can be optimized by the choice of sowing date. Late sowing (SWD 5 and 6) hastened plant development. Sowing in the middle and end of September (SWD 3 and 4) resulted in higher seed yield, acceptable protein, oil, mucilage, and PUFA content, and a higher PUFA/SFA ratio compared to sowing in late October (SWD 5 and 6). Considering the obtained results, sowing dates between middle and end of September (SWD 3 and 4) are recommended to achieve a marketable seed quality and higher yields. This minimizes the possible negative impact of high temperature during vegetative growth and flowering for earlier sowings and of low temperatures during the critical flowering and seed filling periods for later sowings. In future, it should be carefully investigated if higher plant densities or the uses of other genotypes can further increase seed yield in Egypt. Making chia popular in Egypt would require dissemination of information about the crop among the farmers, proper marketing and efficient post-harvest technologies.

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### CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

### SUPPLEMENTAL MATERIAL

An additional supporting file can be found in the online version of this article. The supplemental material contains Supplemental Table S1, Description of developmental stages for chia.

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## 4 Nondestructive Leaf Area Estimation for Chia

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### Publication II

**Mack, L., Capezzone, F., Munz, S., Piepho, H.-P., Claupein, W., Phillips, T., & Graeff-Hönninger, S. (2017). Nondestructive Leaf Area Estimation for Chia. *Agronomy Journal*, 109(5), 1960–1969. doi:10.2134/agronj2017.03.0149.**

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*In Publication 1, results indicated that desert environments in the North of Cairo provided suitable conditions for growth of chia. It was shown that seed yield and quality could be optimized by the choice of sowing date. However, there are several other parameters that need to be optimized in cropping systems to achieve the maximum potential of a crop. To better comprehend this reemerged crop and to better adapt a crop growth model for chia, a nondestructive leaf area estimation model for chia was developed in the second publication. Accurate measurements of the leaf area of a crop are fundamental to understand the relation between crop growth and environmental factors. The leaf area of a crop is relevant for photosynthesis, light interception, water and nutrient use, crop growth, and yield potential and therefore, it is an important model input parameter. Thereby, leaf area can be determined either directly or indirectly and nondestructively by mathematical equations based on linear measurements. The latter method is less costly and much simpler to perform under equipment-limited experimental conditions like Egypt and the plant is not harmed, which is advantageous as plant density is low. For several crops such a leaf area model exists, but so far not for chia.*

# Nondestructive Leaf Area Estimation for Chia

Laura Mack,\* Filippo Capezzone, Sebastian Munz, Hans-Peter Piepho, Wilhelm Claupein, Tim Phillips, and Simone Graeff-Hönninger

## ABSTRACT

Leaf area (LA) is an important agronomic trait but is difficult to measure directly. It is therefore of interest to estimate LA indirectly using easily measured correlated traits. The most commonly used approach to predict LA uses the product of leaf width (LW) and leaf length (LL) as single predictor variable. However, this approach is insufficient to estimate LA of chia (*Salvia hispanica* L.) because the leaves are differently shaped depending on leaf size. The objectives of this study were to develop a nondestructive LA estimation model for chia using LW and LL measurements that can take differences in leaf shape into account and to determine whether population and nitrogen fertilizer level have an effect on the accuracy of a LA estimation model. A total of 840 leaves were collected from five different field experiments in 2015 and 2016 conducted in southwestern Germany. The experiments comprised eight populations of black- and white-seeded chia (07015 ARG, 06815 BOL, 06915 ARG, G8, G7, G3, W13.1, and Sahi Alba 914) and three nitrogen fertilizer levels (0, 20, and 40 kg N ha<sup>-1</sup>). We used meta-regression to integrate the data accounting for heterogeneity between experiments, populations, and nitrogen levels. The proposed LA estimation model with two measured predictor variables (LL and LW) was  $LA = 0.740 \times LL^{0.866} \times LW^{1.075}$  and provided the highest accuracy across populations and nitrogen levels [ $r = 0.989$ ; mean squared deviation (MSD) = 2.944 cm<sup>4</sup>]. The model  $LA = 1.396 \times LW^{1.806}$  with only LW was almost as accurate ( $r = 0.977$ ; MSD = 5.831 cm<sup>4</sup>).

## Core Ideas

- Leaf area in chia cannot be accurately predicted by the product of leaf width and length.
- Regressing leaf area log linearly on width and length accounts for change of shape with size.
- We provide accurate prediction models valid across experiments, populations, and N levels.
- Mixed-model meta-regression allows integrating leaf area data across experiments.

CHIA (*Salvia hispanica* L.) seed production has recently regained attention. The seeds are getting increasingly popular in Europe due to their beneficial nutritional and health-related properties, especially their high concentration of  $\alpha$ -linolenic acid (Ayerza and Coates, 2011; Mohd Ali et al., 2012). Chia seeds are used as a food supplement and are popular in vegetarian and gluten-free diets. In 2015, Europe imported about 11.838 tons of chia seeds worth 26 million Euro. Major suppliers to Europe are Paraguay (34%) and Argentina (24%) (Peperkamp et al., 2015). Chia is an annual herbal crop of the *Salvia* species (Lamiaceae) originating from the regions of west-central Mexico to northern Guatemala (Cahill, 2003; Muñoz et al., 2013). Improving its cultivation and adapting it to other regions requires the understanding of processes related to growth, development, and yield formation.

The leaf area (LA) of a plant is an important factor for radiation interception, processes of water and energy exchange, and crop growth and productivity. Therefore, precise measurements of LA are crucial to comprehend the relation between crop growth and environmental factors. Different methods can be used to determine total LA. Area meter and image analyzer methods offer the best trade-off between accuracy, variability, and required time for measurement (Beerling and Fry, 1990), but measurement equipment is expensive (Schwarz and Kläring, 2001). In addition, plant canopy is harmed (Lu et al., 2004), which might adversely affect subsequent measurements because it is not possible to conduct successive measurements on the same leaf in a nondestructive manner (Rouphael et al., 2006). One of the most often used nondestructive and indirect methods is the assessment of LA by mathematical equations based on linear measurements such as leaf length (LL) and leaf width (LW) and a combination of both (Blanco and Folegatti, 2005). This indirect method is less costly, is much simpler to perform, and produces precise LA estimates (Campbell and Norman, 1990). Especially under equipment-limited experimental conditions, this method enables an accurate in situ determination of LA.

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**Abbreviations:** LA, leaf area; LC, lack of correlation; LL, leaf length; LW, leaf width; MSD, mean squared deviation; NU, nonunity slope; OLA, observed leaf area; PLA, predicted leaf area; SB, squared bias.

In various studies with mathematical models, the leaf is defined as a simple geometric shape, and LA is determined via the product of its linear dimensions (i.e., LL and LW) using the formula (Daughtry, 1990):

$$LA = b \times LL \times LW \quad [1]$$

where  $b$  is a regression coefficient, determined by leaf shape.

Similar approaches are commonly used in prediction models for LA of several plant species, such as hazelnut (Cristofori et al., 2007), radish (Salerno et al., 2005), broccoli (Stoppani et al., 2003), cucumber (Blanco and Folegatti, 2005), eggplant (Rivera et al., 2007), zucchini squash (Rouphael et al., 2006), tomato (Schwarz and Kläring, 2001), and faba bean (Peksen, 2007). For chia, such a LA estimation model has not been developed. In none of the previous mentioned studies were several experiments summarized to perform one regression and to take into account the heterogeneity between the experiments. The integration of data from different experiments requires meta-analysis (Berkey et al., 1995; Van Houwelingen et al., 2002), which is becoming more commonly used in different areas of science (Jackson and Riley, 2014; Madden et al., 2016). Meta-analysis may be implemented using linear mixed models with random effects accounting for heterogeneity. Further, the use of k-fold cross validation (Bernal-Vasquez et al., 2014) leads to a robust assessment of predictive ability (Kohavi, 1995) but has not been performed in previous studies on LA estimation. Therefore, the objectives of this study were (i) to develop an LA estimation model for chia using leaf width and length measurements, (ii) to determine whether population and nitrogen fertilizer level have an effect on the accuracy of a LA estimation model for chia using meta-regression (Borenstein et al., 2009), and (iii) to assess the robustness of the model on an independent set of data by k-fold cross validation.

**Table 1. Details of the experiments conducted for model development and validation.**

Year	Experiment	Population	Nitrogen kg ha <sup>-1</sup>	Number of leaves
2015	1	G8	20	30
	1	07015 ARG	20	30
	1	06815 BOL	20	30
	1	06915 ARG	20	30
	2	G8	0	30
	2	G8	20	30
	2	G8	40	30
2016	3	G8	0	90
	3	G8	20	90
	3	G8	40	90
	3	W13.1	0	30
	3	W13.1	20	30
	3	W13.1	40	30
	4	G7	20	90
	4	G3	20	90
	5	Sahi Alba 914	20	90

## MATERIALS AND METHODS

### Experimental Site and Plant Material

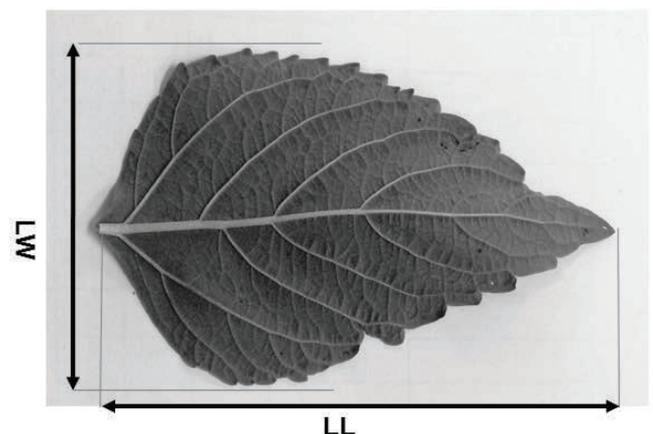
Chia leaves for all measurements and estimations were collected from five different field experiments, which were conducted in 2015 (21 May–11 November) and 2016 (1 June–18 October) at the experimental station Ihinger Hof of the University of Hohenheim (48°74'N, 08°92'E, 475 m a.s.l.) in southwestern Germany. The annual average temperatures were 10.1 and 9.1°C in 2015 and 2016, respectively, with annual precipitation of 545 and 647 mm, respectively. All five experiments were laid out in a randomized complete block design with three replicates. In each plot, eight rows of chia were sown with a row distance of 0.5 m and a sowing rate of 150 seeds m<sup>-2</sup>. Weeds were controlled manually by hoeing twice. The experiments comprised eight populations of black- and white-seeded chia (07015 ARG, 06815 BOL, 06915 ARG, G8, G7, G3, W13.1, and Sahi Alba 914) and three nitrogen fertilizer levels (0, 20, and 40 kg N ha<sup>-1</sup>). The different treatments applied within each experiment and the amount of collected leaves are listed in Table 1.

Experiments in 2015 included four populations with one nitrogen level (Experiment 1) and one population with three nitrogen levels (Experiment 2). In 2016, Experiment 3 had two populations with the same three nitrogen levels. Experiment 4 comprised two different populations and Experiment 5 comprised one population. Nitrogen was applied at a rate of 20 kg ha<sup>-1</sup>.

### Leaf Sampling

One single plant was randomly chosen within each plot at flowering stage. A total of 840 fully expanded leaves (with a minimum of 10 and a maximum of 30 leaves per plant) were randomly collected from the main stem and branches of the plants, resulting in a large range of leaf sizes (Table 1). Fresh leaves were detached carefully, put in plastic bags, and taken directly to the laboratory. Leaf length was measured along the midrib from the lamina tip to the point of intersection of the petiole, whereas LW was gauged from end to end between the widest expansions of the lamina meeting at a 90° angle to the lamina midrib (Fig. 1).

Measurements were performed with a ruler. Values of LL and LW were recorded to the nearest 0.1 cm. Leaf area was determined with a LI-3100 Area Meter (LI-COR) calibrated to 0.01 cm<sup>2</sup>.



**Fig. 1. Chia leaf showing the position of leaf length (LL) and width (LW) measurements.**

## Statistical Analysis

In a first step, the statistical analysis was performed separately for each of the five experiments. Linear models were set up according to the experimental designs of the single experiments with respect to blocks (complete replicates), populations, and nitrogen fertilizer levels. Because plants represented the randomization units, single leaf measures within a plant were pseudoreplicates. Therefore, linear mixed models with random plant effects were used for the prediction of leaf area from the linear measurements (Lawson, 2010; Piepho et al., 2003). The following model was fitted for Experiment 3, which had the form of a multiple linear regression of LA on LL and LW, with replicate- and treatment-specific intercepts and slopes. Response and predictor variables had to be logarithmically transformed for linearization and stabilizing the variance. The fitted model was:

$$\begin{aligned} \log(\text{LA}_{hijkl}) = & \mu + \beta_1 \log(\text{LL}_{hijkl}) + \beta_2 \log(\text{LW}_{hijkl}) + \\ & r_h + \beta_{3h} \log(\text{LL}_{hijkl}) + \beta_{4h} \log(\text{LW}_{hijkl}) + \\ & \tau_i + \beta_{5i} \log(\text{LL}_{hijkl}) + \beta_{6i} \log(\text{LW}_{hijkl}) + \\ & \gamma_j + \beta_{7j} \log(\text{LL}_{hijkl}) + \beta_{8j} \log(\text{LW}_{hijkl}) + \\ & (\tau\gamma)_{ij} + \beta_{9ij} \log(\text{LL}_{hijkl}) + \beta_{10ij} \log(\text{LW}_{hijkl}) + p_{hk} + e_{hijkl} \end{aligned} \quad [I]$$

where  $\log(\text{LA}_{hijkl})$  is the log-transformed LA measurement on the  $l$ -th leaf on the  $k$ -th plant in the  $h$ -th replicate with a combination of the  $i$ -th population and the  $j$ -th fertilizer level,  $\mu$  is the overall intercept,  $r_h$  is the effect of the  $h$ -th complete replicate,  $\tau_i$  is the effect of the  $i$ -th population,  $\gamma_j$  is the effect of the  $j$ -th nitrogen fertilizer level,  $(\tau\gamma)_{ij}$  is the interaction of population and fertilizer,  $\beta_1$  is the fixed slope for LL,  $\beta_2$  is the fixed slope for LW,  $\beta_{3h}$  is the replicate-specific slope for LL,  $\beta_{4h}$  is the replicate-specific slope for LW,  $\beta_{5i}$  is the population-specific slope for LL,  $\beta_{6i}$  is the population-specific slope for LW,  $\beta_{7j}$  is the fertilizer-specific slope for LL,  $\beta_{8j}$  is the fertilizer-specific slope for LW,  $\beta_{9ij}$  is the population- and fertilizer-specific slope for LL,  $\beta_{10ij}$  is the population- and fertilizer-specific slope for LW,  $p_{hk}$  is the random effect of the  $k$ -th plant in the  $h$ -th replicate with  $p \sim N(0, \sigma_p^2)$ ,  $e_{hijkl}$  is the residual error associated with  $\text{LA}_{hijkl}$  with  $e \sim N(0, \sigma_e^2)$ , and  $\text{LL}_{hijkl}$  and  $\text{LW}_{hijkl}$  are the measurements of LL and LW of the  $hijkl$ -th leaf.

The model of Experiment 3 had the most complex structure because it contained different populations and fertilizer levels (Table 1). For Experiments 1 and 4, Model [I] was adjusted by dropping fertilizer-related effects. Population-related effects were dropped for Experiment 2, and all treatment effects were dropped for Experiment 5 comprising only one population and fertilizer level. Models for individual experiments were fitted using the MIXED procedure of SAS 9.4. Variance components were estimated by restricted maximum likelihood. The Kenward–Roger method was used to approximate the denominator degrees of freedom and to adjust standard errors. Error degrees of freedom are adjusted downward and standard errors adjusted upward to account for the fact that variance components are also estimated from the data. This adjustment leads to more accurate  $F$ -tests (Littell et al., 2006). The effects of qualitative treatment factors (fertilizer level, population, and their interactions) were studied by sequential Wald-type  $F$ -tests, which test the significance of an effect accounting for all other effects fitted previously (tests of Type I hypotheses in

SAS). Nonsignificant effects were removed from the models. A significance level of 5% was used ( $\alpha = 5\%$ ). Model assumptions (i.e., normal distribution and homogeneity of variance) were graphically assessed based on studentized residuals by QQ plots, histograms, and the plots of residuals versus predicted values.

To determine a prediction equation for LA derived from linear leaf measurements, the data of all experiments were pooled and analyzed by mixed-model meta-regression (Borenstein et al., 2009; Sauer et al., 2008). Meta-analysis allows combining the results of different studies in one statistical analysis to obtain valid inferences (Van Houwelingen et al., 2002). This analysis was conducted using the HPMIXED procedure because long computation times and convergence problems impeded the use of the MIXED procedure. Random experiment-, treatment-, replicate-, and plant-specific slopes were fitted to account for heterogeneity. The model was defined in the following way:

$$\begin{aligned} \log(\text{LA}_{ghijkl}) = & \mu + \beta_1 \log(\text{LL}_{ghijkl}) + \beta_2 \log(\text{LW}_{ghijkl}) + \\ & \mu_{2g} + \beta_{3g} \log(\text{LL}_{ghijkl}) + \beta_{4g} \log(\text{LW}_{ghijkl}) + \\ & \mu_{3ij} + \beta_{5ij} \log(\text{LL}_{ghijkl}) + \beta_{6ij} \log(\text{LW}_{ghijkl}) + \\ & \mu_{4gh} + \beta_{7gh} \log(\text{LL}_{ghijkl}) + \beta_{8gh} \log(\text{LW}_{ghijkl}) + \\ & \mu_{5ghk} + \beta_{9ghk} \log(\text{LL}_{ghijkl}) + \beta_{10ghk} \log(\text{LW}_{ghijkl}) + \\ & e_{ghijkl} \end{aligned} \quad [II]$$

where  $\log(\text{LA}_{ghijkl})$  is the log-transformed LA measurement on the  $l$ -th leaf on the  $k$ -th plant in the  $h$ -th replicate in the  $g$ -th experiment with the  $ij$ -th combination of population and fertilizer;  $\mu_1$  is the intercept;  $\beta_1$  is the fixed slope for LL;  $\beta_2$  is the fixed slope for LW;  $\mu_{2g}$ ,  $\mu_{4gh}$ , and  $\mu_{5ghk}$  are the experiment-, replicate-, and plant-specific random intercepts, respectively;  $\beta_{3g}$ ,  $\beta_{7gh}$ , and  $\beta_{9ghk}$  are the experiment-, replicate-, and plant-specific random slopes for LL, respectively;  $\beta_{4g}$ ,  $\beta_{8gh}$ , and  $\beta_{10ghk}$  are the experiment-, replicate-, and plant-specific random slopes for LW, respectively;  $\mu_{3ij}$ ,  $\beta_{5ij}$ , and  $\beta_{6ij}$  are the random intercept and slopes for the  $ij$ -th combination of population and fertilizer; and  $e_{ghijkl}$  is the residual error associated with  $\text{LA}_{ghijkl}$ .

Unstructured correlations between random intercepts and slopes for the same type of effect (i.e., experiment, replicate, treatment, or plant) were allowed to guarantee that random regression parameters are invariant to a change in scale of the regressor variables (Piepho and Ogutu, 2002). Details are described in the Supplemental Material S3.

Model [II] was simplified as follows when regressing  $\log(\text{LA})$  on  $\log(\text{LL})$  only:

$$\begin{aligned} \log(\text{LA}_{ghijkl}) = & \mu + \beta_1 \log(\text{LL}_{ghijkl}) + \\ & \mu_{2g} + \beta_{3g} \log(\text{LL}_{ghijkl}) + \\ & \mu_{3ij} + \beta_{5ij} \log(\text{LL}_{ghijkl}) + \\ & \mu_{4gh} + \beta_{7gh} \log(\text{LL}_{ghijkl}) + \\ & \mu_{5ghk} + \beta_{9ghk} \log(\text{LL}_{ghijkl}) + \\ & e_{ghijkl} \end{aligned} \quad [III]$$

The corresponding Model [IV] when regressing  $\log(\text{LA})$  on  $\log(\text{LW})$  alone was derived by replacing  $\log(\text{LL})$  from Model [III] by  $\log(\text{LW})$ .

A fivefold cross-validation procedure was used to estimate the predictive ability of Models [II], [III], and [IV]. The entire dataset, comprising 840 observations, was randomly split into five subsets, each consisting of 168 observations. Sampling was stratified by experiment to ensure that each experiment was equally represented in the five subsets (see SAS code available as Supplemental Material). The prediction equation was estimated using four subsets, and LA was predicted for the remaining subset. In case of convergence problems, which occurred only in some cases with Model [II], the variance components of the analysis of the entire dataset were used, and no iterations were performed (see SAS code available as Supplemental Material). Otherwise, variance components were re-estimated in each of the five validations. In cases where convergence was achieved in the analysis of folds, we verified that results were very similar between analyses with re-estimated variances and with variances fixed at values obtained from analysis of the complete dataset (results not shown; see SAS code available as Supplemental Material). The predictive ability was assessed by Pearson's correlation coefficient ( $r$ ) between predicted leaf area (PLA) and observed leaf area (OLA). In addition, OLA was regressed on PLA. For high predictive ability, intercepts were expected to be 0 and slopes to be 1. Mean squared deviation (MSD) and its constituent components [lack of correlation (LC), nonunity slope (NU), and squared bias (SB)] were computed according to Gauch et al. (2003).

## RESULTS

### Evaluation of Single Experiments

In the present study, LL (cm), LW (cm), their ratio (LL/LW), and LA (cm<sup>2</sup>) of eight different chia populations at different nitrogen levels were recorded (data not shown; see Supplemental

Material S1). The South American populations 07015 ARG, 06815 BOL, and 06915 ARG had the largest leaves, with LA between 22.7 and 18.5 cm<sup>2</sup> and a length to width ratio (LL/LW) ranging from 1.53 to 1.64. The populations G8, G7, and G3 produced more narrow leaves (LL/LW was in the range of 1.71 and 1.91). The white-seeded chia populations Sahi Alba 914 and W13.1 exhibited a LL/LW ratio between 1.71 and 1.77 (data not shown; see Supplemental Material S1).

Equation [1] is the most plausible and commonly used one for the prediction of LA and was therefore initially considered for analyzing the five single experiments. The plot of studentized residuals versus predicted values showed an increased variance with increasing mean (results not shown; see SAS code available as Supplemental Material). Thus, a logarithmic transformation of Eq. [1] was considered to stabilize the variance:

$$\log(\text{LA}) = \log(b) + \log(\text{LL}) + \log(\text{LW}) \quad [2]$$

Equation [2] suggests that a multiple linear regression of  $\log(\text{LA})$  on  $\log(\text{LL})$  and  $\log(\text{LW})$  could be used to estimate the parameter  $b$  in Eq. [1] and that slope estimates near unity are to be expected. We analyzed individual experiments using Model [I], which is an adaption of Eq. [2] accounting for replicate and treatment effects and allowing slopes to differ from unity. The log-transformation stabilized the variance (residual plots not shown) and was used for all single experiments. In Experiment 3, significant main effects for population ( $p = 0.0015$ ) and fertilizer ( $p = 0.0280$ ) were present (Table 2). In Experiment 2, fertilizer-specific slopes for LL ( $p = 0.0411$ ) and for LW ( $p = 0.0450$ ) were found (Table 3).

All other experiments did not show any significant population- and fertilizer-specific slopes in the  $F$ -tests (Supplemental Tables S2.1, S2.2, and S2.3).

**Table 2.** Sequential  $F$ -tests of Experiment 3, including numerator and denominator degrees of freedom and  $F$  and  $p$  values of each effect in the model.

Effect	Explanation of effects	Numerator degrees of freedom†	Denominator degrees of freedom†	$F$ value	$p$ value
$\mu$	overall intercept	1	6.38	268.723	<0.0001
$r_h$	replicate effect	2	5.51	742.12	<0.0001
$\tau_i$	population effect	1	22.8	13.06	0.0015
$\gamma_j$	nitrogen fertilizer effect	2	6.07	6.82	0.0280
$(\tau\gamma)_{ij}$	interaction of population and nitrogen fertilizer	2	17.3	0.49	0.6224
$\beta_1$	common slope for LL	1	277	180.05	<0.0001
$\beta_2$	common slope for LW	1	244	694.01	<0.0001
$\beta_{3h}$	replicate-specific slope for LL	2	324	9.37	<0.0001
$\beta_{4h}$	replicate-specific slope for LW	2	299	11.78	<0.0001
$\beta_{5i}$	population-specific slope for LL	1	339	0.37	0.5446
$\beta_{6i}$	population-specific slope for LW	1	338	0.01	0.9190
$\beta_{7j}$	fertilizer-specific slope for LL	2	307	2.82	0.0612
$\beta_{8j}$	fertilizer-specific slope for LW	2	299	2.71	0.0682
$\beta_{9ij}$	population- and fertilizer-specific slope for LL	2	333	1.39	0.2495
$\beta_{10ij}$	population- and fertilizer-specific slope for LW	2	333	1.30	0.2729

† Restricted maximum likelihood-based mixed model software computes so-called Wald-type  $F$ -tests, which make use of generalized least squares estimators of fixed effects (Littell et al., 2006). Computations involve no sums of squares or mean squares, which is why this table does not provide these statistics. Instead, we report both the numerator and the denominator degrees of freedom. The denominator degrees of freedom are important with mixed models because they need to be approximated and generally vary between different fixed effects. We used the Kenward-Roger method for this purpose.

## Meta-Regression Over All Experiments for Prediction of Leaf Area

The data of all five experiments were pooled to estimate a common prediction equation for LA including random experiment-, replicate- and plant-specific intercepts and slopes. Because Experiment 3 had a significant effect of fertilizer and population and made up almost 40% of the data, random experiment-specific slopes for the combinations of population and fertilizer were added (Model [II]).

### Regression on Leaf Width and Leaf Length

If Eq. [1] holds true, then from Eq. [2] we would expect the two linear slopes of log-transformed LL and LW of Model [II] not to be significantly different from 1. Fitting Model [II], however, the slope for log(LL) was significantly different from 1. The intercept was  $-0.301$  (95% confidence limits,  $-0.385$  to  $-0.217$ ), and the estimated slopes for log(LL) and log(LW) were  $0.866$  ( $0.756$  to  $0.977$ ) and  $1.075$  ( $0.971$  to  $1.180$ ), respectively (see Supplemental Material S3 and SAS code available as Supplemental Material). The significant departure from unity of the slope for log(LL) indicates that Eq. [2] is insufficient to predict LA. Therefore, Eq. [1] was extended as

$$LA = b \times LL^c \times LW^d \quad [3]$$

where  $c$  and  $d$  are additional regression parameters. Equation [3] can be linearized by taking the logarithm:

$$\log(LA) = \log(b) + c \times \log(LL) + d \times \log(LW) \quad [4]$$

The parameters  $c$  and  $d$  in Eq. [3] and [4] provide a more flexible model for the prediction of the LA of chia than Eq. [1] and [2]. Model [II] is a representation of Eq. [4], accounting for random effects of experiments, replicates, treatments, and plants. Fitting Model [II], Eq. [4] was estimated as:

$$\log(LA) = -0.301 + 0.866 \times \log(LL) + 1.075 \times \log(LW)$$

or on the back-transformed scale as:

$$LA = 0.740 \times LL^{0.866} \times LW^{1.075}$$

A final remark on linear model assumptions in the previously shown computations: studentized residuals of fitted Models [I] and [II] had a reasonably symmetric distribution, with slightly heavier tails than the normal. There was no indication of heterogeneity of variance. Thus, distributional assumptions for the residuals in our linear mixed models were deemed approximately tenable (see Supplemental Material S3, S4, and S5).

### Regression on Leaf Width or Leaf Length

The findings presented in the previous section showed that the Eq. [1] or [2] were inappropriate to predict LA, and therefore Eq. [3] or [4] was used instead.

Next, we consider the prediction of LA by only one of the two predictor variables LL and LW. For expressing LA or log(LA) by only one of the two predictors, we need to determine the relationship between them to express LL by LW and vice versa. These expressions can then be used in Eq. [3] or [4] to obtain models regressing LA on LL or on LW only.

The simplest situation occurs when LL and LW are proportional, implying a linear relationship (Fig. 2a).

A linear relationship would imply that leaves can be assumed to have the same shape and symmetry regardless of leaf size and that LA would consequently vary only as a result of proportional enlargement or reduction of this fixed shape. To test whether the relationship of two variables  $y$  and  $x$  is linear, a second-degree polynomial is fitted ( $y = \alpha + \beta x + \beta x^2$ ). If the quadratic term is significant, the relationship of  $y$  and  $x$  is not linear. Thus, the relationship of LL and LW was tested for linearity in a model with untransformed LL as response variable and untransformed LW as predictor variable (Fig. 2a). A quadratic term for LW was introduced into this model. The quadratic term was significant ( $F = 10.59$ ;  $p = 0.001$ ); that is, the relationship between LL and LW was not linear. On the contrary, the quadratic term for LL was not significant ( $F = 0.02$ ;  $p = 0.889$ ) when LW was the response variable. The significant quadratic term in the regression of LL on LW suggests that a simple linear relationship such as

**Table 3.** Sequential  $F$ -tests of Experiment 2, including numerator and denominator degrees of freedom and  $F$  and  $p$  values of each effect in the model.

Effect	Explanation of effects	Numerator degrees of freedom†	Denominator degrees of freedom†	$F$ value	$p$ value
$\mu$	overall intercept	1	2.97	416,713	<0.0001
$r_h$	replicate effect	2	2.97	200.09	0.0007
$\gamma_j$	nitrogen fertilizer effect	2	4.28	0.77	0.5178
$\beta_1$	common slope for LL	1	67.3	22,153.3	<0.0001
$\beta_2$	common slope for LW	1	62.2	363.90	<0.0001
$\beta_{3h}$	replicate-specific slope for LL	2	68.7	0.52	0.5952
$\beta_{4h}$	replicate-specific slope for LW	2	30.9	0.02	0.9781
$\beta_{5j}$	fertilizer-specific slope for LL	2	56.1	3.38	0.0411
$\beta_{6j}$	fertilizer-specific slope for LW	2	54.9	3.28	0.0450

† Restricted maximum likelihood-based mixed model software computes so-called Wald-type  $F$ -tests, which make use of generalized least squares estimators of fixed effects (Littell et al., 2006). Computations involve no sums of squares or mean squares, which is why this table does not provide these statistics. Instead, we report both the numerator and the denominator degrees of freedom. The denominator degrees of freedom are important with mixed models because they need to be approximated and generally vary between different fixed effects. We used the Kenward–Roger method for this purpose.

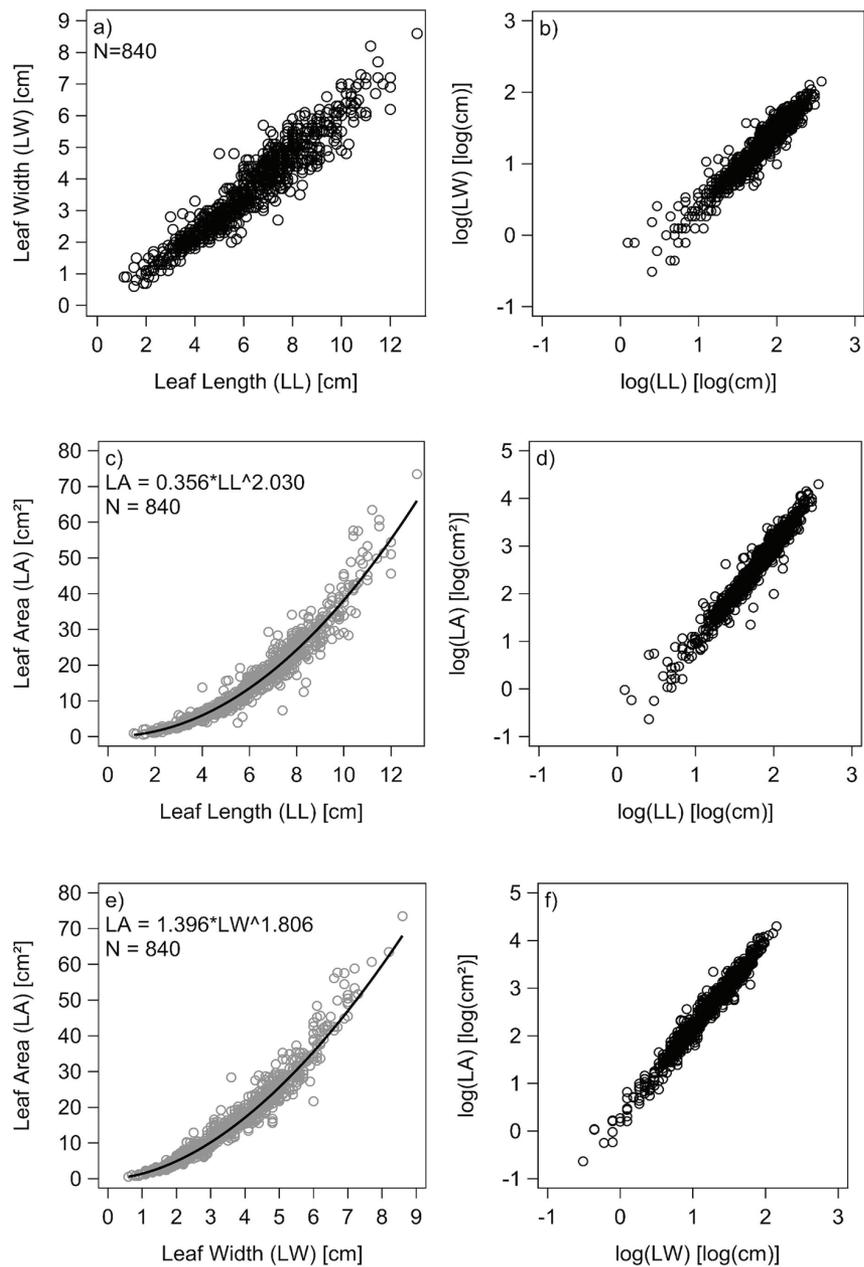


Fig. 2. Relationships of leaf length (LL), leaf width (LW), and leaf area (LA) of chia on the untransformed scale (a, c, and e) and on the log-scale (b, d, and f).

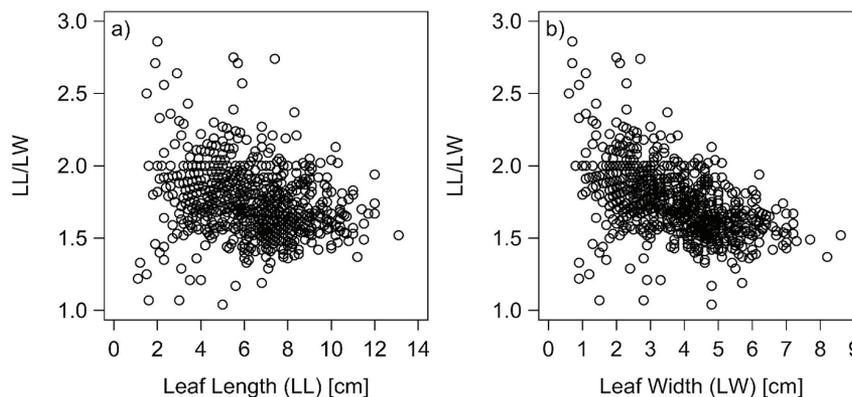


Fig. 3. Change of the leaf length (LL) to leaf width (LW) ratio (LL/LW) with increasing leaf size of chia. The LL/LW ratio is plotted against LL (a) or LW (b) as proxies for leaf size.

$$LW = q \times LL \quad [5]$$

is inappropriate. This is corroborated by fact that the slope of  $\log(LL)$  was found to be significantly different from 1 in Eq. [4]. To allow for nonlinearity, a modification analogous to Eq. [3] may be used, given by

$$LW = q \times LL^p \quad [6]$$

Equation [6] can be linearized by taking the logarithm:

$$\log(LW) = \log(q) + p \times \log(LL) \quad [7]$$

or

$$\log(LL) = \log(m) + n \times \log(LW) \quad [8]$$

First, we fitted Eq. [7] (Fig. 2b) and Eq. [8] and added a quadratic term, which was not significant ( $F = 2.80$ ;  $p = 0.094$  and  $F < 0.001$ ;  $p = 0.975$ ), showing that linearization was successful. This implied a log-linear relationship of LA and LW (Fig. 2f), as can be shown by inserting Eq. [8] into Eq. [4]:

$$\begin{aligned} \log(LA) &= \log(b) + c \times [\log(m) + n \times \log(LW)] + d \\ &\times \log(LW) = [\log(b) + c \times \log(m)] + (d + c \times n) \times \\ &\log(LW) = h + k \times \log(LW) \end{aligned} \quad [9]$$

Analogously, Eq. [7] can be inserted into Eq. [4] to yield:

$$\log(LA) = f + g \times \log(LL) \quad [10]$$

The parameters of Eq. [9] and [10] were estimated by the Models [III] and [IV] (see Supplemental Material S4 and S5; see also SAS code available as Supplemental Material). Equation [9] was estimated as  $\log(LA) = 0.334 + 1.806 \times \log(LW)$  (Fig. 2f), which was back-transformed to  $LA = 1.396 \times LW^{1.806}$  (Fig. 2e). Equation [10] was estimated as  $\log(LA) = -1.032 + 2.030 \times \log(LL)$  (Fig. 2d) and back-transformed to  $LA = 0.356 \times LL^{2.030}$  (Fig. 2c).

These results suggest that leaf shape changes with the size of the leaf. This is also confirmed by observing that the ratio of LL and LW decreased with increasing LW or LL (Fig. 3).

Interpreting LW and LL as proxies for leaf size, we may conclude that the LL/LW changed over time. However, this pattern was much stronger when LL/LW was plotted versus LW (Fig. 3b) than versus LL (Fig. 3a).

### Cross-Validation of the Fitted Models

All three models (LL and LW, LW, and LL) performed well in terms of predictive ability assessed by fivefold cross validation (Table 4).

Correlation coefficients ( $r$ ) indicated that PLA and OLA were strongly correlated in all five validations. All models produced correlation coefficients  $\geq 0.961$ . On average, the correlation of OLA and PLA increased in the order of LL as predictor, to LW, and to LL and LW (0.970, 0.977, and 0.989, respectively). Accordingly, MSD decreased from  $7.455 \text{ cm}^4$  (LL), to  $5.831 \text{ cm}^4$  (LW), and to  $2.944 \text{ cm}^4$  (LL and LW).

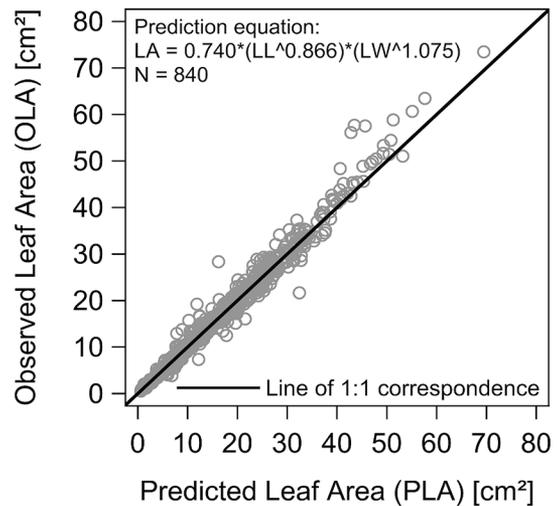


Fig. 4. Observed leaf area versus predicted leaf area using the equation  $LA = 0.740 \times LL^{0.866} \times LW^{1.075}$ , where LA is individual leaf area ( $\text{cm}^2$ ), LL is the leaf length (cm), and LW represents the leaf width (cm). The solid line shows the line of 1:1 correspondence.

Lack of correlation (LC) made up the greatest part of MSD. Recall that MSD consists of three components, one of them being LC, the incongruence between OLA and PLA, which is due to scatter (Gauch et al., 2003). In most cases, the regressions of OLA on PLA had intercepts nonsignificantly different from 0 and slopes nonsignificantly different from 1 (Table 4).

As cross-validation showed, LA can be well predicted by taking LL and LW measurements. Figure 4 shows the high correspondence of OLA and PLA in the prediction of LA by LL and LW.

## DISCUSSION

Leaf area is an important parameter for most physiological and agronomic studies concerning plant growth, light interception, and reaction to fertilizers (Blanco and Folegatti, 2005). In this study, a model that contains both measured predictor variables (LL and LW) resulted in the highest predictive ability for LA (Table 4). This outcome is in agreement with previous studies on nondestructive LA estimation (Cristofori et al., 2007; Kumar and Sharma, 2010; Salerno et al., 2005). From an economic point of view, however, because time is always a limiting factor in experimental work, it would be preferable to reduce measuring time by predicting LA with only one leaf measurement. This was done in previous studies (Cristofori et al., 2007; Kumar, 2009; Rouphael et al., 2006, 2007; Salerno et al., 2005; Stoppani et al., 2003; Williams and Martinson, 2003). Our results were in accordance with the previous findings showing that LL was less adequate for LA prediction ( $r = 0.970$ ) than LW ( $r = 0.977$ ) or the multiple regression on both LL and LW ( $r = 0.989$ ) (Table 4). However, these studies modeled the relationship of LA with LL and LW as linear regressions with non-zero intercept. In the present study, a power model (Eq. [3]), which passes through the origin, was used. Schwarz and Kläring (2001) and Williams and Martinson (2003) used power models as well. Kumar (2009) considered an exponential LA estimation model.

A salient feature of our regression models is that random effects are used to account for heterogeneity due to differences between experiments, replicates, plants, and treatments.

Table 4. Predictive ability of models using leaf length (LL), leaf width (LW), and both measures to predict leaf area (LA) of chia in a fivefold cross-validation: *r* value, intercept, and slope of regression of observed leaf area on predicted leaf area along with their 95% confidence intervals (in parentheses), lack of correlation (LC), mean squared deviation (MSD), and mean value of all parameters.

Model	Calibration run					Mean
	1	2	3	4	5	
Predictors LL and LW	$-0.321 + 0.905 \times \log(\text{LL}) + 1.037 \times \log(\text{LW})$	$-0.296 + 0.852 \times \log(\text{LL}) + 1.091 \times \log(\text{LW})$	$-0.315 + 0.882 \times \log(\text{LL}) + 1.064 \times \log(\text{LW})$	$-0.293 + 0.854 \times \log(\text{LL}) + 1.086 \times \log(\text{LW})$	$-0.285 + 0.857 \times \log(\text{LL}) + 1.078 \times \log(\text{LW})$	
<i>r</i> value	0.995 (0.993, 0.996)	0.986 (0.981, 0.990)	0.985 (0.980, 0.989)	0.983 (0.976, 0.987)	0.996 (0.995, 0.997)	0.989
Intercept†	-0.444 (-0.764, -0.125)	-0.756 (-1.345, -0.166)	-0.018 (-0.547, 0.511)	0.470 (-0.179, 0.957)	-0.314 (-0.601, -0.027)	-0.212
Slope†	1.026 (1.009, 1.042)	1.086 (1.058, 1.115)	1.025 (0.997, 1.052)	0.979 (0.951, 1.008)	1.022 (1.009, 1.036)	1.028
LC‡	1.332	4.427	3.419	2.753	1.034	2.557
MSD, cm <sup>4</sup> ‡	1.410	5.749	3.632	2.815	1.113	2.944
Predictor LL	$-1.050 + 2.040 \times \log(\text{LL})$	$-1.040 + 2.033 \times \log(\text{LL})$	$-1.028 + 2.027 \times \log(\text{LL})$	$-1.061 + 2.046 \times \log(\text{LL})$	$-1.019 + 2.024 \times \log(\text{LL})$	
<i>r</i> value	0.967 (0.956, 0.976)	0.967 (0.956, 0.976)	0.968 (0.956, 0.976)	0.972 (0.962, 0.979)	0.977 (0.969, 0.983)	0.970
Intercept†	-0.647 (-1.459, 0.165)	-0.631 (-1.534, 0.272)	-0.035 (-0.827, 0.757)	0.412 (-0.208, 1.033)	-0.078 (-0.777, 0.621)	-0.196
Slope†	1.036 (0.995, 1.036)	1.075 (1.032, 1.119)	1.014 (0.974, 1.055)	0.965 (0.929, 1.000)	0.994 (0.961, 1.027)	1.017
LC‡	8.136	9.763	7.450	4.371	6.025	7.149
MSD, cm <sup>4</sup> ‡	8.284	10.930	7.511	4.487	6.062	7.455
Predictor LW	$0.351 + 1.793 \times \log(\text{LW})$	$0.308 + 1.824 \times \log(\text{LW})$	$0.320 + 1.816 \times \log(\text{LW})$	$0.345 + 1.799 \times \log(\text{LW})$	$0.315 + 1.816 \times \log(\text{LW})$	
<i>r</i> value	0.990 (0.986, 0.993)	0.972 (0.962, 0.979)	0.973 (0.964, 0.980)	0.961 (0.947, 0.971)	0.988 (0.984, 0.991)	0.977
Intercept†	-0.154 (-0.592, 0.284)	-0.415 (-1.245, 0.415)	0.235 (-0.475, 0.945)	0.879 (0.155, 1.603)	-0.301 (-0.810, 0.209)	0.049
Slope†	1.015 (0.993, 1.037)	1.077 (1.036, 1.117)	1.023 (0.986, 1.060)	0.960 (0.917, 1.002)	1.043 (1.018, 1.068)	1.024
LC‡	2.551	8.449	6.183	6.125	3.194	5.300
MSD, cm <sup>4</sup> ‡	2.585	10.015	6.601	6.341	3.614	5.831

† The intercept should be zero and the slope should have a value of 1 for a good prediction.

‡ Computed according to Gauch et al. (2003). Smaller MSD indicates higher predictive ability.

Addition of such effects is crucial to obtain valid standard errors and significance tests as well as efficient estimates of the intercept and slope. Whereas the full model fitted to the observed data may look a bit complex, the final estimate of the fixed-effects part of the model is just an ordinary regression model with either a single or two predictor variables.

It is worth highlighting that Eq. [1] approximates LA as a fraction  $b$  of the area of the rectangle with side lengths LL and LW. The parameter  $b$  is a coefficient that is determined by leaf shape. A value of 0.5, 0.78, and 1.0 for  $b$  describes the area of a triangle, an ellipse, and a rectangle, respectively (Cristofori et al., 2007). For many grasses, like corn and wheat, the value of  $b$  is approximately 0.75, whereas  $b$  for broadleaf plants (e.g., cotton and sugar beet) is approximately 0.65 (Daughtry, 1990). Values of 0.74 for hazelnut (Cristofori et al., 2007), 0.63 for zucchini (Rouphael et al., 2006), 0.68 for sunflower (Rouphael et al., 2007), and 0.73 for clary sage (Kumar and Sharma, 2010) have been reported. The main advantage of Eq. [1] is that a change in the ratio of LL and LW in differently sized leaves does not negatively affect the predictive quality of the model. For instance, during growth LL/LW might change when bigger leaves tend to be broader in relation to LL than smaller leaves. As long as the general shape is retained, the relationship between LA and the product of LL and LW remains the same. According to Eq. [1], a cartoon model of leaf growth starts with a small picture of a leaf, represented as a rectangular graphical object such as the one shown in Fig. 1. One can stretch (or compress) and expand this object using mouse clicks, altering the values of LL and LW but leaving the general shape of the leaf unchanged. The essence of Eq. [1] is that the factor  $b$  is entirely unchanged by any such stretching or enlarging operations, including pure elongation along the LL axis. In contrast to this cartoon model of leaf growth, in case leaf shape changes (e.g., from a lanceolate shape to a more triangular shape), Eq. [1] would be inappropriate and would fail to predict LA accurately. If we assume Eq. [1] to hold, then taking logarithms on both sides would yield a multiple regression model with slopes equal to 1 for both  $\log(\text{LL})$  and  $\log(\text{LW})$ , as shown in Eq. [2]. However, according to our findings, the slope of  $\log(\text{LL})$  was significantly different from 1 (see section Regression on LW and LL). Hence, Eq. [1] was empirically shown to be inadequate for predicting LA of chia. Allowing the slopes on the log-scale (Eq. [4]) to differ from 1 gave better flexibility in the prediction model. Our use of Eq. [3] and [4] means that in this study, a common shape coefficient  $b$  could not be estimated and compared with values found in the literature. In fact, our observation for chia was that small leaves tended to be of elliptic shape, whereas larger leaves were more ovoid or cordiform. This is in agreement with the findings of Schwarz and Kläring (2001), who reported that tomato leaves do not have a constant shape. Such differences in shape mean that Eq. [1] cannot well predict LA from LL and LW. It is interesting to observe that the assumption of allometric growth, implying that relative growth rates of LA, LL, and LW are proportional to each other (Schwarz and Kläring, 2001), is commensurate with our log-linear models (Batschelet, 1975). Our results show that the extension of Model [1] to the more flexible power Model [3] provides a convenient empirical prediction model that can also account for systematic differences in shape between leaves of different sizes (Fig. 5).



Fig. 5. Chia plant showing different leaf shapes at various leaves sizes.

In only one case did population and nitrogen fertilizer level have an effect on the accuracy of the developed LA estimation model. Taking such treatment effects into account by random-effects meta-regression, we suggest that a universal LA estimation model for chia is plausible. Results indicated that the developed length–width model is highly reliable across a range of populations. All three models showed a high predictive ability in the cross-validation. The model  $\text{LA} = 0.740 \times \text{LL}^{0.866} \times \text{LW}^{1.075}$  provided the best prediction for the LA of chia. However, the model  $\text{LA} = 1.396 \times \text{LW}^{1.806}$ , with only one single leaf measurement (LW), was almost as accurate. The measurement points for LL are easier to locate than those of LW, and consequently more observations can be made per unit time. With LL, the prediction equation  $\text{LA} = 0.356 \times \text{LL}^{2.030}$  can be used because the correlation of OLA and PLA of 0.970 was acceptable. These models enable researchers to make nondestructive or repeated measurements on the same leaves without the necessity of expensive equipment. In conclusion, the models derived in this study are reliable for predicting the LA of chia from different populations and nitrogen levels.

## SUPPLEMENTAL MATERIAL

Additional supporting information can be found in the online version of this article:

Supplemental Material S1, including Table S1 with an overview of the leaf shape (length-to-width ratio, LL/LW), standard deviation (Std Dev), minimum (min) and maximum (max) values for the leaf length (LL) (cm), leaf width (LW) (cm), and leaf area (LA) ( $\text{cm}^2$ ) of eight different chia populations and different nitrogen levels (N-level); Supplemental Material S2, including Table S2.1, S2.2, and S2.3 with sequential  $F$ -tests; and Supplemental Materials S3, S4, and S5, including SAS codes and studentized residuals.

The supplemental ".sas" file contains all the SAS codes used for running the proposed models.

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## **5 Adapting the CROPGRO Model to Simulate Chia Growth and Yield**

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### **Publication III**

**Mack, L., Boote, K.J., Munz, S., Phillips, T., & Graeff-Hönninger, S. (2020). Adapting the CROPGRO Model to Simulate Chia Growth and Yield. *Agronomy Journal*, 1–19. doi.org/10.1002/agj2.20305.**

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*The interest in growing chia in various regions in the world has increased remarkably over the last years. In Publication 1, it was shown that chia could be successfully established in Egypt. Further, the experiments of Publication 2 showed that the temperate climate in Germany provided satisfactory conditions to grow chia, as well. However, there is no broad experience available on the cultivation of chia out of its centers of origin and knowledge of growing chia in temperate climates is missing. Crop simulation models can be helpful instruments for management and decision-making in crop production systems in different locations, testing on a preliminary level the impact of e.g. sowing date, sowing density, fertilizer demand etc. Researchers worldwide have used the process-oriented crop growth simulation model CROPGRO that is integrated in the DSSAT shell. An advantage is that a new crop can be introduced easily via the crop template approach (CROPGRO model). So far, no crop growth model for chia exists. In order to develop a chia model based on CROPGRO, data collection of different chia growth variables was carried out in the field experiments of Publication 2. The initial starting point was the CROPGRO-soybean model as its species file was assumed to be closer to chia than that of any other grain legume. Model adaptation was based on literature information and growth analysis data. The final aim of Publication 3 was to simulate growth and yield of chia and to provide a preliminary tool for improving the production of chia and adapting its cropping system to other regions. Moreover, this publication helped to improve model source codes to simulate plant growth of very small seed.*

## ARTICLE

## Biometry, Modeling, &amp; Statistics

# Adapting the CROPGRO model to simulate chia growth and yield

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## Abstract

Chia (*Salvia hispanica* L.) seeds are becoming increasingly popular as a superfood in Europe. However, broad experience in growing chia in temperate climates is missing. Crop simulation models can be helpful tools for management and decision-making in crop production systems in different regions. The objective of this study was to adapt the CROPGRO model for simulating growth and yield of chia. Data sets from a field experiment conducted over 2 yr in southwestern Germany (48° 74' N, 08° 92' E, 475 m above sea level) were used for model adaptation. The initial starting point was the CROPGRO–soybean [*Glycine max* (L.) Merr.] model as a template for parameterizing temperature functions and setting tissue composition. Considerable iterations were made in optimizing growth, development, and photosynthesis parameters. After model calibration, the simulation of leaf area index (LAI) was reasonable for both years, slightly over-predicting LAI with an average *d*-statistic of 0.95 and root mean square error (RMSE) of 0.53. Simulations of final leaf number were close to the observed data with *d*-statistic of 0.98 and RMSE of 1.36. Simulations were acceptable for total biomass (*d*-statistic of 0.93), leaf (*d*-statistic of 0.94), stem (*d*-statistic of 0.94), pod mass (*d*-statistic of 0.89), and seed yield (*d*-statistic of 0.88). Pod harvest index (HI) showed good model performance (*d*-statistic of 0.96 and RMSE of 0.08). Overall, the model adaptation resulted in a preliminarily adapted model with realistically simulated crop growth variables. Researchers can use the developed chia model to extend knowledge on the eco-physiology of chia and to improve its production and adaption to other regions.

**Abbreviations:** CSM, cropping system model; CUL, cultivar; DSSAT, Decision Support System for Agrotechnology Transfer; ECO, ecotype; HI, harvest index; LAI, leaf area index; N1, nitrogen fertilizer treatment 0 kg ha<sup>-1</sup>; N2, nitrogen fertilizer treatment 20 kg ha<sup>-1</sup>; N3, nitrogen fertilizer treatment 40 kg ha<sup>-1</sup>; PD, photothermal days; PHI, pod harvest index; RMSE, root mean square error; SLA, specific leaf area; SPE, species; Tb, base temperature; TD, thermal days; Tmax, maximum temperature; Tmin, minimum temperature; Topt, optimum temperature.

## 1 | INTRODUCTION

Chia (*Salvia hispanica* L.) seeds are becoming increasingly popular as a superfood in Europe owing to their valuable nutritive and health-related properties, particularly their high concentration of  $\alpha$ -linolenic acid (Ayerza & Coates, 2011; Mohd Ali et al., 2012). Chia seeds can be a food supplement and are widespread in vegetarian and gluten-free diets. An amount of 16,182 Mg of chia seed was imported to

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Europe in 2016 (11,838 Mg in 2015 (Peperkamp, Fitzpatrick, & Salazar, 2015)) with a value of 31 million Euro. Germany is the largest importer of chia seeds in Europe with a share of 40% in 2016 (CBI, 2017).

Chia is an annual herbal crop of the *Salvia* family (Lamiaceae) having its origin in the regions of west-central Mexico to northern Guatemala (Cahill, 2003). In pre-Columbian eras, it represented one of the staple foods of some Central American nations (Ayerza, 2016). Originally, chia is a short-day plant (Jamboonsri, 2010) and matures in tropical and subtropical environments at 400–2500 m above sea level with optimal temperatures between 16–26 °C (Bochicchio et al., 2015). Chia grows well on light to medium clay and sandy soils, and even in dry soils with good drainage (Muñoz, Cobos, Diaz, & Aguilera, 2013). Presently, chia is cultivated in Mexico, Bolivia, Argentina, Colombia, Ecuador, Australia, and Guatemala where latitude ranges from 20°55'N to 25°05'S. New cultivars have been bred that are insensitive to daylength or adapted to regions with daylength greater than 12 h (Hildebrand, Jamboonsri, & Phillips, 2013). There is no broad experience available on the cultivation of chia out of its centers of origin. As no experience exists, crop models may be useful tools to test different management options for growing chia in other regions than its origin. Crop models have gained importance as research tools and augmented in recent decades (Naab, Boote, Jones, & Porter, 2015). Mechanistic crop simulation models are important instruments to improve crop management (Boote, Jones, & Pickering, 1996) and can be beneficial tools for management and decision making in systems in diverse locations with different soils and climatic conditions (Jones et al., 2003). Researchers worldwide have used the Decision Support System for Agrotechnology Transfer (DSSAT) since 1989. DSSAT integrates models of about 46 different crops with software that enables the assessment and application of the crop models for different purposes (Hoogenboom et al., 2017). The DSSAT cropping system model (CSM) design has a modular structure. It includes a soil-water module; an organic-C module; a crop-template module that is able to mimic diverse crops by specifying species input files; a weather module; and a module for simulating light, energy, and water processes shared among the soil, plants, and atmosphere. A benefit is that a new crop can be introduced via the crop template approach (CROPGRO model), which allows model developers to modify values in a species crop-template file without changing any of the FORTRAN code (Boote et al., 1998; Boote, Minguéz, & Sau, 2002; Jones et al., 2003). CROPGRO was developed as a generic approach for modeling crops with one joint source code. It can simulate the growth of several crops {e.g. soybean [*Glycine max* (L.) Merr.], peanut [*Arachis hypogaea* (L.)], dry bean [*Phaseolus vulgaris* (L.)], chickpea [*Cicer*

### Core Ideas

- The CROPGRO model was adapted for chia using 2 yr data from a field trial in Germany.
- Model adaptation was based on literature information and growth analysis data.
- CROPGRO-Chia satisfactorily simulated growth dynamics for leaf area, biomass, and seed yield.
- This model is a preliminary tool for improving the adaptation of chia to other regions.

*arietinum* (L.)], cowpea [*Vigna unguiculata* (L.) Walp.], faba bean [*Vicia faba* (L.)], tomato [*Lycopersicon esculentum* (L.)], cotton [*Gossypium hirsutum* (L.)], and safflower [*Carthamus tinctorius* (L.)] based on weather, plant, soil, and management inputs (Boote, Jones, Hoogenboom, & Pickering, 1998; Hoogenboom et al., 2017). So far, no chia model exists. Enhancing the production of chia and adapting it to new cropping areas demands the comprehension of processes linked to growth, development, and yield formation. Thus, the objective of this study was to adapt the CROPGRO model for simulating chia growth and yield.

## 2 | MATERIALS AND METHODS

### 2.1 | Chia field experiment data for phenology, field, and growth analyses

Data sets from a field experiment conducted over 2 yr were utilized for model adaptation. The experiment was located at the experimental station Ihinger Hof of the University of Hohenheim (48°74' N, 08°92' E, 475 m above sea level) in southwestern Germany during 2016 (1 June–18 October) and 2017 (17 May–9 October). Daily mean maximum and minimum temperatures during the growing period were 21.1 and 10.7 °C in 2016, and 21.6 and 10.8 °C in 2017, respectively. Seasonal cumulative rainfall was higher in 2017 with 350.6 mm compared with 269.2 mm in 2016. Daily mean solar radiation was 16.2 (2016) and 17.4 MJ m<sup>-2</sup> d<sup>-1</sup> (2017). Meteorological data for the two experimental years are shown in Table 1.

Soil properties are shown in Table 2. In 2016, soil texture, soil pH, and mineral nitrogen (N<sub>min</sub>) were measured to 30 cm depth, while in 2017, measurements were taken to a depth of 90 cm. Soil texture was 7.9% sand, 67.0% silt, 22.5% clay, and pH of 7.5 in 2016 and 2.8% sand, 68.1% silt, 29.1% clay, and pH of 6.6 in 2017, respectively. For model purposes, the values of 60–90 cm layer were extended in

**TABLE 1** Monthly meteorological data observed at the experimental site Ihinger Hof in 2016 and 2017

Weather variable	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<b>2016</b>												
Temperature, °C <sup>a</sup>	1.89	3.54	3.32	7.4	12.16	16.11	18.52	17.97	16.22	7.86	3.48	0.63
Solar radiation, MJ m <sup>-2</sup> d <sup>-1</sup> <sup>b</sup>	0.8	0.9	9.6	13.8	17.5	17.7	19.5	18.3	14.4	7.0	3.7	3.9
Rainfall, mm <sup>c</sup>	64.7	54.2	29.3	47.4	88.0	108.3	64.8	29.3	50.6	53.3	48.7	5.0
ET <sub>O</sub> , mm <sup>c,d</sup>	2.8	3.3	41.6	70.2	109.4	119.0	136.3	119.0	87.8	34.1	13.0	12.6
<b>2017</b>												
Temperature, °C <sup>a</sup>	-3.75	3.3	7.15	7.12	13.62	18.34	18.24	18.05	11.81	10.31	4.0	1.1
Solar radiation, MJ m <sup>-2</sup> d <sup>-1</sup> <sup>b</sup>	4.2	6.2	11.6	15.6	18.9	23.5	18.1	16.2	11.3	8.2	3.0	2.5
Rainfall, mm <sup>c,d</sup>	23.5	40.8	63.2	29.0	47.0	72.2	109.9	69.3	52.2	51.1	63.0	32.7
ET <sub>O</sub> , mm <sup>c,d</sup>	7.0	23.8	58.6	73.7	115.9	152.8	117.7	104.5	58.1	40.1	10.5	6.3

<sup>a</sup>Monthly mean.<sup>b</sup>Daily mean.<sup>c</sup>Monthly sum.<sup>d</sup>Priestley-Taylor method was used to calculate potential ET<sub>O</sub>.**TABLE 2** Soil profile characteristics defined in the model for 2016 and 2017 field sites, at the experimental station Ihinger Hof of the University of Hohenheim (48°74' N, 08°92' E, 475 m a.s.l.)

Depth	SLOC	NO <sub>3</sub>		SLLL	SDUL	SSAT	SRGF	SSKS
		2016	2017					
cm	%	g Mg <sup>-1</sup>		cm <sup>3</sup> [water]/cm <sup>3</sup> [soil]				cm h <sup>-1</sup>
30	1.94	1.49	8.73	0.205	0.385	0.481	1.000	0.68
60	0.83	1.02	3.09	0.239	0.406	0.482	0.638	0.68
90	0.03	2.42	1.14	0.239	0.406	0.465	0.472	0.68
120	0.03	2.20	1.10	0.239	0.406	0.465	0.372	0.68
150	0.03	1.10	1.10	0.239	0.406	0.465	0.272	0.68
180	0.03	1.10	1.10	0.239	0.406	0.465	0.172	0.68

<sup>a</sup>Note. SLOC, soil organic carbon; NO<sub>3</sub>, nitrate in soil layer L; SLLL, soil water content in soil layer L at lower limit of plant extractable soil water; SDUL, soil water content at drained upper limit in soil layer L; SSAT, Soil water content in layer L at saturation; SRGF, soil root growth factor, 0.0 to 1.0, modified for deeper shape after derivation by SBUILD program in DSSAT model; SSKS, Sat. hydraulic conductivity.

the soil input file to 180 cm as the accepted soil profile depth at the research station is 180 cm.

The field experiment was carried out over two consecutive years as randomized complete block design with three replications. The experiments included three N fertilizer treatments (0 [N1], 20 [N2], and 40 [N3] kg N ha<sup>-1</sup>). In each plot, eight (2016) and six (2017) rows of the black-seeded chia genotype G8 were sown with plot length of 15 m (2016) and 10 m (2017). Row-distance was 0.5 m and sowing density was 150 seeds m<sup>-2</sup> (Mack et al., 2017). Seeds of a flowering mutant genotype (G8) were supplied through an agreement between the University of Hohenheim and the University of Kentucky. Different from the wild type chia plants, this genotype matures under long day conditions as it is considered insensitive to photoperiod and adapted to daylengths greater than 12 h. It was produced by gamma-ray mutagenesis as described by Hildebrand et al. (2013). Weeds were controlled manually by hoeing during early vegetative stage and mechanically using a row chop-

per (CHOPSTAR) 33 d (2016) and 28 d (2017) after sowing. In 2016, the previous crop was silage maize [*Zea mays* (L.)], while in 2017 it was winter wheat. Fertilization (calcium ammonium nitrate) was broadcast with a box spreader 9 d (2016) and 14 d (2017) after sowing. In 2016, there was a severe rainfall event directly after sowing, which resulted in a more heterogeneous crop stand, and a lower final plant density (17 plants m<sup>-2</sup>) compared to 2017 (43 plants m<sup>-2</sup>).

Non-destructive measurements were conducted on 10 plants per plot weekly after emergence (6 d after sowing) to determine plant height (top of the plant), canopy width (orthogonal to the row), number of leaves (tip appearance), and phenological stage. Destructive samples were taken at 14-d intervals during the growing period starting 46 d (2016) and 41 d (2017) after sowing (beginning of branching). Three randomly selected plants per plot were sampled and separated into stems, petioles, leaves, and inflorescences. The number of branches, main stem leaves, and inflorescences were counted and leaf area was determined

with a LI-3100 Area Meter (LI-COR). Then, plant components were dried separately to constant weight in an oven at 60 °C and dry weight was determined. At final maturity, the hand-harvest area was 1 m<sup>2</sup> in 2016 and 2 m<sup>2</sup> in 2017. In 2017, a subsample of randomly selected 25 plants was taken out of this hand-harvested sample for detailed measurement of biomass, seed yield, harvest index (HI), and thousand-kernel weight. At each sampling, N concentration of all dried plant components was determined. Nitrogen (protein) concentration of the seeds (at harvest), and seed oil concentration (at harvest) were determined as described by Mack et al. (2018). Light-saturated photosynthetic rate was determined in 2017 with the LCPro-SD portable photosynthesis system (ADC Bioscientific Ltd) operating at 375 μL L<sup>-1</sup> CO<sub>2</sub>, 30 °C and 2000 μmol m<sup>-2</sup> s<sup>-1</sup>. Measurements were made on the youngest fully developed leaf on two to three plants per plot. Leaf area and leaf dry weight of each leaf were determined to calculate specific leaf area (SLA).

A brief validation of the model was conducted with an additional experiment conducted in 2017 to evaluate the effect of 0.35, 0.50, and 0.75 m row spacing. The sowing date and management were the same as the 2017 experiment described above, conducted on an adjacent field with similar soil, using an N rate of 20 kg N ha<sup>-1</sup>. The growth analysis sampling was conducted in a similar manner.

## 2.2 | Model adaptation

The CROPGRO model uses external data files that specify species (SPE), ecotype (ECO), and cultivar (CUL) traits (Hoogenboom et al., 2017; Jones et al., 2003). In the SPE file, base temperatures (*T<sub>b</sub>*) and optimum temperatures (*T<sub>opt</sub>*) are defined for developmental processes (rate of emergence, leaf appearance, and rate of progress toward flowering and maturity), and growth processes (photosynthesis, leaf expansion, pod addition, seed growth, and N mobilization). Further, the file includes data for photosynthesis, tissue composition, and growth and maintenance respiration coefficients. Phenology is an essential part of the CROPGRO crop template approach, which takes information from both the CUL and ECO files. The CROPGRO model adaptation for chia required parameterizing of the previously described parameters. This was done by a combination of (i) values and relationships reported in literature and (ii) inverse model calibration by comparison of simulated outputs with the observations from the field experiments. The DSSAT version 4.7 (Hoogenboom et al., 2017) was used for the adaptation of chia. The initial starting point was the CROPGRO–soybean model as its SPE file was assumed to be closer to chia than that of any other grain legume. Soybean is a crop with high protein

content, short-day daylength sensitivity, and has a multiple branching behavior. In this study the Priestley–Taylor method was used to calculate potential evapotranspiration, the CERES Godwin module for soil organic matter, and the Ritchie two-stage method for soil evaporation. A systematic procedure was used, similar to that explained by Boote et al. (2002). First, information on tissue composition and *T<sub>b</sub>* and *T<sub>opt</sub>* for photosynthesis and development were obtained from the literature and entered directly into the SPE file. Some relationships taken from the literature were successively adjusted based on observed data. Next, model simulations were compared to experimental data. In a second step, life cycle was adjusted to correctly simulate bud formation (first pod in CROPGRO) and physiological maturity dates. Third, simulated biomass accumulation and leaf area index (LAI) were compared to observed data, and used for calibration of photosynthesis and leaf growth parameters. Fourth, a comparison of simulated vs. observed pattern of leaf and stem dry matter, and timing of pod growth and rise in pod HI were made to parameterize dry matter partitioning among leaf, stem, and pod components. Fifth, leaf N concentration and N decrease during grain fill were assessed and adjustments made on parameters influencing N mobilization. As a last step, seed size, seed growth rate, and duration were adapted to predict observed seed growth period and threshing percentage (ratio of seed/pod-plus-seed). Comparison between simulated and observed growth were made visually, followed by statistical evaluations of degree of model fit. Considerable iterations occurred between the last four steps. Simulations were made with water and N balance turned on, because N fertilizer treatments were present and there were water limitations in the year 2016. Initial simulation showed too much N stress for all treatments, because seasonal net mineralization was only 61.19 (2016) and 69.82 kg ha<sup>-1</sup> (2017). Prior crop residue was not measured, but it was estimated at 6000 kg ha<sup>-1</sup> with 80% incorporation to 6-cm depth on 15 April (2016) and 16 April (2017). With the entry of prior crop residue, the mineralization increased to 104.16 (2016) and 110.16 kg N ha<sup>-1</sup> (2017), which allowed simulations in agreement with observed growth for the 0 N (N1) treatment. The experimental data showed only relatively small effects of the N fertilization treatments. Statistical evaluation of repeated measures indicated a significant N effect at ( $\alpha = 5\%$ ) for the following variables: plant height (2016), LAI (2016), leaf mass (2016 and 2017), stem mass (2017), biomass (2017), seed yield (2017), pod mass (2017), HI (2017), stem N concentration (2016), and seed N concentration (2016 and 2017). Since observed and simulated N effects were small, less attention was paid to N effects, as the primary aim of this study was to adapt the model to account for effects of species traits and response to climatic factors.

In this study, sample size of destructive measurements was much smaller than for final harvest. The three-plant sample size had an upward bias in mass per plant associated with unknown bias in land-area involved, caused by tendency to ignore small unproductive plants in random sampling and multiplying by plant population. Singh et al. (2016) stated that biomass samples from larger sample size are more consistent and reliable due to less variation than small sample sizes. The calculated bias factor was then applied to land-area dependent observations like LAI, leaf mass, stem mass, biomass, seed yield, and pod yield. Bias-adjustment factors (Equation 3) of 0.63 for 2016 and 0.56 for 2017 were computed based on comparing per plant weight (TWPM6, TWPM7) of relatively late-cycle samples (two sample dates M6 and M7) when plant growth had nearly plateaued, to the per plant weight (TWPHA) computed from the final samples where final harvest was done on a 1 or 2 m<sup>2</sup> land area basis and plant count recorded:

$$\text{bias}(M6) = \frac{\text{TWPM6}}{\text{TWPHA}} \quad (1)$$

$$\text{bias}(M7) = \frac{\text{TWPM7}}{\text{TWPHA}} \quad (2)$$

$$\text{bias adjustment factor} = \frac{\text{bias}(M6) + \text{bias}(M7)}{2} \quad (3)$$

### 2.3 | Statistics for evaluating model performance

Model performance during calibration and after final adaptation was evaluated based on root mean square error (RMSE) (Equation 4), and the Willmott agreement index (*d*-statistic; Equation 5) (Willmott, 1982; Willmott et al., 1985).

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^N (Y_i - \hat{Y}_i)^2} \quad (4)$$

where *N* is the total number of data points for comparison, *Y<sub>i</sub>* are the observed values, and  $\hat{Y}_i$  are the simulated values. A smaller RMSE represents a better model prediction. The Willmott agreement index is given by

$$d = 1 - \left[ \frac{\sum_{i=1}^N (Y_i - \hat{Y}_i)^2}{\sum_{i=1}^N (|\hat{Y}_i - \bar{Y}| + |Y_i - \bar{Y}|)^2} \right], \quad 0 \leq d \leq 1 \quad (5)$$

where *N* is the total number of data points for comparison, *Y<sub>i</sub>* are the observed values,  $\hat{Y}_i$  are the simulated values, and  $\bar{Y}_i$  is the mean of the observed data. For good model prediction the *d* index should be near 0.90, for example, close to 1. Those statistical model parameters and the related evaluation statistics are frequently used in model calibration and validation studies (Malik, Boote, Hoogenboom, Cavero, & Dechmi, 2018; Singh et al., 2016).

For statistical analysis of the experimental growth and yield data, the following model was fitted:

$$y_{ijk} = \mu + b_i + (b\lambda)_{ik} + \tau_j + \lambda_k + (\tau\lambda)_{jk} + e_{ijk} \quad (6)$$

where *y<sub>ijk</sub>* is the observed value for the *i*th block, the *j*th *N* fertilizer level at the *k*th time point,

$\mu$  is the overall intercept,

$b_i$  is the effect of the *i*th complete block,

$(b\lambda)_{ik}$  is the time-specific block effect

$\tau_j$  is the effect of the *j*th *N* fertilizer level,

$\lambda_k$  is the effect of the *k*th time point,

$(\tau\lambda)_{jk}$  is the interaction of time and fertilizer level, and

$e_{ijk}$  is the residual error associated with *y<sub>ijk</sub>* with  $e_{ijk} \sim N(0, \sigma_e^2)$ .

Statistical analysis of the experimental data was done using restricted maximum likelihood (REML) and the MIXED procedure in SAS 9.4 (Lawson, 2010). The fixed effects in the model were tested for significance using partial Wald-type *F* tests. A significance level of 5% was used ( $\alpha = 5\%$ ).

## 3 | RESULTS AND DISCUSSION

### 3.1 | Model adaptation–Based on literature and experimental data

#### 3.1.1 | Cardinal temperatures for development, growth processes and photosynthesis

The phenological processes in the CROPGRO model depend on cardinal temperature (°C) parameters (Table 3) including base temperature (*T<sub>b</sub>*), first optimum (*T<sub>opt1</sub>*), second optimum (*T<sub>opt2</sub>*), and maximum temperature (*T<sub>max</sub>*), defined for rate of leaf appearance as well as both early and late reproductive development (Boote et al., 2002). Several references were used to set *T<sub>b</sub>* and *T<sub>opt</sub>* for vegetative and reproductive development. Paiva et al. (2016) reported that seeds of chia germinated satisfactorily under conditions of temperatures between 20–30 °C. The highest germination percentage and shortest average germination time occurred under constant temperature (25 °C) and alternating temperature

**TABLE 3** Cardinal temperatures (°C): base ( $T_b$ ), first optimum ( $T_{opt1}$ ), second optimum ( $T_{opt2}$ ), and maximum ( $T_{max}$ ) used for development, photosynthesis, pod addition, and seed growth rate of chia and soybean in the CROPGRO model

Growth/development process	Chia				Soybean			
	$T_b$	$T_{opt1}$	$T_{opt2}$	$T_{max}$	$T_b$	$T_{opt1}$	$T_{opt2}$	$T_{max}$
Vegetative development	10	25	35	45	7	28	35	45
Early reproductive development, Progress to anthesis	7	25	30	45	6	26	30	45
First seed to maturity	10	25	30	45	-15	26	34	45
Light saturated leaf photosynthesis	8	40	44	48	8	40	44	48
Pod addition and seed addition	9	21	27	40	14	21	27	40
Seed growth rate	6	21	24	41	6	21	24	41

(25–30 °C). Stefanello, Neves, Abbad, and Viana, (2015) found out that a temperature of 20 °C is optimal for germination of chia. Ayerza and Coates (2005) stated that chia is very sensitive to frost in any development phase and that the minimum and maximum growth temperatures are 11 and 36 °C with an optimum range between 16 and 26 °C. Baginsky et al. (2016) and Silva et al. (2018) suggested  $T_b$  to be 10 °C. There is a lack of cardinal temperatures for early and late reproductive development, which were set as given in Table 3. The  $T_b$  for early reproductive phase was dropped from 10 to 7 °C based on a better fit to observed phenology in the two seasons which differed in temperature during early season.  $T_{max}$  (upper temperature for zero rate) was kept the same as soybean. The model simulates development rate based on hourly air temperature applied to these four-point functions.

Information regarding the photosynthetic response of chia, for example, the effect of temperature on leaf and canopy photosynthesis, is scarce (Bochicchio et al., 2015; Silva et al., 2016). Hence, soybean values were used for temperature parameterization of hourly light-saturated leaf photosynthesis for chia (Table 3). Chia and soybean are presumed to have comparable temperature sensitivities as both crops can tolerate high temperatures as well as relatively cool nights. There are two temperature functions affecting leaf photosynthesis in CROPGRO: one for response of leaf photosynthesis (electron transport rate) to instantaneous hourly temperature which has a  $T_b$  of 8 °C and  $T_{opt1}$  of 40 °C (Table 3), and a second for response to cold night ( $T_{min}$ ) temperature. For the latter, soybean has an asymptotic function response starting to reduce photosynthetic rate below 19 °C and approaching zero rate at  $T_{min}$  of 0 °C. Both functions were adapted from soybean for use with chia. Jiao and Grodzinski (1996) recorded that under ambient CO<sub>2</sub> and O<sub>2</sub> conditions, photosynthesis and export rates of scarlet sage [*Salvia splendens* (L.)] were similar at 15 and 25 °C, but both decreased as leaf temperature was elevated from 25 to 40 °C.

### 3.1.2 | Life cycle and phenology

The CROPGRO model can simulate either long or short day genotypes. Although chia is originally a short-day plant, this study used the daylength-insensitive flowering mutant genotype G8, which can flower under daylengths greater than 12 h (Hildebrand et al., 2013). Therefore, photoperiod sensitivities in the SPE file were set to "NON" for all life cycle phases. The CUL file specifies the critical short daylength (CSDL), at which progress to anthesis is most rapid, and a slope of daylength sensitivity that reduces rate of progress at longer daylengths (PP-SEN). In this study, daylength-insensitive chia was simulated using a CSDL of 14.1 h and a PP-SEN insensitivity of 0.001 (meaning insensitive) (Table 4). Initially, the timing of pod growth and pod HI were early by a few days, thus the parameters for phase durations shown in Table 4 were modified. Time between emergence and flower appearance (EM-FL) was increased from 16.8 (Soybean Maturity Group 0) to 25.0 photothermal days (PD) for a better match with observed data. For chia, pod formation occurs prior to flowering (Note: botanically, chia fruit are in spikes. Each chia flower produces up to four fruit (called nutlets) in the calyx "capsule". Commercially, each of these fruitlets is named "seed" [Capitani, Ixtaina, Nolasco, & Tomas, 2013; Kochhar, 2016]). However, in this study the term "pod" refers to "spike" or "inflorescence". Thus, to get first pod to occur on time, a "false" anthesis was simulated as a dummy variable, while real-anthesis of chia occurs after first pod, and closer to simulated first seed. In the CROPGRO model, pods are added at the beginning-pod stage for photothermal-dependent duration (PODUR) at a rate that is dependent on current canopy assimilation rate and temperature. Time from "false" anthesis to beginning pod (FL-SH) and beginning seed (FL-SD) were calibrated to match onset of observed reproductive dry matter accumulation. After a certain duration within each new pod cohort, seeds are added in the cohorts as the pod reaches age defined by FL-SD minus FL-SH. To attain a good simulation of HI and grain, FL-SD was increased to 15.2 PD to delay beginning

**TABLE 4** Genetic coefficients of chia as defined in the cultivar (CUL) file of the CROPGRO model, after calibration, and compared to soybean

Genetic coefficients	Chia	Soybean (MG 0)
Critical short daylength below which reproductive development progresses as rapidly as possible with no daylength effect (CSDL), h	14.10	14.10
Slope of the relative response of development vs. photoperiod (PP–SEN), 1 h <sup>-1</sup>	0.001	0.171
Time between emergence and flower appearance (EM–FL) (PD) <sup>a</sup>	25.0	16.8
Time between first flower and beginning rachis/bud/pod (FL–SH) (PD) <sup>a</sup>	4.8	6.0
Time between first flower and beginning seed (FL–SD) (PD) <sup>a</sup>	15.2	13.0
Time between beginning seed and (beginning) physiological maturity (SD–PM) (PD) <sup>a</sup>	18.20	30.80
Time between first flower and last leaf expansion (FL–LF) (PD) <sup>a</sup>	28.00	26.00
Maximum leaf photosynthetic rate at 30 °C, 350 μL L <sup>-1</sup> CO <sub>2</sub> , and high light (LFMAX), mg CO <sub>2</sub> m <sup>2</sup> s <sup>-1</sup>	1.53	1.03
Specific leaf area of cultivar under standard growth conditions (SLAVAR), cm <sup>2</sup> g <sup>-1</sup>	340.0	375.0
Maximum size of fifth full leaf (SIZLF), cm <sup>2</sup>	12.6	180.0
Maximum fraction of daily growth that is partitioned to seed + shell (XFRT)	0.95	1.00
Maximum weight per seed (WTPSD), g	0.001	0.19
Seed-filling duration for seed cohort under standard conditions (SFDUR) (PD) <sup>a</sup>	18.0	23.0
Duration of pod addition under standard conditions (PODUR) (PD) <sup>a</sup>	23.0	10.0
Threshing percentage [seed (seed + shell) <sup>-1</sup> ] (THRSH)	34.0	77.0
Fraction protein in seeds (SDPRO), kg (protein) kg (seed) <sup>-1</sup>	0.210	0.405
Fraction oil in seeds (SDLIP), kg (oil) kg (seed) <sup>-1</sup>	0.410	0.205

<sup>a</sup>Note.MG, Maturity Group; PD, photothermal days.

of seed set (as timing of grain weight was initially too early in 2017). Time between beginning seed and (beginning) physiological maturity (SD–PM) was shortened to match the time of senescence of leaf mass near the end of the life cycle. The simulated shelling percentage (seed divided by pod wall plus seed) depends on the cultivar parameters of threshing percentage (THRSH) (final genetic potential, same definition) and seed-filling duration (SFDUR) which affects rate of single seed growth (Boote et al., 2002; Singh et al., 2016). The final mass per seed at harvest relies on genetic potential seed size (maximum weight per seed, WTPSD) (Table 4) and environmental conditions. Model seed size was calibrated based on observed data by setting WTPSD to 0.001 g seed<sup>-1</sup> [Note: model code was modified to allow seed size this small; change to be made in future DSSAT release]. WTPSD, THRSH, SFDUR, and SD-PM were defined in the CUL file based on the observed data, where SD–PM is the time from beginning seed to beginning maturity. Maximum fraction of daily growth that is partitioned to seed plus shell (XFRT) was calibrated and set in the CUL file (Table 4). The partitioning to reproductive parts is an indication of a degree of determinacy in chia.

### 3.1.3 | Tissue composition, growth, and conversion costs

Chia seeds consist of proteins (15–25%), lipids (30–33%), ash (4–5%), and carbohydrates (26–41%) and have high fiber content. The lipids contain one of the highest known percentage of  $\alpha$ -linolenic acid (62–64%) in plants (Coates, 2011). Reyes-Caudillo, Tecante, and Valdivia-Lopez. (2008) recorded that the total digestible fiber of chia seed ranges from 36 to 41%. Insoluble fiber varies between 23 and 46%, and soluble fiber between 3 and 7%. The compositions for seed and vegetative tissues are specified in the SPE file, except for seed protein (SDPRO) and seed lipid (SDLIP), which are CUL traits. Seed protein value (SDPRO) in CUL file should be consistent (same as) with standard protein concentration of seed (SDPROS) and growth protein concentration of seed (SDPROG) in the SPE file. Tissue composition of different organs in the model is defined by fractions of six types of compounds: protein, lipid, lignin, carbohydrate-cellulose, organic acids, and minerals for leaf, stem, root, nodule, shell, and seed tissue (Wilkinson, Jones, Boote, Ingram, & Mishoe, 1983). The sum of the different compounds for a specific plant organ, such

TABLE 5 CROPGRO tissue composition parameters, definitions, initial (soybean) values and calibrated chia values for leaf (LF), stem (ST), shell (SH), and seed (SD)

Compound		Tissue—Initial values from soybean			
		LF	ST	SH	SD
PRO__	Protein	0.356	0.150	0.250	0.405
PCAR__	Carbohydrate-cellulose	0.405	0.664	0.380	0.315
PLIP__	Lipid	0.025	0.020	0.020	0.205
PLIG__	Lignin	0.070	0.070	0.280	0.020
POA__	Organic acid	0.050	0.050	0.040	0.040
PMIN__	Mineral	0.094	0.046	0.030	0.025
Compound		Tissue—Calibrated values for chia			
		LF	ST	SH	SD
PRO__	Protein	0.306	0.185	0.250	0.210
PCAR__	Carbohydrate-cellulose	0.455	0.629	0.380	0.295
PLIP__	Lipid	0.025	0.020	0.020	0.410
PLIG__	Lignin	0.070	0.070	0.280	0.020
POA__	Organic acid	0.050	0.050	0.040	0.040
PMIN__	Mineral	0.094	0.046	0.030	0.025

as protein (PROLFI), carbohydrate (PCARLF), lipid (PLIPLF), lignin (PLIGLF), organic acid (POALF), and mineral (PMINLF) must sum up to 1.00 (Singh et al., 2016). Growth respiration (and conversion) cost for tissue synthesis is calculated by the Penning de Vries, Brunsting, and van Laar, (1974) method. In the model three main values for protein concentrations are defined: (a) leaf (PROLFI), stem (PROSTI), root (PRORTI), and storage (PROSRI) for new tissue developed under luxurious supply of N; (b) for the minimum concentration for growth (PROLFG, PROSTG, PRORTG, PROSRG) for tissue grown during limited N; and (c) for the concentration after complete N remobilization/exhaustion (PROLFF, PROSTF, PRORTF, PROSRF) when, for example, leaves fall off. Tissue carbohydrate concentration parameters (PCARLF, PCARST, PCARSH, PCARSD) were calculated as the remaining difference between all other tissue composition parameters for a given tissue (and it includes cellulose). As a starting point, parameters from the CROPGRO soybean SPE file were used for setting chia tissue composition due to the close similarity and the lack of measured data. Subsequently, some parameters were modified by comparison of simulated and observed values. The final parameters for tissue composition are presented in Table 5. The protein concentration of the seeds of observed data is in agreement with data from the literature (Ayerza & Coates, 2009, 2011; Bushway, et al., 1981; Coates, 2011; Peiretti & Gai, 2009). The lipid concentration in the seeds (SDLIP) was based on the following references (Ayerza, 1995; Ayerza & Coates, 2009; Ixtaina et al., 2011; Jambroonsri, 2010) and on observed data (data not shown) and set to 0.410 (Table 5) to simulate final oil content of 32%. Actual sim-

ulated lipid concentration can be less than the genetic potential depending on environmental conditions, especially temperature.

The protein composition of new tissue is affected by N availability from root uptake. CROPGRO simulates protein mobilization from vegetative tissues toward reproductive and new vegetative tissues as a function of thermal age (Malik et al., 2018). The function for maximum rate of protein mobilization from vegetative tissues during reproductive growth (NMOBMX) was set to a value of 0.120, thereby mobilizing most of the available protein to the exhausted level by maturity. A second parameter, NVSMOB set at 0.45, defines the fractionally slower rate of N mobilization from vegetative tissues (relative to NMOBMX) prior to the beginning of seed growth. Leaf senescence is simulated based on remobilization of N in addition to crop age and drought stress. The amount (grams) of leaf mass (SEN RTE) lost per gram of protein mobilized due to N mobilization (controlled by NMOBMX and NVSMOB) was increased slightly from 0.80 to 0.85 to cause more senescence in later stages of the life cycle to match observed values. The amount of stem mass (petioles) abscised as a fraction of the leaf mass abscised (PORPT) was set to 0.08, which is much lower than soybean (0.58) given the considerably smaller petioles of chia.

### 3.1.4 | Photosynthesis, leaf appearance, leaf area expansion, and canopy expansion

Chia is a C<sub>3</sub>-plant (Lovelli, Valerio, Phillips, & Amato, 2018). Stomatal conductance declines as an effect of

**TABLE 6** Leaf area growth parameters as defined in the species file of the CROPGRO model for chia and soybean, after calibration process

Variable	Chia	Soybean
Specific leaf area of leaves at plant emergence (FINREF)	180.0	180.0
Specific leaf area of the standard reference cultivar at peak vegetative growth (SLAREF)	340.0	350.0
Upper limit for specific leaf area (in low light), thinnest leaves (SLAMAX), cm <sup>2</sup> g <sup>-1</sup>	950.0	950.0
Lower limit of specific leaf area (in saturated light and optimum conditions), thickest leaves (SLAMIN), cm <sup>2</sup> g <sup>-1</sup>	340.0	250.0
Leaf area per trifoliate leaf for the leaf at the V-stage 5 position (SIZREF)	6.3	171.4

**TABLE 7** V-stage dependencies: Maximum limited leaf area growth (derived from Equation 7); vegetative partitioning to leaf, stem, and root; and canopy internode height and width parameters as a function of main stem leaf number (XVGROW, XLEAF, and XVSHT)

XVGROW	0.0	1.0	2.0	4.0	6.0	8.0				
YVREF <sup>a</sup>	0.0	1.53	2.40	5.88	14.44	35.46				
XLEAF	0.0	1.5	3.3	5.0	7.8	12.5	30.0	40.0		
YLEAF <sup>b</sup>	0.43	0.44	0.44	0.43	0.39	0.34	0.30	0.30		
YSTEM <sup>b</sup>	0.09	0.13	0.21	0.29	0.36	0.48	0.55	0.55		
YROOT <sup>b</sup>	0.48	0.43	0.35	0.28	0.25	0.18	0.15	0.15		
XVSHT	0.00	1.00	4.00	6.00	8.00	10.00	14.00	16.00	20.00	40.00
YVSHT <sup>c</sup>	0.0100	0.0200	0.0200	0.0340	0.0540	0.0660	0.0740	0.0740	0.0740	0.0600
YVSWH <sup>c</sup>	0.0100	0.0220	0.0240	0.0300	0.0360	0.0360	0.0360	0.0360	0.0360	0.0360

<sup>a</sup>cm<sup>2</sup> per plant.<sup>b</sup>Fraction partitioning.<sup>c</sup>Internode length, m.

low water availability, but stomata remain partially open (Nobel, 2005). Silva et al. (2016) showed that chia plants implement osmotic adjustment mechanisms in response to water stress. Lovelli et al. (2018) reported that chia of the less daylength-sensitive flowering mutant genotype G8 showed a high unit photosynthetic rate having a range of 1.1–1.6 mg CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> depending on water availability. Silva et al. (2016) reported maximum photosynthesis of 1.1 mg CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (chia seeds from Bolivia). Based on literature and measurement from the field experiments, the maximum leaf photosynthesis rate (LFMAX) in the CUL file was increased from 1.03 (initial value used for soybean) to 1.53 mg CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> for chia. The specific leaf weight (SLWREF), at which LFMAX is defined, was based on measured data and set to 0.0036 g cm<sup>-2</sup>. Leaf growth parameters such as specific leaf area (SLA) at emergence (FINREF), at peak vegetative growth (SLAREF and SLAVAR), and leaf area per trifoliate leaf at vegetative stage (V-stage meaning leaf number on main stem) (SIZREF) were adjusted to better simulate the rate of leaf area expansion (Table 6).

Initial leaf area per plant is set by seed size (0.001 g) multiplied by fraction of seed mass converted to plant mass (WTFSD) (0.55), fraction partitioning to leaf, and SLA. Leaf area expansion is generally driven by photosynthesis but can be limited during the first five nodes, using the following described function. The upper limit of leaf area expansion

for the first five nodes is described by a function of leaf area per plant function vs. V-stage based on observed data (destructive measurements from 2 up to 12 leaf stage). Leaf area of plants at early V-stages (through 8) was estimated on the basis of digital photographs (with ruler scaling) and leaf area estimation function described by Mack et al. (2017) assuming an exponential function that resulted in:

$$y = 0.977 \exp^{0.449x} \quad (7)$$

where  $y$  is the leaf area per plant and  $x$  is V-stage (Table 7, see YVREF), but is applied only until V-stage 5 in the model.

Several processes in the model are linked to progressive V-stage increase (continued main stem node production) including very early leaf area expansion, vegetative partitioning, canopy height, and canopy width growth parameters (Table 7). V-stage is a type of thermal accumulator, and very early leaf area expansion, and vegetative partitioning are linked to it. Likewise, height and width depend on progressive node production and internode length. These processes and their linkage to V-stage were calibrated to match observed data as described later.

In the SPE file of the CROPGRO model an upper and lower limit of SLA is described (SLAMAX and SLAMIN, representing SLA against limiting low and saturating high solar radiation, respectively) to set the SLA of newly grown

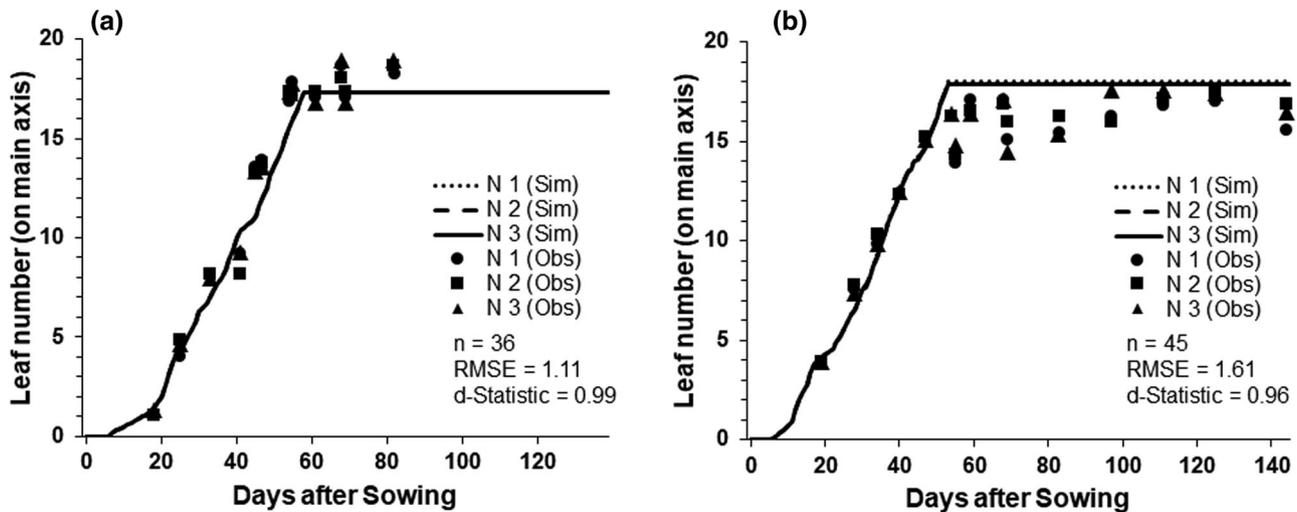


FIGURE 1 Comparison of simulated (lines) and observed (symbols) V-stage (leaf number on main axis) as a function of days after sowing for chia at Ihinger Hof, Germany, in (a) 2016 and (b) 2017

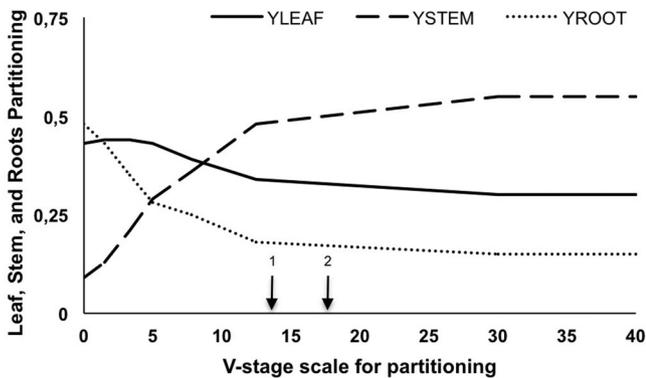


FIGURE 2 Fraction instantaneous biomass partitioning to vegetative components (leaf, stem, and roots) of chia as a function of V-stage progression (1 = leaf number at “false” anthesis; 2 = leaf number at first bud)

leaves for a given species. These limits were set at 950 and 340  $\text{cm}^2 \text{g}^{-1}$  based on calibration to SLA data. The SLA of cultivar (SLAREF) was set to 340  $\text{cm}^2 \text{g}^{-1}$  (Table 6). The rate of leaf appearance on the main stem (TRIFL) in the ECO file was augmented from 0.32 (soybean) to 0.72 leaves per thermal day. One reason for this is that chia forms two opposed leaves per node (thus counting twice the number of leaves). Therefore, this parameter had to be approximately doubled. Figure 1 shows observed and simulated V-stage as a function of days after sowing. At harvest a final leaf number of 18 (2016) and 16 (2017) on the main stem was observed. Simulations were very close to the observed data with  $d$ -statistic of 0.99; RMSE of 1.11 (2016) and  $d$ -statistic of 0.96; RMSE of 1.61 (2017).

Maximum size of the full leaf (SIZLF) was standardized to 12.6  $\text{cm}^2$  based on the leaf size taken from the trials conducted at Ihinger Hof (Table 4). Since main-stem leaf num-

ber is based on tip appearance (rather than fully expanded leaves), the time from emergence to first leaf (EM–V1) had to be reduced from 6.0 to 3.0 thermal days (TD), and other functions based on rate of leaf appearance (partitioning) had to be modified. To simulate end of main stem leaf appearance (FL–VS) the time from first flower to last leaf on the main stem was set to terminate leaf appearance on the main stem at 0.50 photothermal days (PD) after first flower. In addition, end of leaf area expansion on branches after “false” first flower (FL–LF) was set to 28.00 PD (Table 4). For simulating canopy height and width increase over time as V-stage increases, the primary look-up of internode length (and canopy width) per V-stage in the SPE file, were adjusted by increasing YVSHT (equals length per internode) and decreasing YVSWH (equals width per internode) (Table 7). Realistic values of plant height were simulated with these calibrations. The main stem leaf number ceases when leaf appearance ends, thus the model does not simulate total chia height associated with continued inflorescences extension. Other variables in the ECO file were modified, such as time required for growth of individual podwalls (here rachis) (LNGSH), which was increased from 10.0 to 13.0 PD to better predict indeterminate nature of observed inflorescence/rachis data. Effect of daylength sensitivity after R1 (R1PPO) was decreased from 0.189 to zero, because this chia cultivar is not daylength sensitive. Life cycle sensitivity to water deficit was adjusted to simulate response to the occurrence of water stress in 2016. The sensitivity of reproductive phase progression to water stress was increased from 0.70 (soybean) to 0.80, which function allows water deficit to shorten the life cycle during grain filling. In addition, in 2017 the sensitivity of N stress was evident, and the sensitivity of reproductive phase progression to N stress was set to 0.3, which also

caused shortening of the time from first seed to physiological maturity for the more N-stressed treatments.

### 3.1.5 | Dry matter partitioning

The instantaneous daily partitioning among leaf, stem, and root components depends on vegetative stage (V-stage) progression (Boote et al., 2002; Malik et al., 2018). In CROPGRO, the partitioning parameters are organized in an array containing values for plant V-stages (XLEAF); and YLEAF, YSTEM, and YROOT as the corresponding parameters for fractional partitioning to leaf, stem, and root, respectively (Malik et al., 2018). In the literature, no values exist for partitioning of dry matter to organs at different vegetative stages of growth for chia, thus the partitioning of soybean was used as a starting point, and calibrations were solved (Table 7) to match dry matter accumulations in leaf and stem. Partitioning to root was unknown, and therefore, maintained similar to soybean. The partitioning to roots (YROOT) is attained by difference, as the sum of partitioning coefficients to all organs must be 1.00. Figure 2 illustrates the partitioning to all plant organs (leaf, stem, and roots), where the  $x$  axis, XLEAF, represents the V-stage progression.

Biomass partitioning to leaves was increased in the early crop life cycle compared to soybean, in part because of a higher rate of leaf appearance and shorter EM–V1. Concurrently, biomass partitioning to stem was augmented in the middle and particularly toward the end of crop life cycle. With the onset of buds and seeds later in the crop life cycle, which have first priority in CROPGRO, actual realized partitioning to vegetative parts is automatically reduced. The partitioning fractions are captured “in the model” at first flower (57 and 53 d in 2016 and 2017, respectively), and make a linear interpolated shift of fractions to FRLFF and FRSTMF that occur at NDLEAF (105 and 100 d in 2016 and 2017, respectively). NDLEAF is a physiological stage set by the value of FL–LF, while FRLFF and FRSTMF represent the “final partitioning” fractions to leaf and stem, respectively, that occur at the NDLEAF stage. Thermal rate of progress to NDLEAF determines the rate of shift in partitioning.

## 3.2 | Evaluation of the model simulations of growth and yield

The emphasis of model adaptation for chia is the adjustments to improve simulations by comparison to observed development, growth, dry matter accumulation, and partitioning. The figures and tables illustrate simulations after

final adaptations, as the goal was to calibrate and adapt a new model for a preliminary release and use.

### 3.2.1 | Leaf area index

The model’s capability to simulate LAI depends on the ability to simulate leaf mass (from daily growth and current partitioning to leaf) and SLA. Solar irradiance, temperature, and water stress affect the SLA of new leaves. The time of the maximum LAI in the model is affected by the start of pod and seed growth, partitioning between vegetative components (leaf, stem, and root), and by the end of leaf area expansion (Boote et al., 2002). After model calibration, the predictions of LAI were good for both years yet a bit over-predicted with an average  $d$ -statistic was of 0.95 and RMSE of 0.53 over both years. In 2016 (Figure 3a), the averaged LAI over dates and three treatments was 1.53 while in 2017 (Figure 3b) an average LAI of 2.84 was computed. This difference was associated with a lower realized plant density in 2016. The quicker leaf senescence in 2016 due to N stress (N1 treatment) was not captured by the model.

### 3.2.2 | Biomass accumulation, yield attributes, and yield

Contrast between simulated and observed total biomass showed minor under-prediction of biomass in 2016 with RMSE of 1383 and  $d$ -statistic of 0.93 (Figure 4a), and small over-prediction of biomass in 2017 with RMSE of 1536 and  $d$ -statistic of 0.93 (Figure 4b). The relatively small differences between three treatments within a given year are related to the N-fertilization treatments and different actual sowing densities. The adapted model predicted total biomass response to N in both years (Figures 4a, 4b), although significant differences in observed total biomass ( $p = .0004$ ) due to the different N fertilization only occurred in 2017. Simulated pod mass for 2016 (Figure 4c) was under-predicted by the model with  $d$ -statistic of 0.82 and RMSE of 1302, while simulation for 2017 (Figure 4d) showed a minor over-prediction with good  $d$ -statistic of 0.96 and RMSE of 621. Model predicted pod mass response to N in both years, although significant difference in observed pod mass ( $p = .0082$ ) only occurred in 2017.

Overall, the 2 yr were different in growth patterns and only minor improvements for year effect were possible based on minor modifications of a few temperature-dependent parameters. A respectable agreement was found between observed and predicted pod HI. This trait

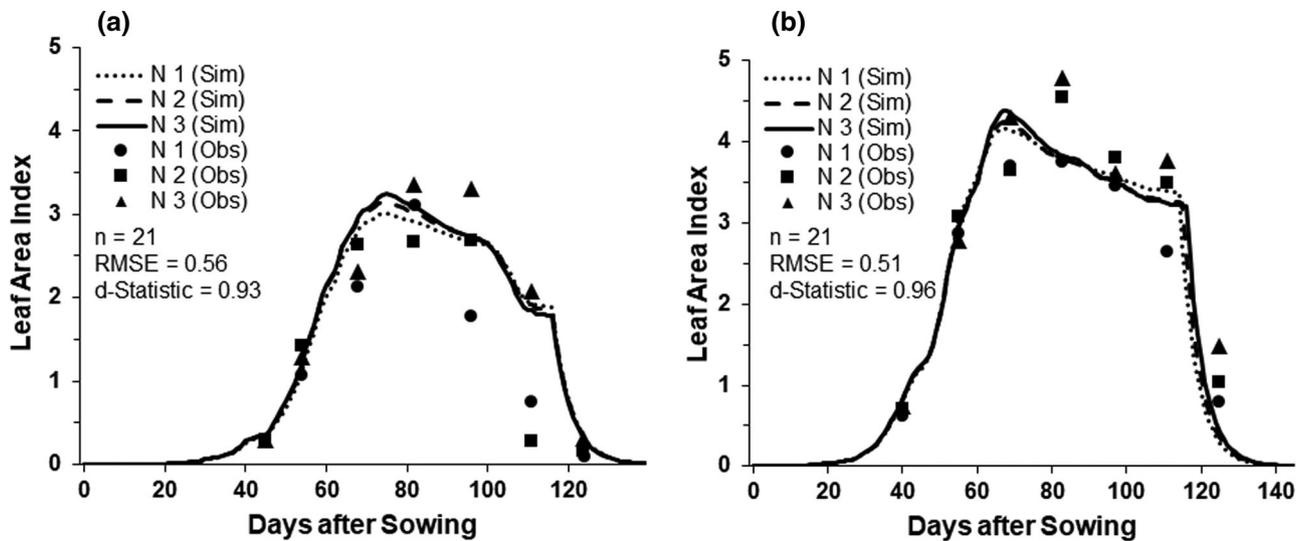


FIGURE 3 Comparison between simulated (lines) and observed (symbols) leaf area index as a function of days after sowing for chia at Ihinger Hof, Germany, in (a) 2016 and (b) 2017

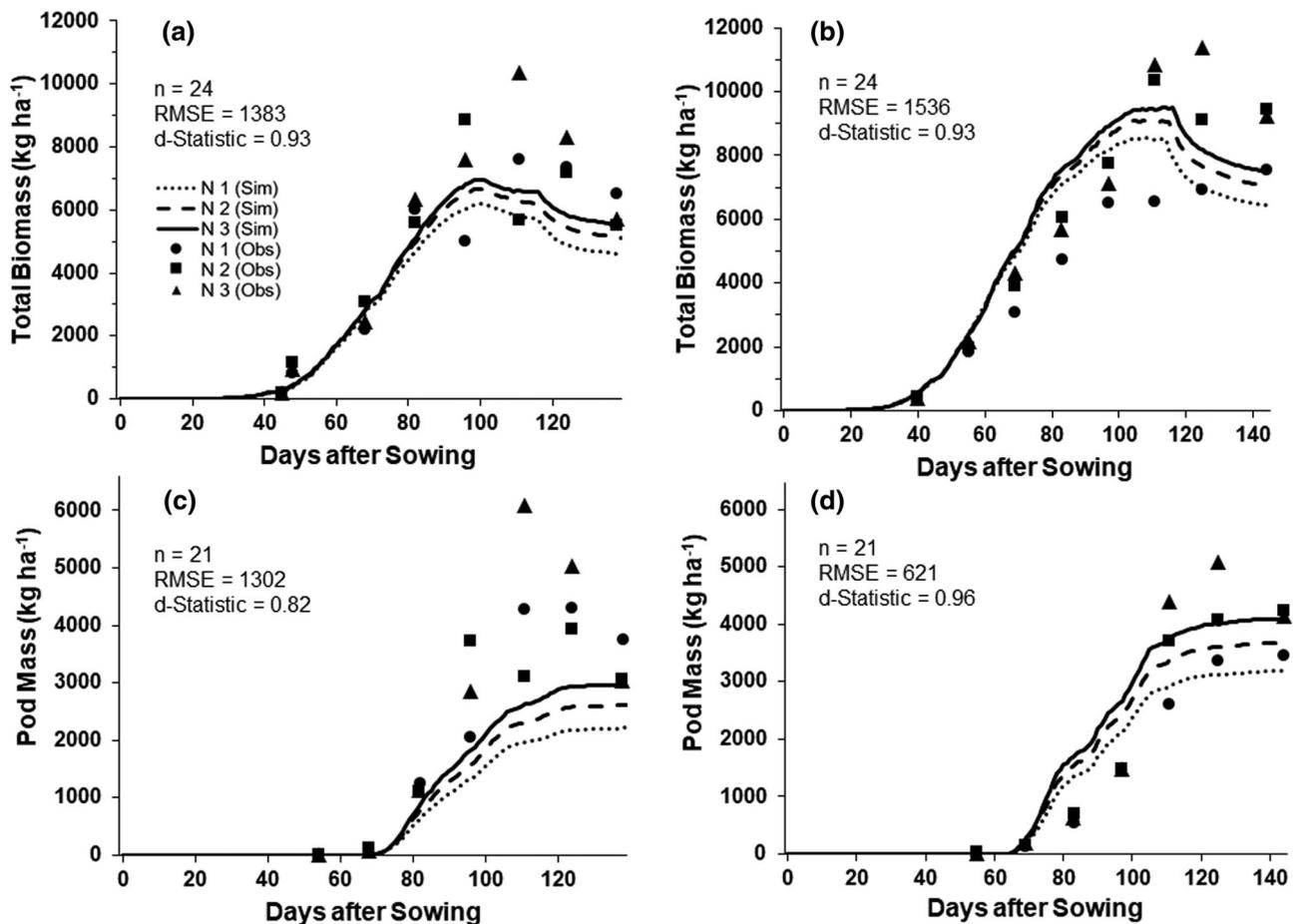


FIGURE 4 Comparison between simulated (lines) and observed (symbols) total biomass (kg DM ha<sup>-1</sup>) in (a) 2016 and (b) 2017 and pod mass (kg DM ha<sup>-1</sup>) in (c) 2016 and (d) 2017 as a function of days after sowing for chia at Ihinger Hof, Germany

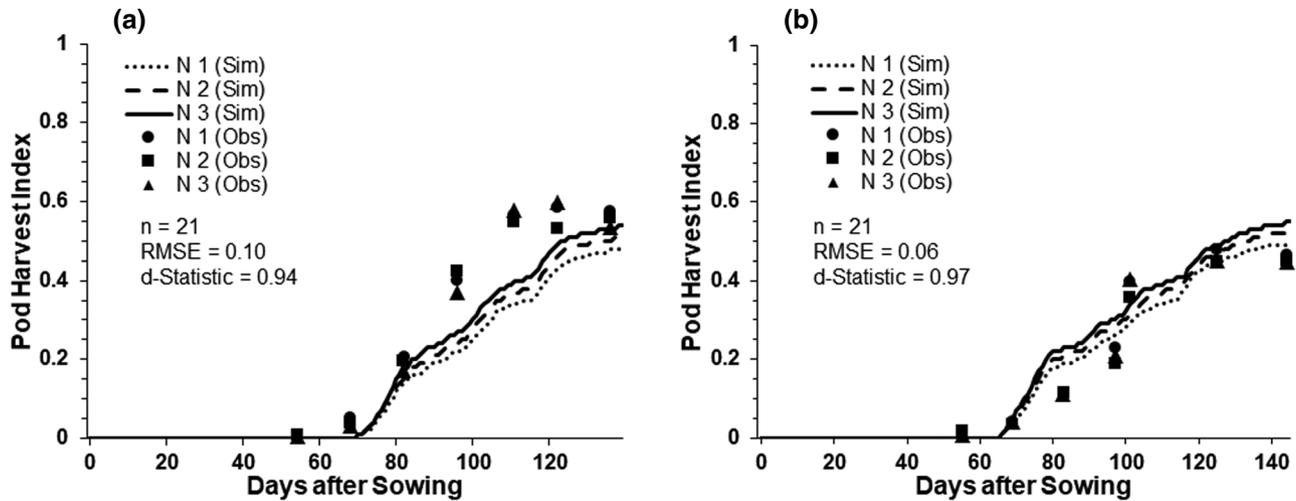


FIGURE 5 Comparison between simulated (lines) and observed (symbols) pod harvest index as a function of days after sowing for chia at Ihinger Hof, Germany, in (a) 2016 and (b) 2017

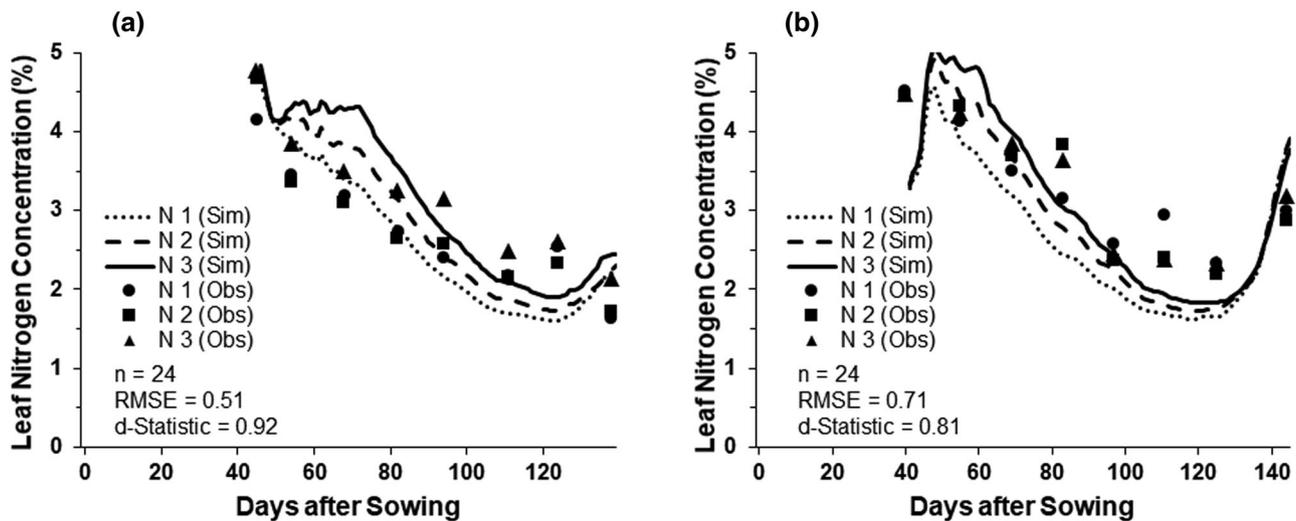


FIGURE 6 Comparison between simulated (lines) and observed (symbols) leaf nitrogen concentration as a function of days after sowing for chia at Ihinger Hof, Germany, in (a) 2016 and (b) 2017

was well simulated, with a negligible early lag, the mostly linear middle phase, and the slight curvilinear phase during late pod growth. Pod HI was to some extent under-predicted in 2016 ( $d$ -statistic of 0.94, RMSE of 0.10) (Figure 5a), while it was over-predicted in 2017 ( $d$ -statistic of 0.97, RMSE of 0.06) (Figure 5b). The grain HI was considered less reliable because of possible seed shattering. In 2016, the simulated grain HI was very close to the observed HI with  $d$ -statistic of 0.84 and RMSE of 0.05 averaged over three treatments, whereas for 2017, simulated HI was over-predicted with  $d$ -statistic of 0.83 and RMSE of 0.04.

### 3.2.3 | Nitrogen concentrations of plant organs

The decrease in stem and leaf mass during late reproductive phase is reliant on the mobilization rate of proteins and associated abscission of leaf and petiole. Rate of protein mobilization was calibrated to mobilize about two-thirds of the total protein from vegetative tissues by the time of maturity and to result in a final leaf N concentration of 3%. The simulations of leaf-N concentration predictions were better for 2016 (Figure 6a) ( $d$ -statistic of 0.92, RMSE of 0.51) than for 2017 (Figure 6b) ( $d$ -statistic of 0.81, RMSE

**TABLE 8** Comparison between means of observed and simulated crop variables over all time-series measurements through to final harvest) for the 2 yr, root mean square error (RMSE), and *d*-statistic

Crop variable	Observed mean	Simulated mean	RMSE	<i>d</i> -statistic
Leaf area index (LAI)	2.18	2.26	0.53	0.95
Specific leaf area (SLA), cm <sup>2</sup> g <sup>-1</sup>	259	262	40.4	0.29
Total plant biomass, kg DM ha <sup>-1</sup>	5,248	5,034	1,459	0.93
Stem mass, kg DM ha <sup>-1</sup>	2,664	2,707	691	0.94
Leaf mass, kg DM ha <sup>-1</sup>	743	810	240	0.94
Pod mass, kg DM ha <sup>-1</sup>	2,158	1,783	962	0.89
Seed yield, kg DM ha <sup>-1</sup>	777	701	283	0.88
Seed harvest index	0.11	0.11	0.04	0.84
Pod harvest index	0.29	0.26	0.08	0.96
Threshing percentage (THRSH), %	23.6	23.8	9.11	0.74
Leaf N, % of DM	3.11	2.98	0.61	0.87
Stem N, % of DM	1.27	1.22	0.63	0.85
Grain N, % of DM	3.52	3.52	1.12	0.37

Note. DM, dry matter.

of 0.71). The model showed N treatment effects on leaf N concentration, although there was no significant effect determined in the observed data ( $p = .3175$  [2016] and  $p = .5666$  [2017]). The fact that simulated N concentration in leaf (and stem) was lower than observed during the later part of the life cycle is caused by carbohydrate accumulation as a result of N stress, which decreases apparent N concentration (this excessive carbohydrate accumulation under N stress is a model feature that needs improvement, such as creating feedback inhibition on photosynthesis rather than accumulating assimilate).

Stem N concentration showed a good prediction as well as response to N treatment. Nitrogen effect on observed stem N concentration was significant only for 2016 ( $p < .0001$ ). Simulation was better for 2016 (*d*-statistic of 0.94, RMSE of 0.41) than for the 2017 season (*d*-statistic of 0.75, RMSE of 0.85), which showed an under-prediction. Simulated average grain-N concentration over both years and three treatments was 3.52%, which was close to the observed grain-N concentration of 3.52%, but *d*-statistic was low (Table 8). In 2016, the model under-predicted grain N concentration, while in 2017 it was over-predicted.

### 3.2.4 | Statistical evaluation

The model predictions and statistics of the in-season crop variables measured and averaged over three treatments and 2 yr after model adaptation are summarized in Table 8. Principally, good outcomes were attained for the means of observed and simulated values and associated RMSE

and *d*-statistic. The LAI was slightly over-predicted with *d*-statistic of 0.95 and RMSE of 0.53. Although observed and simulated mean of SLA were close, *d*-statistic was low. Total plant biomass, pod mass, and seed yield were slightly under-predicted by the model but showed good *d*-statistic of 0.93, 0.89, and 0.88, respectively. Stem and leaf mass were slightly over-predicted by the model but *d*-statistic was very good (0.94). Pod HI showed the best model performance with high *d*-statistic of 0.96 and low RMSE of 0.08. In addition, leaf N and stem N concentrations over time were satisfactorily simulated (Table 8).

The model over-estimated the observed threshing percentage (*d*-statistic of 0.74, RMSE of 9.11, Table 8), using a value of THRSH of 34% that is low compared to soybean (Table 4). It should be lower than soybean because of inclusion of rachis tissue, but it is also suspected that some seed shattering may have occurred, and contributed to the low observed threshing percentage.

Table 9 sums up the model predictions and RMSEs for the crop variables measured at final harvest and averaged over 2 yr. Total plant biomass, pod mass, HI, and threshing percentage were under-predicted while seed yield was over-predicted. The model responded to N treatments, whereas repeated measure of observed time-series data did not show any significance in some cases. However, field data showed significant N response for final seed yield, total biomass, pod mass, and HI only in 2017. The model predicted N3 to obtain highest yield. In the field data, N2 achieved the highest yield traits. A possible reason could be the high initial nitrate concentration in the soil in 2017 that resulted in lodging problems and reduced yield traits.

**TABLE 9** Comparison between means of observed and simulated crop variables measured at final harvest for the three N levels (N1–N3) averaged over the 2 yr, and root mean square error (RMSE), and *d*-statistic

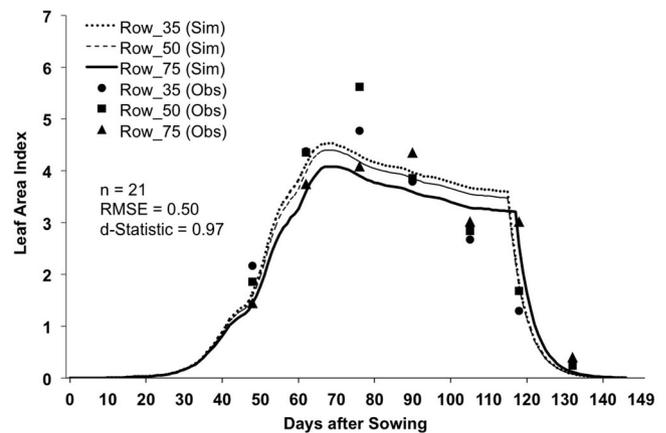
Crop variable	Observed mean	Simulated mean	RMSE	<i>d</i> -statistic
Total plant biomass, kg DM ha <sup>-1</sup>	6,901	6,027	1,491	0.74
N1, 0 kg ha <sup>-1</sup>	6,142	5,506	1,236	
N2, 20 kg ha <sup>-1</sup>	7,292	6,071	1,904	
N3, 40 kg ha <sup>-1</sup>	7,270	6,502	1,231	
Pod mass, kg DM ha <sup>-1</sup>	3,748	3,124	915	0.62
N1, 0 kg ha <sup>-1</sup>	3,632	2,700	1,300	
N2, 20 kg ha <sup>-1</sup>	3,900	3,142	868	
N3, 40 kg ha <sup>-1</sup>	3,714	3,532	262	
Seed yield, kg DM ha <sup>-1</sup>	1,107	1,250	270	0.55
N1, 0 kg ha <sup>-1</sup>	934	924	299	
N2, 20 kg ha <sup>-1</sup>	1,212	1,076	143	
N3, 40 kg ha <sup>-1</sup>	1,036	1,204	226	
Seed harvest index	0.16	0.18	0.04	0.41
N1, 0 kg ha <sup>-1</sup>	0.15	0.17	0.03	
N2, 20 kg ha <sup>-1</sup>	0.18	0.18	0.03	
N3, 40 kg ha <sup>-1</sup>	0.15	0.19	0.05	
Threshing, %	26.29	34.28	8.33	0.34
N1, 0 kg ha <sup>-1</sup>	25.05	34.34	9.29	
N2, 20 kg ha <sup>-1</sup>	26.60	34.26	4.65	
N3, 40 kg ha <sup>-1</sup>	24.20	34.20	10.01	

Note. DM, dry matter.

### 3.3 | A preliminary model validation

An independent data set from a row spacing experiment conducted in an adjacent field in 2017 allowed preliminary model validation, using the model parameterization described above. The observed row spacing and plant populations were entered into the model for 0.35, 0.50, and 0.75 m row spacing treatments. Figure 7 illustrates excellent simulation of LAI with RMSE of 0.50 and *d*-statistic of 0.97 averaged over three treatments.

The model well predicted the pattern of early exponential growth and timing of subsequent leaf area senescence with maturity. As expected, the wider row spacing caused a small penalty on growth as simulated by the hedgerow light interception model with slower completion of full canopy cover, especially for the widest row spacing. Simulated total crop biomass and pod mass were slightly over-predicted (Figures 8a, 8b) with RMSE of 985 and *d*-statistic of 0.95 for total biomass and RMSE of 800 and *d*-statistic of 0.90 for pod mass. The growth pattern was reasonable and the widest row spacing caused lower simulated biomass accumulation as expected, but not much effect on pod mass.



**FIGURE 7** Comparison between simulated (lines) and observed (symbols) leaf area index of row spacing experiment as a function of days after sowing for chia at Ihinger Hof, Germany, in 2017

The pod harvest index (PHI) and HI were well simulated with RMSE of 0.088 for PHI and 0.017 for HI and *d*-statistic of 0.93 for PHI and 0.96 for HI (Figures 9a, 9b).

Table 10 shows the summary of statistics for several other time-series observations including LAI, SLA, leaf mass, stem mass, seed yield, HI, and threshing percent.

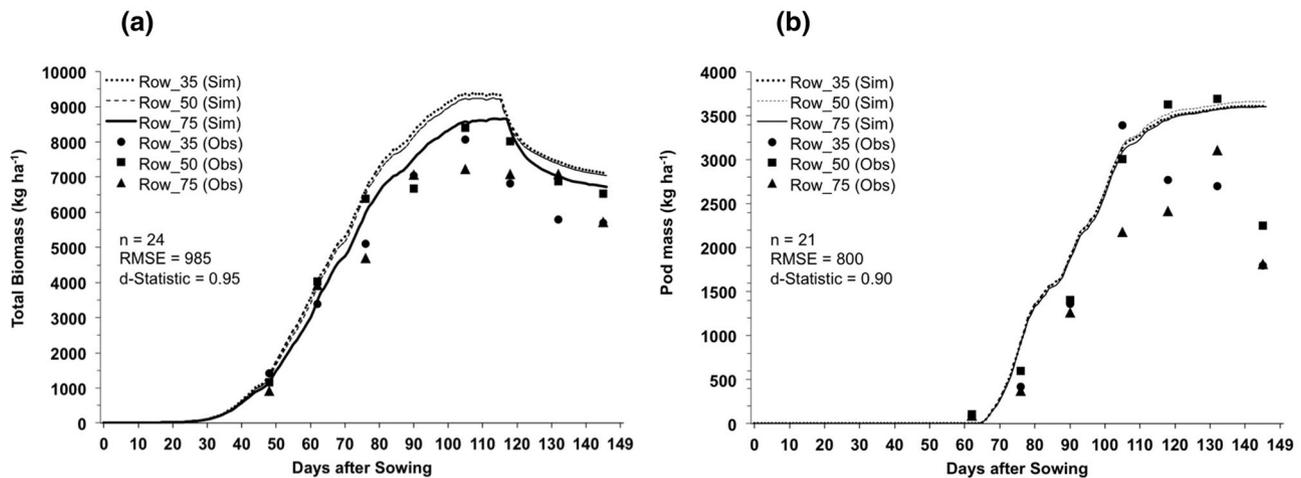


FIGURE 8 Comparison between simulated (lines) and observed (symbols) (a) total biomass and (b) pod mass as a function of days after sowing for chia at Ihinger Hof, Germany, for row spacing experiment in 2017

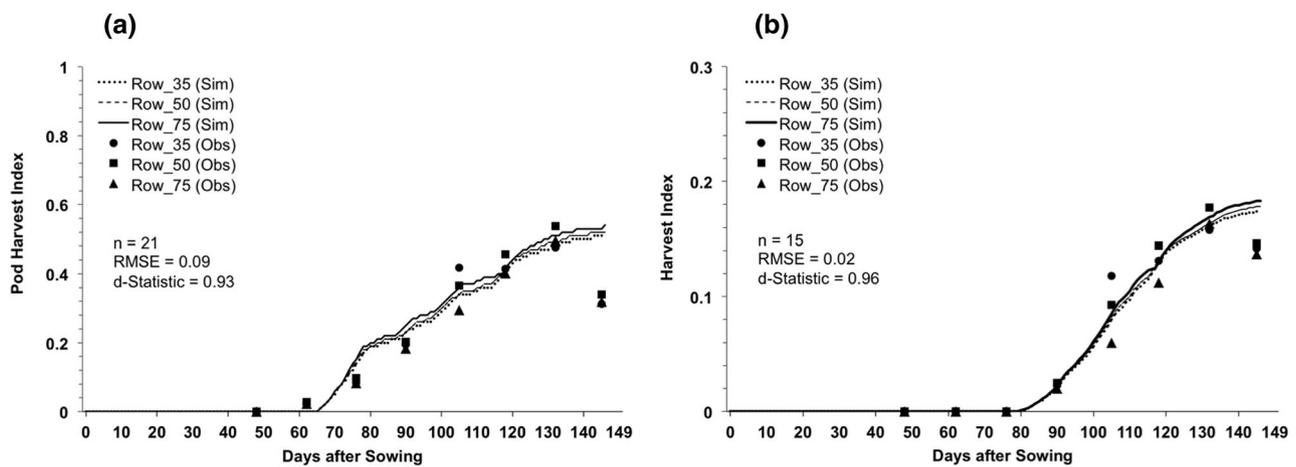


FIGURE 9 Comparison between simulated (lines) and observed (symbols) (a) pod harvest index (PHI) and (b) harvest index (HI) as a function of days after sowing for chia at Ihinger Hof, Germany for row spacing experiment in 2017

TABLE 10 Comparison between means of observed and simulated crop variables over all time-series measurements through to final harvest for row spacing experiment (0.35, 0.50, and 0.75 m) in 2017, root mean square error (RMSE), and *d*-statistic

Crop variable	Observed mean	Simulated mean	RMSE	<i>d</i> -statistic
Leaf area index (LAI)	2.78	2.69	0.50	0.97
Specific leaf area (SLA), cm <sup>2</sup> g <sup>-1</sup>	291	247	57	0.37
Total plant biomass, kg DM ha <sup>-1</sup>	5,599	6,449	985	0.95
Stem mass, kg DM ha <sup>-1</sup>	3,063	3,546	468	0.96
Leaf mass, kg DM ha <sup>-1</sup>	832	958	268	0.95
Pod mass, kg DM ha <sup>-1</sup>	1,850	2,421	800	0.90
Seed yield, kg DM ha <sup>-1</sup>	739	912	238	0.88
Seed harvest index (HI)	0.11	0.12	0.02	0.96
Pod harvest index (PHI)	0.27	0.32	0.09	0.93
Threshing percentage (THRSH), %	28	27	4.58	0.94

Note. DM, dry matter.

Although additional validation data would have been desirable, such data were not available, and we made the decision to develop a more robust model with two seasons of data rather than to calibrate to 1 yr and validate to a second season. Validation with the row spacing experiment data indicates adequate model performance, but further evaluation by others in diverse environments would be desirable.

#### 4 | CONCLUSION ON MODEL ADAPTATION AND PERFORMANCE

The CROPGRO model was adapted for chia to simulate development, growth, and final yield of this newly emerged superfood under temperate European climate conditions. The adaptation process was conducted by adjusting cardinal temperatures for growth and development, by setting plant tissue compositions, and by parameterizing species and cultivar traits for phenology and vegetative and reproductive partitioning. This model adaptation has succeeded to provide a preliminarily adapted model and at the same time to point out future research requirements and issues requiring model improvement such as N response. Several parameters may be relatively simple to adjust as new knowledge becomes available. For upcoming researchers, this developed chia model can be a first useful tool to extend knowledge and to implement chia modeling for improving the production of chia and adapting it to other regions. Because the model was adapted using two field experiments, carried out in 2 yr at a single site in Germany, it should be verified for different pedo-climatic conditions, agronomic managements and genotypes to improve the robustness of the model. In conclusion, the model simulates reasonably the responses to management and seasonal climatic variation and can be used as a preliminary tool. Code issues within the model dealing with very small seed sizes, N uptake, and soil N availability should be addressed in future steps of model code improvement. In addition, new code needs to be added to the (parent) CROPGRO model to account for the effect of N on SLA, canopy height and width, rate of leaf senescence, and rate of N mobilization. Additional evaluation and improvement of this model will be facilitated by the inclusion of the model in a future DSSAT software release, and as new data become available.

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#### CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

#### AUTHOR CONTRIBUTIONS

L. Mack, S. Graeff-Hönniger, and S. Munz conceived the research plans. L. Mack performed the experiments and measurements. L. Mack and K.J. Boote analyzed the data and adapted the model. L. Mack wrote the article with contributions of all the authors.

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## 6 General Discussion

The main objective of this thesis was to develop and transfer cultivation practices for the ancient grain chia in other regions than its origin. The intended transfer necessitates the expansion of existing knowledge on management strategies for new environments to ameliorate overall yields and seed quality. **Publication 1** provided first results of chia cultivation in an arid region out of its center of origin to expand on the knowledge of chia cultivation practices. **Publication 2** formed the basis for a simple LA estimation model under equipment-limited conditions. The LA of a crop is an important input parameter for process-oriented crop models. It determines light capture and potential photosynthetic rates (Boote et al. 1998a; UNFCCC 2016). Data collected during the field experiments of Publication 2 helped to develop a crop growth model for chia. In **Publication 3**, the adaptation process of the CROPGRO model simulating growth and yield of chia was described in detail. A comprehensive discussion of the results was incorporated into each scientific publication.

The general discussion will therefore link all aspects of the previously presented articles. Keeping in mind, that CC and water scarcity will considerably put pressure on agriculture and sustainable development (UNFCCC 2016; Zeibisch et al. 2005), this chapter will also discuss future challenges for cropping systems. Particularly, in arid and semi-arid regions, crop yields will be reduced resulting in food shortages. Therefore, crops with a low water demand are needed. A promising alternative could be the drought-tolerant chia with high nutritional value and high revenues in international markets (Ayerza and Coates 2005; Coates and Ayerza 1996). However, management practices for chia out of its center of origin are missing and a future cultivation of chia especially in drought environments necessitates the development of suitable cropping systems and management guidelines for farmers. Further, this chapter presents perspectives of a modeling approach using the process-oriented plant growth simulation model CROPGRO for chia in new environments and discusses the model's applicability and additional improvements and suggestions on integrating chia into existing crop rotation.

### 6.1 Future challenges for agricultural cropping systems

As mentioned previously, the demand for water will become one of the tenacious resource problems of the 21<sup>st</sup> Century (Abdin and Gaafar 2009). Particularly, developing countries with arid and semi-arid climate will face extreme problems regarding water availability. For example, the water use in Egypt has increased within last years and continues to rise rapidly (El-Sadek 2010; Kijne 2006). Egypt is already one of the world's most water-scarce countries and is unable to produce enough food for its population (Abdin and Gaafar 2009). The desert covers about 96% of Egypt's total area. Most of the cultivated land is located close to the banks of the Nile River, its main branches and canals, and in the Nile Delta (FAO 2016). The soil is highly fertile and can be cropped more than once a year. The

agricultural sector is important for tackling poverty in developing countries as a large share of Egypt's population works in the agricultural sector. In the future, Egypt's agriculture, which mainly depends on irrigation, has to fulfill the demands of a larger population with rising living standards (Abdin and Gaafar 2009). Egypt's fast growing population has forced an intensification of cropping systems. Farmers can choose the crops they cultivate since 1992 (Gersfelt 2007 in FAO 2016). Consequently, some changes in cropping patterns were made supporting the production of high value-added crops e.g. rice and sugarcane with the highest water requirements among the crops cultivated in Egypt. The crop choice is profit-driven and is relevant if the poverty levels in rural areas of Egypt are considered. Rice is a high value crop and is likely to be an important contributor to income rising (FAO 2016). Thus, the cropping patterns that sometimes lead to water shortages serve the welfare of rural families. The cropping patterns have an important role in controlling the irrigation water demand (Abdin and Gaafar 2009). Chia has also been recommended as a highly economic substitute for common field crops supporting diversification and stabilization of the local economy (Coates and Ayerza 1996). Especially, in arid and semiarid regions, where water availability is the main limitation to crop production (Ayerza and Coates 2009; Peiretti and Gai 2009), the cultivation of chia may be attractive. For this reason, chia could be an adequate alternative as temperature and photoperiod demands of chia are fulfilled in Egypt. In Publication 1, preliminary results indicated that chia could be successfully cultivated under desert environments in Egypt achieving a marketable seed quality (Mack et al. 2018). Chia offers the opportunity to save the scarce source water in Egypt because it is considered to be drought tolerant. Further, these high value seeds generate foreign exchange for reimporting e.g. wheat that is characterized by a higher water demand (Kijne 2006). Beside these opportunities, targeted assistance and dissemination of information on how to cultivate are required, so that, e.g., existing irrigation-intensive crops can also be replaced. This will lead to more sustainability if, e.g., wheat is imported from countries where there is sufficient water, while in countries with water scarcity, drought-tolerant but high value crops are cultivated. For all those reasons, the increasing production of chia seeds could also be valuable for both contrasting environments, Germany and Egypt.

## **6.2 Crop modeling - CROPGRO**

Several studies have reported that CC will have different effects on cropping systems (UNFCCC 2016). Crop models summarize essential information on how crops respond to changing climatic conditions at site-specific locations. These process-oriented crop models include several simple relationships that sufficiently represent actual plant processes in contrast to descriptive models. The majority of crop models were initially developed as instruments for improving crop management by providing information on the optimal amounts of input (irrigation, fertilizer, and pesticides) and the optimal scheduling of the application and integrated in decision support systems (DSS) to assess alternate management practices (Boote et al. 1998b). Thus, today, scientists and farmers broadly use a variety of crop models to assess agricultural alternatives at a given location under different conditions

e.g., years of drought, changes in application of agrochemicals and in irrigation (Banterng et al. 2010). For example, process-based crop models, such as DSSAT CROPGRO are commonly used (UNFCCC 2016). The CROPGRO model is intended to predict the response of a given crop to specific weather, soil, and management, even when experimental data for that specific field do not exist (Boote et al. 1996). The Cropping System Model (CSM)-CROPGRO model is inserted in the DSSAT (Hoogenboom et al. 2017; Jones et al. 2003). The CSM was established to adapt cropping systems (Jones et al. 2003). A new crop can easily be implemented via the CROPGRO template. This was a favorable argument for choosing CROPGRO for developing a growth model for chia. The emphasis was to obtain detailed physiological information to define the functions and parameters for the Species (SPE) file, which was done in Publication 3. To define a new crop, there are three “genetics files” that are entered as external input files (Boote et al. 1998a): Cultivar (CUL), Ecotype (ECO), and Species (SPE) coefficients (Hoogenboom et al. 2003; Jones et al. 2003). The SPE file contains a range of temperature functions for development, photosynthesis, partitioning, and several other physiological functions, the effect of water or N-deficit on rate of life cycle progress, and the basic tissue compositions (proteins, lipids, fiber, carbohydrates, and other compounds) of different plant organs, like leaves, stems, roots, and reproductive structures (Boote et al. 1998a; Hoogenboom et al. 2003). The ECO file includes 17 parameters that describe the distinctive characteristics of a group of cultivars (Thorp et al. 2014). The CUL file describes a minimum set of 18 important genetic attributes that determine life cycle characteristics (time from emergence to flowering, time from flowering to first pod and first seed, time from first seed to physiological maturity) but also include seed size and fill duration, leaf photosynthesis, and oil and protein composition of the seeds (Boote et al. 1998a).

The tiny seed-size of chia was initially a problem for model simulation. Very small seed size caused two types of problems. The first was with a “lower limit protection” statement in the DEMAND.FOR and PODS.FOR subroutines that caused failure to grow pods and seeds. This was easily fixed by a single change in DEMAND.FOR and PODS.FOR subroutines, changing from “IF (GRRAT1 .GE. 0.001) THEN ADDSH = GRRAT1 \* SHELN(NPP)\*.....” to “IF (GRRAT1 .GE. 0.00001) THEN ADDSH = GRRAT1 \* SHELN(NPP)\*.....”. GRRAT1 is shell growth rate, but it is coupled to the parameter (WTPSD) that sets potential seed size. The second problem was more complex and only partly solved because the very low weight per seed caused very low initial plant mass, LAI, and root mass. The model has minimum protections for LAI, sunlit LAI, and shaded LAI in the ETPHOT.FOR subroutine. This “protection” resulted in higher LAI than observed, and too much photosynthesis which caused excessive assimilate supply for which N-supply was not met by the initial small seed N amount and small root length density for N-uptake. As a result, there was very severe computed N-stress (NSTRES) exactly on the day after emergence, along with low leaf N-concentration, and associated extreme high total non-structural carbohydrate (TNC) accumulation. The initial N in leaf, stem, and root depends on initial weights (computed from seed size and fractions to organs) and initial

tissue N-concentrations. These three N-balance problems occurred if WTPSD <0.050 g. This problem was mostly solved with the following code changes in ETPHOT.FOR to reduce the lower limit protections on LAI, sunlit LAI (LAISL), and shaded LAI (LAISH). The old code

```
“IF (XLAI.GT.0.0 .AND. XLAI.LT.0.01) XLAI = 0.01
    LAISL = MAX(LAISL,0.02)
    ELSE
    LAISL = 0.02
LAISH = XLAI - LAISL
LAISH = MAX(LAISH,0.02)”
```

was changed to

```
“IF (XLAI.GT.0.0 .AND. XLAI.LT.0.006) XLAI = 0.006
    LAISL = MAX(LAISL,0.005)
    ELSE
    LAISL = 0.005LAISH = XLAI - LAISL
LAISH = MAX(LAISH,0.001)”;
```

with XLAI = Leaf area (one side) per unit of ground area.

These code changes are not necessarily next to each other in the ETPHOT.FOR subroutine, but reduced greatly the temporary NSTRES computation, apparent low N-concentration the day after emergence, and the TNC accumulation. There is a small remaining effect on these outputs depending on the sowing density and seed size, but the effect is small and negligible for two reasons. In a practical sense, these simulated outputs are not very important, because the N-concentration used for photosynthesis does not include the TNC pool, so leaf N-effect on photosynthesis is not affected by the computed NSTRES or high TNC. In addition, other features of the model limit the rate of LAI increase for the first five nodes produced, thus controlling the real LAI which thus responds very little to the higher computed assimilate supply from “supposed” LAI protection in the ETPHOT.FOR subroutine. These changes may also be relevant for other tiny seed crops such as quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus* sp.) or buckwheat (*Fagopyrum esculentum*).

Crop models can mimic many main food, feed and fiber crops. Nevertheless, models for specialty crops are rare. So, the intention was to first calibrate the CROPGRO model for chia under European conditions and in a future step to apply this model to other regions.

### **6.3 Application of the CROPGRO-Chia model to other regions**

There are several factors that influence crop production, e.g., management practices such as choice of cultivar, planting date, plant density, irrigation, and fertilizer, herbicide, and pesticide applications. Finding optimum crop management practices could help to increase crop yield for a specific production area. Nevertheless, it is time consuming and expensive because it requires several years of experimental data collection. Therefore, the combination of experimental field research and crop

modeling has become popular to determine optimum farming practices under varying weather conditions (Banterng et al. 2010). The CSM model has been evaluated across several different soil and climate conditions (Jones et al. 2003) and the CROPGRO model has been used in the research to create understanding of growth physiology, to offer genetic improvement and policy recommendation, and to develop production strategies to optimize yield (Hoogenboom et al. 2012).

In the prevailing literature, there are no examples to use a chia model to analyze the environmental effects or to test other management strategies in other regions than its origin. One limitation might be the missing data on genetic and physiological information. Considering the production of chia, producers should be able to make site-specific recommendations, preseason and in-season decisions related to cultivar choice, planting date, row spacing, fertilization, irrigation, crop rotation, and minimization of soil and agrochemical losses. The results presented in Publication 3 indicated that the model is a suitable tool to simulate chia growth and yield for temperate climates. In a next step, this developed model should be validated using an independent data set, e.g., another experiment with different treatments or a different location (Wang et al. 2003) (e.g. Egypt).

Life cycle progress through any given phase depends on a physiological day (PD) accumulator as a function of temperature and daylength. A new stage is triggered, when the physiological day accumulator reaches a value defined by a PD threshold given in the CUL file. In CROPGRO, crop development is sensitive to temperature, photoperiod, water deficit, and N-stresses during different growth stages. Therefore, different critical daylength values and base and optimal temperatures are used in a generic phenology module (Boote et al. 1998a). For chia, there is a lack of knowledge of those temperature and daylength sensitivities. However, as more information becomes available, those findings could be easily implemented in the model. For model adaptation, seeds of a flowering mutant genotype (G8) were used. Therefore, the critical short daylength below which reproductive development progresses as rapidly as possible with no daylength effect (CSDL) was set to 14.10 hours, and the slope of the relative response of development vs. photoperiod (PP-SEN) in CUL file was set to 0.001 (to create no daylength sensitivity/ effect). During the model adaptation of chia, water stress was simulated in one year. Adding soil layers and extending root length density solved the water stress problem caused by the model.

The differences between cultivars and major groups of cultivars can be quantified in the CUL and ECO files with respect to durations of the life cycle phase durations, daylength sensitivities, number of seeds per pod, seed size, determinacy of both pod addition and leaf area growth, leaf photosynthesis rate, relative internode length, and relative canopy width. Nevertheless, local varieties, cultivars or hybrids are uncertainties for many crop models and a challenge for model developers. To apply the CROPGRO-chia model to Egypt, several genetic coefficients that conceptually represent each crop variety need to be adjusted such as PD requirements for life cycle phases such as emergence to flower

(EM-FL), first flower to first pod (FL-SH), first flower to first seed (FL-SD), and beginning seed to physiological maturity (SD-PM) and degree of determinancy (related to duration of pod addition (PODUR), node appearance after flowering (FL-VS), and leaf expansion after flowering (FL-LF). As described above, PP-SEN = 0.001 assures a daylength-insensitive cultivar. Those parameters (PP-SEN and CSDL) need to be adapted for the cultivar used in Egypt, which was commercial short-day flowering genotype with charcoal seeds available in the local supermarket (Mack et al. 2018). Data on flowering may be good enough to solve CSDL, PP-SEN, and EM-FL for that Egyptian experimental cultivar and trying to simulate this could be suggested as a future action. The CUL coefficients that describe each variety are only initial values and should be further adjusted during the calibration process to simulate crop growth and development of the selected variety under the climate and management conditions of the specific region. Parameters may be easily adjusted as new knowledge (life cycle phase durations, daylength sensitivity, senescence etc.) becomes available.

To identify the optimum management practice for chia production for Egypt, the following theoretical analysis may be conducted based on the crop simulation model. Different simulation scenarios, e.g., different plant density (50 plants m<sup>-2</sup>) could be suggested over all combinations of N-fertilizer rates, planting dates, when compared with lower plant densities of 20 and 30 plants m<sup>-2</sup> to subsequently recommend the optimal planting density for chia production in Egypt. Furthermore, it could be tested if the model predicted that different rates of N-fertilizer application would show much difference in average values over all combinations of planting.

#### **6.4 Model improvements and future research**

The simplifications of crop models cause uncertainties of the outputs. Although crop models can simulate the impact of abiotic stresses, especially drought stress, very well, they are weak with respect to simulating the interaction with biotic stresses, such as pests, diseases, and weeds. It is assumed that weeds, diseases, and insect pests are managed and that there are no problematic soil conditions, such as high salinity or acidity. Further, devastating weather events, such as heavy storms are excluded, as well. Additionally, crop models simulate the current agricultural technologies available worldwide, but do not contain potential improvements like new technology or new cultivars (UNFCCC 2016).

Another problem is the calibration of the soil N-release parameters for predicting N-supplying rate when only poor quality information on initial soil conditions and soil variables exists. However, the larger problem is defining the initial conditions (residue, nitrate, ammonium) and especially the N-supplying capability of the soil when using the different soil organic carbon modules. To confirm the assumption in the model, additional rooting data would be needed. Considering the calibration of parameters influencing response to water deficit, water-deficit treatments to well-watered treatments should be compared (Hoogenboom et al. 2012). The relative sensitivity of leaf senescence to water deficit and the relative delay or acceleration of life cycle stages could be calculated with adequate data.

Another step would also be to test the temperature parameters over a wider range of conditions to improve the usefulness of the model, as the temperature response is not well understood because of a shortage of data on relationships. However, that requires experiments that have different temperature environments and although daylength is an issue, so those studies would need to be on a daylength-insensitive cultivar. Additionally, early-season growth dynamics of LAI increase over time versus leaf appearance rate and partitioning of mass to leaf, stem, root are not well covered with the present experiments. Different sowing dates are useful because of varying temperatures.

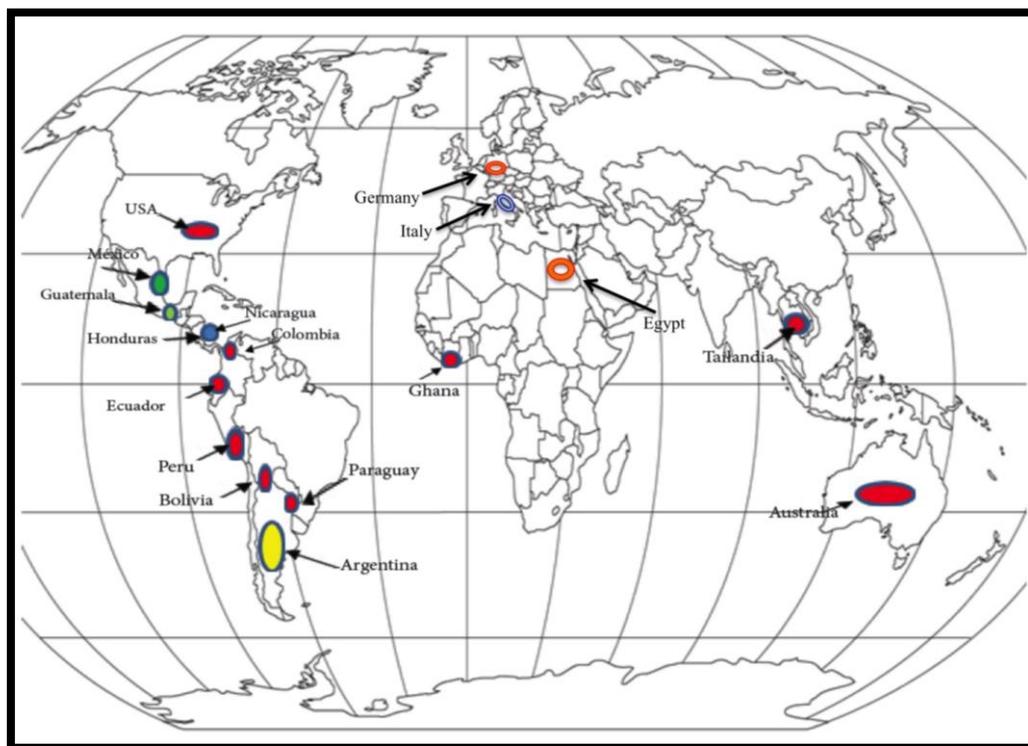
Improving the model also depends on the potential users. Addressing chia producers then, the priorities of the model should consider their needs. In addition to high yields, the seed quality of chia is of high importance. Therefore, the integration of a tool that can simulate parameters of seed quality could improve plant growth models for chia. If the model would be able to simulate quality parameters, such as  $\alpha$ -linolenic acid (ALA), fatty acid (FA) profile, or oil content or to estimate those quality compounds under different crop management strategies, the field of application could be increased in the future. In future, another expected benefit of chia simulation models would be achieved by integrating the models with other software and hardware components for whole system optimization. For example, chia simulation models could be integrated with equipment control systems (e.g. irrigation controllers), which use actual telemetry data that describe environmental conditions and crop status to automatically adjust crop inputs both spatially and temporally for optimum crop production.

Another possibility to study and optimize a cropping system is to make use of the management module, integrated in the DSSAT. It provides opportunity to define several crops and management strategies for crops and sequencing for simulating optional management systems for different applications. To address cropping systems and particularly crop rotations, the CSM was established (Jones et al. 2003). However, crop rotation was not part of this work but can encourage researchers to further work on.

## **6.5 Expansion of chia cropping to other regions - Chia as a crop on the world**

The most essential trait for expansion of dispersal is photoperiod insensitivity. Further, domestication syndrome traits such as yield components, vigor, plant architecture, uniform maturation, and non-shattering, are needed to support expansion beyond the Mesoamerican center of chia diversity (Cahill 2003; Jamboonsri et al. 2012). *Salvia* is a large genus and has species that differ in their photoperiod requirements for anthesis. The geographic restrictions for domesticated chia across its traditional range in Mesoamerican are mainly defined by photoperiodic sensitivity. The northern border is limited by the lack of types that reach maturity of seeds prior to frost, whereas human selection for later flowering enabled the expansion of the southern boundary into tropical areas where the growing season extends longer into short-days. In general, the domesticated chia variety 'Pinta' dominates

cultivation (Cahill and Provance 2002; Jamboonsri et al. 2012). Domesticated chia germplasm has a photoperiod of about 12 h for anthesis induction, so in the northern hemisphere up to 25°, anthesis of chia takes place in October (Jamboonsri et al. 2012). Modified chia lines through chemical mutagenesis which flower under long-day photoperiods of about 12–15 h offer significant potential to expand the range of production to regions with shorter growing seasons as shown in Figure 4. However, uniform maturation succeeding anthesis initiation is important for temperate zones with a shorter crop cycle in order to maximize yield (Jamboonsri et al. 2012). The agricultural areas (Figure 4) shaded in green, blue, yellow, and red characterize the area of chia cultivated on Early pre-Columbian times from N. central Mexico into Guatemala. (3500 BC-1000 AC); Late pre-Columbian time (1000AC-1500 AC); Post Columbian time (1500 AC- 2000 AC); and Modern time (2010 until now) (Sosa et al. 2016). The areas, marked with orange circles, represent the new contrasting environments, Egypt and Germany.



**Figure 4: Chia cultivation and dispersal of chia crop around the world** (Bochicchio et al. 2015b; Sosa et al. 2016; Modified).

The importance of chia as crop is very high in countries such as the U.S. (Jamboonsri et al. 2012), Chile (Baginsky et al. 2016; Cortés et al. 2017), Argentina (Ayerza 1995), and Italy (Bochicchio et al. 2015b). Several researchers investigated different agronomic practices to adapt it despite difficult climatic conditions for chia cultivation. These countries face the problem that grain production of chia

seeds is originally restricted to latitudes of 22°55' N to 25°05' S (Jamboonsri et al. 2012; Hildebrand et al. 2013) because of being adapted to short-day conditions. Therefore, when chia is cultivated outside of latitude, a low SY and nutritional quality are achieved or chia did not even produce seeds (Jamboonsri et al. 2012). Thus, researchers of the U.S. developed cultivars able to flower in locations where the daylength is longer than 12.5 hours which were also cultivated in the experiments conducted in Germany (Mack et al. 2017).

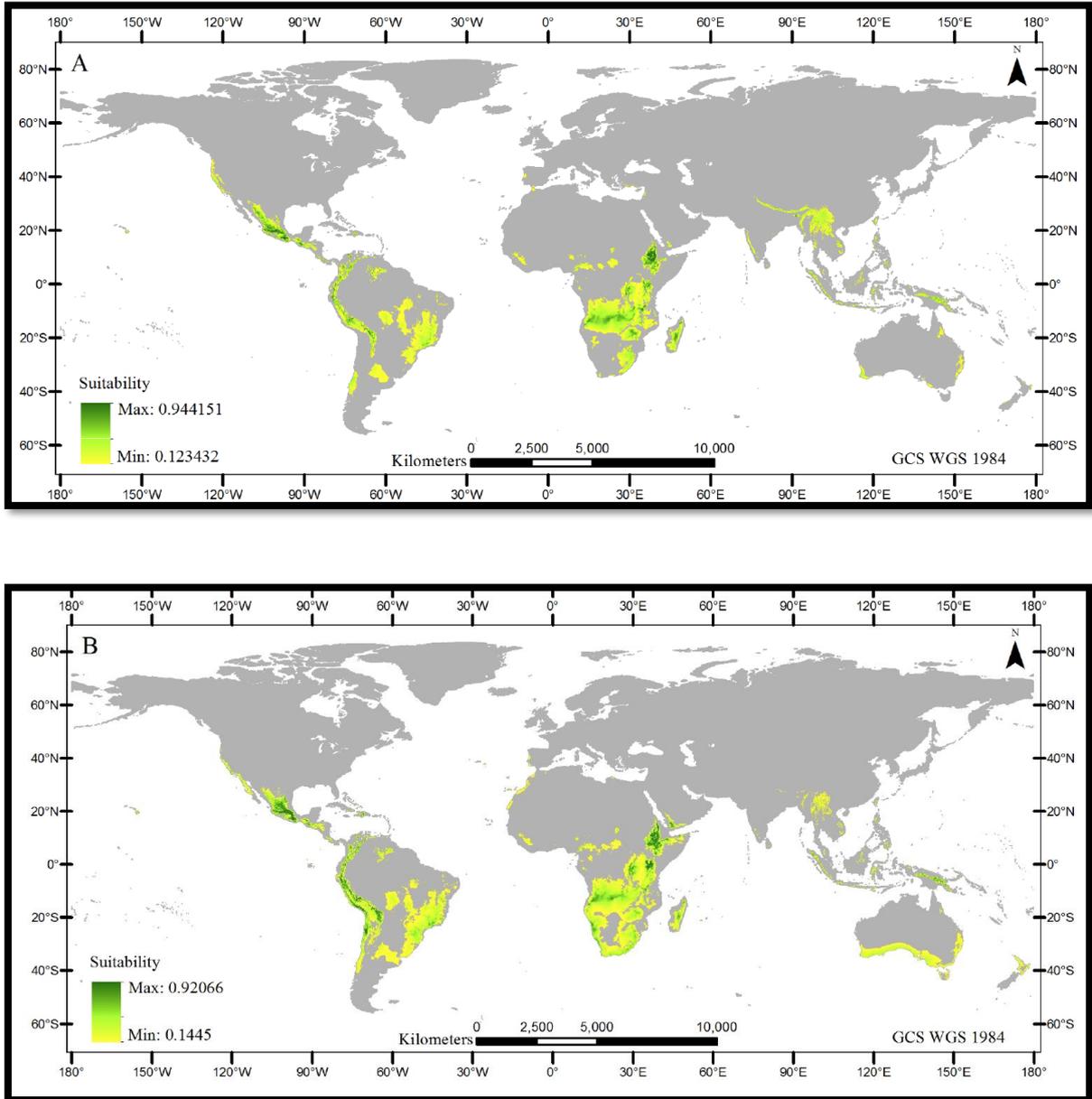
In agricultural regions located in the tropical zone (between latitude 23° 30'N and 23° 30'S) such as Jalisco (Mexico) and Ghana (Africa) it is possible to achieve high yields (1305-2605 kg ha<sup>-1</sup>), especially under irrigation. Sosa et al. (2016) reported that it is feasible in Jalisco to grow chia twice per year, and achieving in both seasons high yields and seed quality. It would be also theoretically possible to grow traditional chia in Egypt twice a year because there are not any weather restrictions or daylength limitations, but management practices are missing. It has to be noted that chia established in the summer season could face very high temperatures that has to be considered by farmers and researchers (Mack et al. 2018). Table 2 shows growing regions and seasons of chia worldwide.

**Table 2. Global growing regions and seasons of chia.**

<b>Country</b>	<b>Hemisphere</b>	<b>Season</b>
Mexico	Northern	March-October
Argentina	Southern	October-May
Kenya	Southern	September-June
Egypt	Northern	September-June
Germany	Northern	May-October/November

However, the yield gap between 500 kg ha<sup>-1</sup> and 1305 kg ha<sup>-1</sup> indicates the potential productive of chia in Mexico has not been fully exploited. To transfer and develop cropping systems for chia in Egypt and Germany, the developed CROPGRO-Chia model (Publication 3) could assist farmers to implement a chia cropping system.

Cortés et al. (2017) simulated worldwide cultivation potential distribution of *Salvia hispanica* based on the Maxent model (Figure 5). However, this method did not consider the photoperiod layer due to lack of information. This would have been valuable to model the niche of chia. Further, data on solar radiation, relative humidity, evapotranspiration or soils were not available. This led to incongruence and lack of representation of the topoclimates (Cortés et al. 2017). It was assumed that chia as a drought-tolerant species, requires a minimum of 500 mm precipitation annually to develop, having a minimum temperature demand of 10°C, an optimum temperature of 18°C and maximum temperature of 29°C. Although there are estimates that chia can withstand up to 32-33°C. Cortés et al. (2017) reported that high temperatures and long photoperiods accelerate plant growth and decrease SY, which was also observed in Egypt (Mack et al. 2018), thus this is an important factor to consider.



**Figure 5: Worldwide potential distribution of *Salvia hispanica* cultivation (A, rainfed; B, irrigated) based on the Maxent model (Cortés et al. 2017).**

## 6.6 Crop rotation or how chia can be integrated into crop sequence

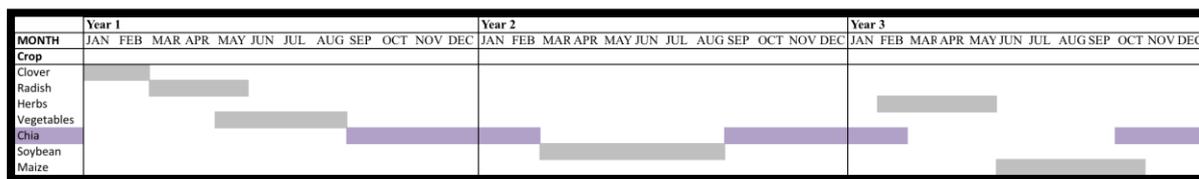
As mentioned in the introduction section, this project dealt also with water scarcity in Egypt. The importance of integrating chia as new crop in the Egyptian cropping structure required (i) the development of cropping and management systems for chia, (ii) the assessment of interactions between drought stress, physiological response, and relevant quality traits, and (iii) the establishment of practical management recommendations regarding choice of cultivar, sowing date, and sowing density for optimization of yield, quality, and economic returns. To study and investigate cultivation

of chia as a solution for water scarcity in Egypt, the question, how could chia fit generally into an Egyptian cropping sequence should also be considered.

Beside the previously mentioned management strategies, good cultivation practices also include crop rotations (CR). CR will generate positive economic and environmental benefits to soil ecosystems (Arriaga et al. 2017). Crop rotation is described as growing a different crop on a given land area every cropping cycle and season. Crops are rotated for different purposes, such as to reduce disease and pest risks (Arriaga et al. 2017), to improve or maintain soil fertility, and to increase the resilience of the agricultural system (Kollas et al. 2015; Ouda and El-Hafeez Zohry 2015). Ouda and El-Hafeez Zohry (2015) reported that crop rotation could also save irrigation water. The current common agricultural policy (CAP) of the European Commission regards the diversification of crop rotations as an important instrument for more agricultural sustainability (Kollas et al. 2015). In general, crops can be rotated for two, three years or longer periods. A good crop rotation should offer alternatives of different crop rotations with less water requirements (cereals, sugar crops, oil crops, and forage crops), as compared to the prevailing crops rotations, which exhaust the soil and have high water requirements (Ouda and El-Hafeez Zohry 2015). Agriculture in Egypt largely depends on irrigation. Policies are aimed at substituting water-intensive crops like wheat and cotton by water efficient crops with high revenues in international markets (Abdin and Gaafar 2009). In Egypt, 80% of the arable land is cultivated with wheat, clover, cotton, rice, and maize. Wheat and clover represent the main winter crops (September-March). In summer (April-September), cotton and rice are important cash crops, whereas maize and sorghum are major subsistence crops. The cultivation of sugar beet in winter season displaced other winter legumes like faba bean and lentil. Chia has been suggested as a favorable economic alternative for common field crops sustaining diversification and stabilization of the local economy (Coates and Ayerza 1996). The cultivation of chia may be attractive, especially in arid and semiarid regions, where water is the limiting factor for crop production (Ayerza and Coates 2009; Peiretti and Gai 2009). In Table 3, several crop rotations over a period of three years could be suggested for cultivation of chia in Egypt. Chia could be theoretically cropped twice a year because of its short crop cycle and climatic conditions in Egypt. It may be rotated with winter crops, such as legumes (clover, faba bean or lentil) or with summer crops like maize, sorghum, soybean, and vegetables. Figure 6 shows a potential cropping calendar in Egypt.

**Table 3. Integrating chia into existing crop rotation in Egypt.**

	<b>Year 1</b>	<b>Year 2</b>	<b>Year 3</b>
Winter (September-March)	Sugar beet	Clover	Faba bean
Summer (April-September)	Chia	Maize	Chia
	Chia	Chia	Clover
	Soybean	Vegetables (Tomato)	Peanut



**Figure 6. Cropping calendar for integrating chia into existing crop rotation in Egypt.**

In other countries, such as Mexico, chia grows naturally in juniper, oak, and pine forests. In the state of Jalisco (Mexico), chia is grown on farmland from late spring to early summer. The produced seeds are more profitable than the cultivation of beans. In northwestern Argentina and southern Bolivia, chia can be a substitute for tobacco crops (Sept/Oct to May), where they can be rotated in the fields so that soil nutrients are not depleted (Muñoz et al. 2013). In European countries, it is grown during summer from May/June to October/November and harvested before frost (Bochicchio et al. 2015a; Mack et al. 2017). In Germany, e.g., it can be cultivated after silage maize or winter wheat following a cover crop (Publication 3). It depends how much N is left in the soils. However, chia has low requirements for pesticides, fertilizer, and irrigation (Jamboonsri et al. 2012).

## 6.7 Future potentials

In light of CC and water scarcity that will significantly threaten agriculture and sustainable development in future, it is essential to focus on the optimization of management strategies. Egypt's agriculture, which mainly depends on irrigation, has to fulfill the demands of a larger population with rising living standards (Abdin and Gaafar 2009). A large share of Egypt's population works in the agricultural sector. Therefore, the agricultural sector is important for tackling poverty in developing countries (FAO 2016). The drought tolerance of chia and the possibility of export make chia attractive for cultivation in Egypt. Chia seeds are becoming increasingly popular for human nutrition due to their nutritional and health properties. If legislation changes in future, a meaningful market growth for chia seeds is expected. However, the current production of chia seeds is not able to fully meet the world market demand (Coates and Ayerza 1996; Mohd Ali et al. 2012). The production of chia has to develop from formerly small-scale production systems to a sustainable and larger scale production with modernized management practices. As broad experience in growing chia in new environments is missing, this thesis provides further information and expanded knowledge on chia cultivation in two contrasting environments (including a desert region) out of its center of origin. Overall, the current doctoral thesis presents a combined approach of experimental field research and crop modeling to support the optimization of farming practices of chia in new environments. The approaches presented in this thesis may contribute to testing new environments for chia cultivation and to improve its production.

## 7 Summary

Chia (*Salvia hispanica* L.) seeds have been revived as functional “superfood” for human nourishment especially for vegan and vegetarian diets and are becoming increasingly widespread and present in new food products in Europe. The seeds are beneficial because of being gluten-free, containing antioxidants and a high concentration of  $\alpha$ -linolenic acid, and having a high content of dietary fiber and high-quality protein. Chia is originally adapted to short-day conditions and grows naturally in tropical and subtropical environments. Nevertheless, it can survive under water stress and could, therefore, be cultivated in arid regions.

Egypt has been classified as a water-scarce state. Due to its drought tolerance, chia might contribute to saving the scarce source “water” in Egypt and offer the chance to export these high value seeds, generating foreign exchange for reimporting e.g. wheat characterized by a higher water demand. Worldwide, the biggest problems and key challenges under climate change (CC) are water and food security in arid and semiarid regions. In the future, CC and water scarcity will significantly threaten agriculture and sustainable development. A rising population requires on the one hand an increase in food grain production, but also a change toward environmentally sound sustainable agriculture. Chia has been suggested as a favorably economic alternative for common field crops sustaining diversification and stabilization of the local agricultural economy. However, broad experience in growing chia in new environments is missing. The agronomic management has not been improved from formerly small-scale production systems. Most of the previous studies focused on seed characteristics. Information on fertilization, plant protection, and improved varieties is scarce, which are reasons for its low productivity in the countries of origin.

In order to expand the existing knowledge on management strategies, to transfer cultivation practices for chia in new environments, and to overall ameliorate yields and seed quality, the following hypotheses were examined within this thesis:

- Different sowing dates (SWD) have an effect on (i) agronomic traits and (ii) seed quality. Seed yield (SY) is reduced by late sowing. The choice of SWD influences also seed quality (protein and oil content; content of polyunsaturated fatty acids (PUFA)). The optimal SWD for chia cultivation in Egypt can be determined.
- Leaf area (LA) of chia cannot be accurately predicted by the product of leaf width (LW) and length (LL). LA of chia can sufficiently be predicted by developing a new LA estimation model by extending the model to a more flexible power model and taking into account different populations and N-fertilizer levels.
- The crop growth simulation model CROPGRO can be adapted for chia. The developed CROPGRO-Chia model is a suitable tool for predicting chia yield and growth under temperate European climate conditions.

In consideration of the above mentioned goals, field experiments were conducted at the experimental station “Ihinger Hof” of the University of Hohenheim in southwestern Germany from 2015 to 2017 and in Egypt during the cropping season 2015 to 2016 at SEKEM’s experimental station located 50 km Northeast of Cairo. The present doctoral thesis was based on a project embedded in the graduate school Water-People-Agriculture (WPA) at the University of Hohenheim funded by the Anton-&-Petra-Ehrmann foundation that focuses on key water issues and water related challenges of today's society.

Preliminary results indicated that chia could be successfully cultivated under desert environments in Egypt achieving a marketable seed quality. SY and seed quality could be optimized by the choice of SWD. Under the latest SWD (SWD 6; 31 Oct.) a significantly lower SY ( $125.91 \text{ kg ha}^{-1}$ ), harvest index (HI) (0.11), oil content (27.08%), content of polyunsaturated fatty acids (PUFA) (81.46%), and ratio of PUFA to saturated fatty acids (SFA) (7.24), but higher thousand kernel weight (TKW) (1.51 g), protein content (26.03%), and higher content of saturated fatty acids (SFA) (8.21%) were achieved in comparison to the other SWDs. The optimal sowing date to achieve high yields and good seed quality of chia took place between middle and end of September (SWD 4) because of highest HI and SY ( $664.94 \text{ kg ha}^{-1}$ ) with high PUFA/SFA ratio and content of PUFA, oil, and protein. Bivariate linear mixed models were used to estimate correlations between traits in designed experiments with blocking and treatment factors. It was shown that increases in protein content were at the expense of SY.

Leaf area (LA) is an essential agronomic attribute because it is relevant for photosynthesis, light interception, water and nutrient use, crop growth, and yield potential and therefore it is an important input parameter for process-oriented crop models. However, it is problematic to measure LA directly. Thus, it is of high interest to estimate LA indirectly using easily measured correlated traits. The most commonly used method to predict LA is to use the product of LW and LL as single predictor variable. However, the LA of chia cannot be accurately predicted by this approach due to leaf shape variations of chia leaves with different leaf sizes. A new model was developed by regressing LA log linearly on LW and LL accounting for variation in shape with leaf size. Mixed-model meta-regression allowed integrating LA data across experiments. Results indicated that the developed length–width model was highly reliable across a range of populations and N-fertilizer levels. The suggested LA estimation model with two measured predictor variables (LL and LW) was  $LA = 0.740 \times LL^{0.866} \times LW^{1.075}$  which provided the highest accuracy across populations and N-levels [ $r = 0.989$ ; mean squared deviation (MSD) =  $2.944 \text{ cm}^4$ ]. The model  $LA = 1.396 \times LW^{1.806}$  with only LW was acceptable as well ( $r = 0.977$ ; MSD =  $5.831 \text{ cm}^4$ ). These models support scientists to make repeated measurements on the same leaves not harming the plant and without expensive equipment.

Crop simulation models can be helpful instruments for management and decision-making in crop production systems in different locations and climatic conditions. The crop growth model CROPGRO was adapted for chia (*Salvia hispanica* L.) using growth data collected over 2 yr in southwestern

Germany. Model adaptation was based on literature information and growth analysis data. The CROPGRO-Chia model satisfactorily simulated growth dynamics for LAI ( $d$ -statistic of 0.95 and root mean square error (RMSE) of 0.53), final leaf number ( $d$ -statistic of 0.98 and RMSE of 1.36) biomass ( $d$ -statistic of 0.93 and RMSE of 1459), seed yield ( $d$ -statistic of 0.88 and RMSE of 283), and pod harvest index ( $d$ -statistic of 0.96 and RMSE of 0.08) under temperate European climate conditions. Researchers can use the developed chia model to extend knowledge of chia and to improve its production and adaption to other regions.

On a final note, the main results of this thesis provide further information and expanded knowledge on chia cultivation in two contrasting environments (including a desert region) out of its center of origin. Overall, the current doctoral thesis presents a combined approach of experimental field research and crop modeling to support the optimization of farming practices of chia in new environments. A universal and nondestructive LA estimation model for chia was developed. Further, the CROPGRO model was adapted for chia to provide a preliminary model for a realistic simulation of crop growth variables. The approaches presented in this thesis may contribute to testing new environments for chia cultivation and to improving its production. Moreover, this study helped to develop further general model source codes to simulate the growth of tiny seeds. The adaptation to other *Salvias* should be much easier with this developed model. Future research requirements and issues requiring model improvement such as N-response and the development of code relationships that can simulate parameters of seed quality could improve the plant growth model for chia.

## 8 Zusammenfassung

In den letzten Jahren entwickelte sich um Chiasamen (*Salvia hispanica* L.) ein regelrechter Hype. Als sogenanntes “Superfood” für die menschliche Ernährung, vor allem für Veganer und Vegetarier, wurden Chiasamen wiederentdeckt. Daher gibt es immer mehr Lebensmittelprodukte mit Chiasamen. Diese Samen sind vorteilhaft, weil sie frei von Gluten sind, ein ausgewogenes Verhältnis von Makronährstoffen aufweisen und einen hohen Anteil an Omega-3-Fettsäuren, Mikronährstoffen, Antioxidantien und Ballaststoffen enthalten. Chia ist ursprünglich an Kurztagbedingungen angepasst und in tropischen und subtropischen Gebieten verbreitet. Dennoch kommt es mit Bedingungen wie Wasserstress zurecht und kann daher auch in trockenen Regionen angebaut werden.

Ägypten wurde als wasserknappes Land eingestuft. Chia kann aufgrund seiner Trockenstresstoleranz dazu beitragen die knappe Ressource „Wasser“ in Ägypten einzusparen und bietet gleichzeitig Exportmöglichkeiten. Im Gegenzug kann dadurch z.B. Weizen mit einem höheren Wasserbedarf importiert werden. Der Klimawandel (CC) und die damit verbundene mangelnde Wasser- und Ernährungssicherheit stellen eine der weltweit größten Herausforderungen in ariden und semiariden Regionen dar und gefährden in Zukunft nicht nur die landwirtschaftliche Produktion, sondern auch die nachhaltige Entwicklung. Auf der anderen Seite, erfordert eine steigende Bevölkerung einerseits eine Steigerung der Nahrungsmittelproduktion, andererseits auch den Wandel zu einer umweltverträglichen und nachhaltigen Landwirtschaft. Chia könnte eine wirtschaftliche Alternative zu den herkömmlichen Feldfrüchten darstellen und so die Diversifizierung und Stabilisierung der lokalen Agrarwirtschaft unterstützen. Es fehlt jedoch die Erfahrung zum Anbau von Chia in neuen Regionen. Die Mehrheit der bisherigen Studien zu Chia legte den Schwerpunkt auf Qualitätseigenschaften der Samen. Das traditionelle Anbausystem von Chia wurde bislang nicht modernisiert und Informationen über den Einsatz von Düngemitteln, Pestiziden und verbesserten Sorten sind nur vereinzelt zu finden, welches Gründe für eine geringe Produktivität sind. Um die gestiegene Nachfrage an Chiasamen zu decken, werden neue Erzeugerländer hinzukommen müssen, für die Anbaukonzepte fehlen.

Um das vorhandene Wissen zum Anbau von Chia auf neue Regionen zu erweitern und damit die Erträge und die Kornqualität von Chia zu verbessern, wurden im Rahmen dieser Arbeit folgende Hypothesen untersucht:

- Unterschiedliche Saatzeitpunkte (SWD) wirken sich auf (i) agronomische Eigenschaften und (ii) Saatgutqualität aus. Der Samenertrag (SY) wird durch eine verspätete Aussaat reduziert. Die Kornqualität (Protein- und Ölgehalt; Gehalt an mehrfach ungesättigten Fettsäuren (PUFA)) kann durch den SWD optimiert und der optimale Aussaattermin für Chia in Ägypten bestimmt werden.
- Die Blattfläche (LA) von Chia kann durch das Produkt aus Blattbreite (LW) und Länge (LL) nicht genau vorhergesagt werden. Die LA von Chia kann durch die Entwicklung eines neuen

Blattflächenmodells zufriedenstellend vorhergesagt werden, indem das Modell zu einer Exponentialfunktion erweitert wird und verschiedene Populationen sowie N-Düngerstufen berücksichtigt werden.

- Das Pflanzenwachstumsmodell CROPGRO kann an Chia angepasst werden. Das entwickelte CROPGRO-Chia-Modell ist geeignet, um den Ertrag und das Wachstum unter gemäßigten europäischen Klimabedingungen vorherzusagen.

Zur Prüfung dieser Hypothesen wurden im Zeitraum von 2015 bis 2017 Feldversuche an der Versuchsstation „Ihinger Hof“ der Universität Hohenheim im Südwesten Deutschlands und in Ägypten von 2015 bis 2016 an der 50 km nordöstlich von Kairo gelegenen Versuchsstation von SEKEM durchgeführt. Die vorliegende Dissertation basiert auf einem von der Anton-&Petra-Ehrmann-Stiftung geförderten Projekt, das sich auf die Graduiertenschule Wasser-Menschen-Landwirtschaft (WPA) an der Universität Hohenheim stützt und sich mit zentralen Fragen zum Thema Wasser und wasserbezogenen Herausforderungen der heutigen Gesellschaft befasst.

Erste vielversprechende Ergebnisse zeigten, dass Chia erfolgreich in Ägypten angebaut werden konnte und eine marktfähige Kornqualität erzielt wurde. SY und Kornqualität konnten durch die Wahl des Saatzeitpunktes optimiert werden. Beim letzten SWD im Oktober (SWD 6) wurden ein deutlich niedrigerer SY ( $125,91 \text{ kg ha}^{-1}$ ), Ernteindex (HI) (0,11), Ölgehalt (27,08%), Gehalt an mehrfach ungesättigten Fettsäuren (PUFA) (81,46%) und ein geringeres Verhältnis von PUFA zu gesättigten Fettsäuren (SFA) (7,24), aber ein höheres Tausendkorngewicht (TKW) (1,51 g), Proteingehalt (26,03%) und ein höherer Gehalt an gesättigten Fettsäuren (SFA) (8,21%) im Vergleich zu den anderen Saatzeitpunkten erreicht. Der vierte Saatzeitpunkt (SWD 4) stellte sich im Hinblick auf Ertrag und Kornqualität als optimal heraus, da HI sowie SY ( $664,94 \text{ kg ha}^{-1}$ ) am höchsten waren und zudem ein hohes PUFA/SFA-Verhältnis sowie hohe Protein- und Ölgehalte erzielt wurden. Um Korrelationen zwischen Merkmalen wie SY und Proteingehalt aufzuzeigen, sollten, wie in dieser Arbeit dargestellt, bivariate lineare gemischte Modelle verwendet werden. Dabei wurde gezeigt, dass der Proteingehalt auf Kosten des Ertrags gesteigert wurde.

Die Blattfläche (LA) ist ein wesentliches agronomisches Merkmal, da sie für Prozesse wie Photosynthese, Lichtaufnahme, Wasser- und Nährstoffbedarf, Pflanzenwachstum und Ertragspotenzial relevant ist. Aus diesem Grund stellt sie einen wichtigen Inputparameter für prozessorientierte Pflanzenwachstumsmodelle dar. Allerdings ist es schwierig, die LA direkt zu bestimmen. Daher ist es vorteilhaft, die LA indirekt mit Hilfe von leicht messbaren und korrelierten Merkmalen zu schätzen. Die am häufigsten verwendete Methode zur Vorhersage der Blattfläche ist die Verwendung des Produkts aus LW und LL. Allerdings kann die LA von Chia aufgrund von Blattformveränderungen mit diesem Ansatz nicht genau vorhergesagt werden. Ein neues Modell wurde entwickelt, indem die LA logarithmisch auf LW und LL abgebildet und zugleich die Blattformveränderung unterschiedlicher Blattgrößen berücksichtigt wurde. Die Meta-Regression mit gemischten Modellen ermöglichte es,

Daten aus verschiedenen Experimenten gemeinsam auszuwerten. Die Ergebnisse zeigten, dass das entwickelte Längen-Breiten-Modell zur Schätzung der LA von Chia unabhängig von Sorte und N-Düngerstufen sehr zuverlässig war. Das vorgeschlagene Blattflächenmodell mit zwei Variablen (LL und LW) mit der höchsten Genauigkeit [ $r = 0,989$ ; mittlere quadratische Abweichung (MSD) = 2,944 cm<sup>4</sup>] lautete  $LA = 0,740 \times LL^{0,866} \times LW^{1,075}$ . Das Modell mit nur einer Variablen (LW),  $LA = 1.396 \times LW^{1,806}$ , war ebenfalls akzeptabel ( $r = 0,977$ ; MSD = 5,831 cm<sup>4</sup>). Diese Modelle unterstützen Wissenschaftler dabei, wiederholte Messungen an gleichen Blättern durchzuführen, ohne die Pflanze zu beschädigen oder teure Ausrüstung zu verwenden.

Pflanzenwachstumsmodelle können hilfreich sein, die Anbaustrategien an verschiedenen Standorten und klimatischen Bedingungen zu verbessern und zu optimieren. Daher war ein Teilaspekt dieser Doktorarbeit, das prozessorientierte Pflanzenwachstumsmodell DSSAT CROPGRO für Chia (*Salvia hispanica* L.) anzupassen. Die Modellanpassung basierte auf Literaturangaben und Wachstumsdaten, die über einen Zeitraum von zwei Jahren im Südwesten Deutschlands erhoben wurden. Das CROPGRO-Chia-Modell simulierte den Blattflächenindex (LAI) ( $d$ -Statistik von 0.95 und Root Mean Square Error (RMSE) von 0,53), die Blattzahl ( $d$ -Statistik von 0.98 und RMSE von 1,36), die Biomasse ( $d$ -Statistik von 0.93 und RMSE von 1459), sowie den Korntrug ( $d$ -Statistik von 0.88 und RMSE von 283) unter gemäßigten, europäischen Klimabedingungen zufriedenstellend. Das entwickelte Chia-Modell kann genutzt werden, um das bestehende Wissen über Chia zu erweitern und den Anbau in neuen Regionen zu unterstützen.

Abschließend ist festzuhalten, dass die Ergebnisse dieser Arbeit neue zentrale Informationen über den Anbau von Chia in zwei unterschiedlichen Regionen (einschließlich einer Wüstenregion) außerhalb des ursprünglichen Verbreitungsgebietes liefern. Die vorliegende Dissertation stellt einen ersten und vielversprechenden Ansatz zur Entwicklung eines Blattflächenmodells für Chia dar. Darüber hinaus kombiniert die Arbeit experimentelle Ansätze in Feldversuchen mit Pflanzenwachstumsmodellen, um die Anbaumethoden von Chia in neuen Regionen zu unterstützen und zu optimieren. Dafür wurde erstmals das CROPGRO-Modell für Chia angepasst, um Ertrag und Wachstumsparameter abzubilden. Darüber hinaus trug diese Arbeit dazu bei, den allgemeinen Quellcode des Modells zu verbessern, um das Wachstum sehr kleiner Samen zu simulieren. Die Anpassung an andere *Salvias* sollte mit diesem entwickelten Modell ebenfalls wesentlich einfacher sein. Zukünftige Modellverbesserungen und die Integration eines Tools, das ebenfalls die Kornqualität simulieren kann, könnten das Pflanzenwachstumsmodell für Chia weiter verbessern.



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## Appendix



Figure A 7. Mechanical harvesting of chia in Germany. © Picture by Laura Mack.



Figure A 8. Traditional field drying of chia in Egypt. © Picture by Laura Mack.



**Figure A 9. Traditional and manual sieving of field dried chia seeds in Egypt. © Picture by Laura Mack.**

### List of all publications and publications embedded in the dissertation

Mack, L., Munz, S., Capezzone, F., Hofmann, A., Piepho, H.-P., Claupein, W., & Graeff-Hönninger, S. (2018). Sowing Date in Egypt Affects Chia Seed Yield and Quality. *Agronomy Journal*, 110(6), 2310–2321. doi:10.2134/agronj2018.05.0324.

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Mack, L., Boote, K.J., Munz, S., Phillips, T., & Graeff-Hönninger, S. (2020). Adapting the CROPGRO Model to Simulate Chia Growth and Yield. *Agronomy Journal*, 1–19. doi.org/10.1002/agj2.20305.

### Conference proceedings

- Conference Tropentag 2015 “Management of land use systems for enhanced food security - conflicts, controversies and resolutions”, September 2015, Humboldt-Universität zu Berlin, Berlin, Germany. Attendance.
- Conference 5<sup>th</sup> International EcoSummit “Ecological sustainability”, September 2016, Le Corum, Montpellier, France. Attendance.
- Gesellschaft für Pflanzenbauwissenschaften e.V. – 59<sup>th</sup> Annual Conference “Climate Change and Quality”, 26<sup>th</sup> -28<sup>th</sup> September 2016, University of Gießen. Mack, L., Munz, S., Hendawy, S.F., Hofmann, A., Höping, A., & Graeff-Hönninger, S. (2016). Landwirtschaft in der Wüste– Entwicklung eines Anbau- und Bewässerungssystems für die Alte Körnerfrucht Quinoa (*Chenopodium quinoa* Willd.) in Ägypten. *Mitt. Ges. Pflanzenbauwiss*, 28, 252-253. Poster presentation.

### Oral Presentations

- World Water Day 2017 “Wastewater – A Threat or Resource for Global Water Supply?” – Colloquium, 22<sup>th</sup> March 2017, University of Hohenheim. Water scarcity in Egypt– Irrigation strategies for quinoa. Oral presentation.
- Sommertagung AG Landwirtschaftliches Versuchswesen 29<sup>th</sup>-30<sup>th</sup> June 2017, DLR Rheinlandpfalz, Neustadt an der Weinstraße. Wie lässt sich die Blattfläche von Chia (*Salvia hispanica* L.) anhand mathematischer Gleichungen bestimmen? Oral presentation.

### Other Contributions

- <https://water4use.uni-hohenheim.de/mack>
- <http://www.sekem.com/de/trendy-und-nachhaltig-quinoa-und-chia-forschung-in-sekem/>  
(Figure A 10)
- <http://peoplefoodandnature.org/blog/trendy-and-sustainable-quinoa-and-chia-research-in-egypt/>



**Figure A 10. Monitoring of cultivated chia plants at the SEKEM experimental station (Adeja farm) in Egypt.**

© Picture by Laura Mack.

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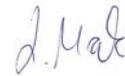
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