Growth and yield of chia (*Salvia hispanica* L.) in the Mediterranean and desert climates of Chile

Cecilia Baginsky[1], Jorge Arenas[2], Hugo Escobar[3], Marco Garrido[1], Natalia Valero[1], Diego Tello[1], Leslie Pizarro[3], Alfonso Valenzuela[4], Luís Morales[1], and Herman Silva[1*]

ABSTRACT

Chia (*Salvia hispanica* L.) is a species with seeds that have high essential fatty acid content, which has encouraged increased crop production worldwide. However, the expansion of chia is limited because it is a photoperiod-sensitive plant adapted to areas without cold. The objective of the present study was to determine the effect of different climatic conditions on the growth, grain yield and oil production of chia under irrigation in three geographic areas of Chile: Valle de Azapa (18°30' S lat) with a coastal desert climate, normal desert climate in Canchones (20°26' S lat), and Las Cruces (33°30' S lat) with dry Mediterranean climate with marine influence, and two chia phenotypes: white and dark. Results indicated that desert conditions in the Valle de Azapa (VA) and Canchones (CH) provided better conditions for plant growth; the highest yield (> 2900 kg ha^{-1}) and oil production (> 550 L ha^{-1}). In Las Cruces (LC), at higher latitude, low temperatures present beginning in April coincided with the reproductive stage, affecting yield which was no more than 129 kg ha^{-1}; thus this zone is not recommendable for chia cultivation. This study also determined an 11.8 h day length threshold for the beginning of flowering; when plants are exposed to shorter days flower initiation is more precocious, but when day length is not adequate plants only begin to flower when they have accumulated 600-700 °C d.

Key words: Chia, date sowing, grain yield, photoperiod.

[1]Universidad de Chile, Facultad de Ciencias Agronómicas, Santa Rosa 11315, La Pintana, Santiago, Chile.
[*]Corresponding author (hsilva@uchile.cl).
[2]Universidad Arturo Prat, Facultad de Recursos Naturales Renovables, Av. Arturo Prat 2120, Iquique, Chile.
[3]Universidad de Tarapacá, Facultad de Ciencias Agronómicas, 18 de Septiembre 2221, Arica, Chile.
[4]Universidad de Chile, Instituto de Nutrición y Tecnología de los Alimentos, El Líbano 5524, Santiago, Chile.

INTRODUCTION

Chia (*Salvia hispanica* L.) belongs to the Lamiaceae family and its center of origin is in mountain areas of Mexico and Guatemala (Cahill, 2004). It was traditionally one of the four basic elements in the diet of Central American civilizations in the pre-Columbian epoch. Today chia is being re-introduced into western diets because of its numerous positive nutritional characteristics. These include 1) a high concentration of essential fatty acids (29%-32% extractable), 2) polyunsaturated fatty acids omega 3 and omega 6 (Peiretti and Gai, 2009), and 3) mucilaginous fiber content (27% of the grain) that expands when hydrated and has a low cation exchange capacity that increases availability of several minerals, proteins, and vitamins required by humans (Reyes-Caudillo et al., 2008; Ayerza, 2013). These characteristics are helping to rapidly increase its production worldwide.

Chia is currently cultivated in Australia, Bolivia, Colombia, Guatemala, Mexico, Peru, and Argentina (Busilacchi et al., 2013). The largest production center is located in Mexico; it currently exports seeds to Japan, USA, and Europe (Alenbrant et al., 2014). Chia grows naturally in tropical and subtropical environments; it is optimally established from 400 to 2500 m a.s.l., but conditions below 200 m elevation are not adequate for its cultivation (Orozco et al., 2014). It is intolerant to freezing in all development stages (Lobo et al., 2011; Bochicchio et al., 2015). Its minimum and maximum growth temperatures are 11° and 36 °C, respectively, with an optimum range of 16-26 °C (Ayerza and Coates, 2009). It is considered to be a short-day plant with a threshold of 12-13 h (Jamboonsri et al., 2012; Busilacchi et al., 2013), and as such, its period of growth and fruiting depend on the latitude where it grows. Jamboonsri et al. (2012) indicated that domesticated chia germplasm has a flowering induction photoperiod of approximately 12:12 h. Thus in the Northern Hemisphere chia begins to flower in October and in the Southern Hemisphere in April. Hildebrand et al. (2013) indicated that with existing plant germplasm, distribution of chia for grain production is restricted to 22°55' N-25°05' S. At higher latitudes, the probability of the crop reaching maturity is low, since plants die due to early frosts (Ayerza and Coates, 2005). Efforts to induce chia to flower with day lengths greater than 12 h have failed (Cahill, 2004), with the idea of widening cultivated area to temperate zones and regions such as the Mediterranean basin. Jamboonsri et al. (2012) demonstrated the existence of new chia varieties with early flowering, which were able to flower with a photoperiod 15:9 h in greenhouse and in the field with a photoperiod of 14 h 41 min.

There is a new photoperiod-insensitive variety ('Sahi Alba 914'; Sorondo, 2014), however it is not yet a commercial variety. The sowing date is an extremely relevant variable, since it determines the duration of the development period of the crop due to variations in environmental temperature and day length to which it is exposed (Lobo et al., 2011). These conditions are mostly responsible for the potential yield and seed quality (Ayerza, 2010; 2011).

Grain oil yield ranges from 29.4% to 33.5% depending on the area of origin of the chia, climatic conditions and the technique used for its extraction (Ayerza and Coates, 2009). Studies have shown that seed oil content tends to increase with altitude in which seeds are grown; however, the environment also modifies oil composition. The temperature affects the type of fatty acids present in the oil; increase of temperature during grain development produces a reduction in the production of polyunsaturated fatty acids. A relation has been found between altitude, fatty acid composition, and oil saturation. Ayerza and Coates (2009) found that in different inter-Andean valleys of Ecuador, the lower altitude environment (1600 m a.s.l.) showed a higher percentage of α-linolenic acid, but lower values for linoleic and oleic acids, indicating that oil saturation decreased at higher altitude (Ayerza, 2011). This would be related to the negative relation between altitude and temperature, which produces changes in the metabolic processes as has been demonstrated in other oil crops such as jojoba (*Simmondsia chinensis* (Link) C.K. Schneid.; Ayerza, 2001). Colder temperatures generally increase the level of unsaturation of fatty acids (Thomas et al., 2003).

The objective of this study was to determine the effect of climatic conditions in regions of coastal desert, normal desert, and dry Mediterranean with marine influence climates of Chile on the growth, yield and oil composition of chia grown with irrigation. This study also proposes to determine the most promising sowing date in terms of yield and oil quality in chia in the different study areas.

MATERIALS AND METHODS

The study was performed from January to June 2013 in Valle de Azapa in the Arica y Parinacota Region, Canchones in Alto Hospicio, Tarapacá Region, and Las Cruces in Valparaiso Region, Chile. In the Valle de Azapa (18°30' S lat) prevailing conditions are a coastal desert climate with abundant cloudiness, no frost, high humidity and high solar radiation throughout the year (González et al., 2013). Canchones Experiment Station has a desert climate, hot with absence of rainfall and strong thermal oscillation and low relative humidity. It is located at an altitude of 990 m a.s.l. and therefore exposed to frost that begins in winter (Lanino, 2005). Las Cruces has a mild Mediterranean climate with very low incidence of frost, located at an altitude of 12 m a.s.l. Table 1 summarizes climate and soil characteristics of the experimental sites, and Table 2 shows thermal sum and number of freezes recorded during January-June 2013 in each experimental site.

Configuration of the experiment and agricultural management

We used phenotypes of white and dark (black spotted) chia from Santa Cruz de la Sierra (Bolivia) provided by the company Benexia S.A. Five sowing dates were used for each locality (Table 2), constituting 10 treatments per locality (5 sowing dates × 2 phenotypes). The experimental design was randomized complete blocks with six replicates. The experimental unit was defined as a plot 5.0 m long and 2.4 m wide, with 0.6 m between rows.

Seeds were sown by hand at 1 cm depth with 5 kg seeds ha^{-1}, producing plant densities between 120 and 160 plants m^{-2}. Once the first pair of leaves had completely unfolded, plants were thinned to a density of 80-90 plants m^{-2} (50 to 60 plants m^{-1}). Plants were fertilized by fertirrigation

Table 1. Location climatic characteristics, and soil and water quality from sowing to harvest in the sites where chia was sown.

Characteristics		Location		
		Valle de Azapa	Canchones	Las Cruces
General				
Climate		Coastal Desert	Normal Desert	Mediterranean[*]
Latitude		18°30' S	20°26' S	33°30' S
Longitude		70°00' W	69°32' W	71°36' W
Elevation (m a.s.l.)		230	996	12
Temperature, °C	Max.	24.1	33.8	22.5
	Min.	13.3	3.7	1.8
	Mean	18.6	19.2	12.2
Precipitation, mm	Mean	0.0	0.0	166.0
VPD, kPa		1.1	1.2	0.9
Soil				
Order		Aridisol	Aridisol	Mollisol
pH		7.8	8.1	7.8
Salinity, dS m^{-1}		7.9	28.4	0.8
Nutrient, mg kg^{-1}	N	123.0	117.0	10.0
	P	37.0	20.0	92.0
	K	914.0	1060.0	264.0
Organic matter, %		1.4	1.5	2.3
Irrigation water				
pH		7.8	8.2	7.5
Salinity, dSm^{-1}		1.1	8.2	7.8
Carbonate, mmol L^{-1}	HCO$_2^{-3}$	2.1	1.4	5.3
Sulfate, mg L^{-1}	SO^{-2}	239.0	286.0	262.0
Chloride, mg L^{-1}	Cl$^-$	146.0	113.0	258.0
Boron, mg L^{-1}	B	0.7	nd	nd
Sodium, mmol L^{-1}	Na$^+$	4.2	7.7	7.5

[*]Dry Mediterranean with maritime influence (Csb).
VPD: Vapor pressure deficit.
nd: not detected.

Table 2. Accumulated growing degree-days (GDDi) and frost number.

	Locality					
	Valle de Azapa		Canchones		Las Cruces	
Month	GDDi	Frost number	GDDi	Frost number	GDDi	Frost number
January	332.3	0	363.2	0	194.2	0
February	350.2	0	346.0	0	181.6	0
May	310.2	0	337.7	0	113.9	0
April	196.0	0	223.1	0	54.3	0
May	183.5	0	207.8	4	44.3	0
June	176.9	0	161.0	9	22.0	5
Total	1549.1	0	1638.8	13	610.3	5

GDDi: Accumulated growing degree days (> 10 °C).

to maintain 45, 60, and 100 units N, P_2O_5, and K_2O, respectively, in the soil. We used drip irrigation controlled by a Rain Bird programmer (Rain Bird Corporation, Tucson, Arizona, USA). Water requirements were programmed to replenish 70% of reference evapotranspiration demand (ET_0), calculated daily using the Penman Monteith method. Weeds were controlled by hand when they appeared; no pests or diseases were observed.

Sampling and analysis

Plants were evaluated every 2-3 d to determine their phenological stages of emergence (visible cotyledons and completely expanded over the soil), initiation of branching (aerial branches with a node), flower initiation (inflorescence of the central stalk with first flower open), physiological maturity (inflorescence of the central stalk with brown color), and harvest maturity (grains with 14% humidity). The development stage was defined when 50% plants of the experimental unit reached that stage.

At the beginning of flowering the accumulation of aerial biomass was measured by sampling 0.5 m of plants per experimental unit. These were dried in an oven at 70 °C. This measurement was repeated at harvest, when we also evaluated plant height and length, and number of inflorescences. Yield was measured along 3 m per experimental unit by separating inflorescences from the rest of the plant. The inflorescences were dried in a forced air oven at 40 °C until constant weight. Dry inflorescences were threshed, separating the grains. Yield per plant was estimated and 500 grains sampled randomly were weighed. We calculated the harvest index (HI) as the quotient of dry weight to total plant weight.

Growing degree days (GDDi) were calculated with the following function:

$$GDDi = \sum_{ti}^{tf}(T_i - T_b) \text{ if } GDDi < 0 \text{ then } GDDi = 0 \qquad [1]$$

where $GDDi$ is the accumulated growing degree days, Ti is the mean air temperature on day i and Tb is the base development temperature, in this case 10 °C, given the area of origin of the species.

The day length for each locality was estimated as:

$$DL = \left(\frac{24}{\pi}\right) ACOS\left[- TAN(LAT)TAN\left(23.45 \right.\right.$$
$$\left.\left. SIN(\frac{360}{365}(d + 284))\right]\right. \qquad [2]$$

where DL is day length, LAT is the latitude of the locality, and d is the corresponding calendar day.

The meteorological variables were obtained from weather stations closest to the study sites.

To quantify oil content and lipid profile after harvest we sampled 500 g seeds per treatment; we used the modified cold method of Folch et al. (1957) to extract oil and determine lipid profile of each oil sample. To each 100 g chia seeds ground in a processor we added 500 mL 2:1 (v:v) mixture of chloroform:methanol and mixed for 5 min. Mixtures were then filtered twice, obtained filtrate was placed in a decantation funnel and 40-50 mL distilled water was added, agitated vigorously for 2 min and then left to stand until phases were completely separated (1.5-3 h). Two phases were obtained,

the lower phase is chloroform, which was rescued by rotary evaporation at 60 °C in a vacuum. This separated the solvent, remaining chia oil was 25%-39% total seed weight. The oil samples of each treatment were homogenized and then total fatty compounds were extracted in methanol:chloroform 2:1 with 0.5 N Mg_2Cl. Total fatty compounds and chloroform were recovered; the latter was evaporated using N_2, thus obtaining the total lipid fraction available in seeds. To determine fatty acid profile of fatty fraction extracted it was first methylated with boron trifluoride (BF_3) in 12% methanol and then with NaOH in methanol to obtain free methylated esters of fatty acids. These were suspended in hexane in order to be injected into a gas chromatograph.

Statistical analyses

The results obtained for each locality were submitted to ANOVA using the InfostatGenprogram (Balzarini et al., 2011); when significant differences were found among treatments ($P < 0.05$), Duncan *post hoc* test was used to identify significantly different means. We also performed a combined ANOVA to evaluate the effect of locality on sowing date and chia accession, and a principal components analysis to associate growth parameters (biomass at flowering and harvest, plant height, number and length of inflorescences and yield) with the combination of locality and sowing date.

RESULTS

Climatic condition and crop development

The day lengths to which the crops were exposed were similar at VA and CH, which are at similar latitudes (18° and 20° S lat, respectively). At 33°30' S lat, LC had longer days before the autumn equinox and shorter days afterwards. Canchones had the greatest temperature oscillation and LC the least. Mean temperatures were similar in VA and CH, and lower in LC (Figure 1). Since there were nonsignificant differences between white and dark chia phenotypes in their growing cycle length, values of each type were averaged. Sowing date did not modify cycle length in VA or CH. However, in VA flowering and maturity were more rapid when plants were sown later (Figure 2). In CH, frosts affected plants and did not allow them to reach maturity on the latest sowing dates (Figures 1 and 2). The low thermal accumulation in LC did not allow plants to reach and complete adequately their phenological development (Figures 1, 2, and 3). In VA, the fifth sowing date (F5) reached physiological maturity with a thermal sum of 728.7 °C d, while in LC the earliest sowing (F1) only accumulated 585.1 °C d at harvest. Plants did not accumulate sufficient temperature in LC, due to which they could not adequately complete their phenological development. This is shown by the lack of yield, since there was not an adequate phase for grain filling. Chia thermal requirements were affected by day length. Photoperiods of less than 11.8 h define the lower thermal requirement of plants to start flowering, which corresponded to about 500 °C d, while longer day length increases the thermal time necessary to initiate flowering (Figures 1 and 3).

Figure 1. Variation of photoperiod (A), minimum temperature (B), maximum temperature (C), and accumulated evapotranspiration (ET_0) (D) from January to June 2013 in each experimental site.
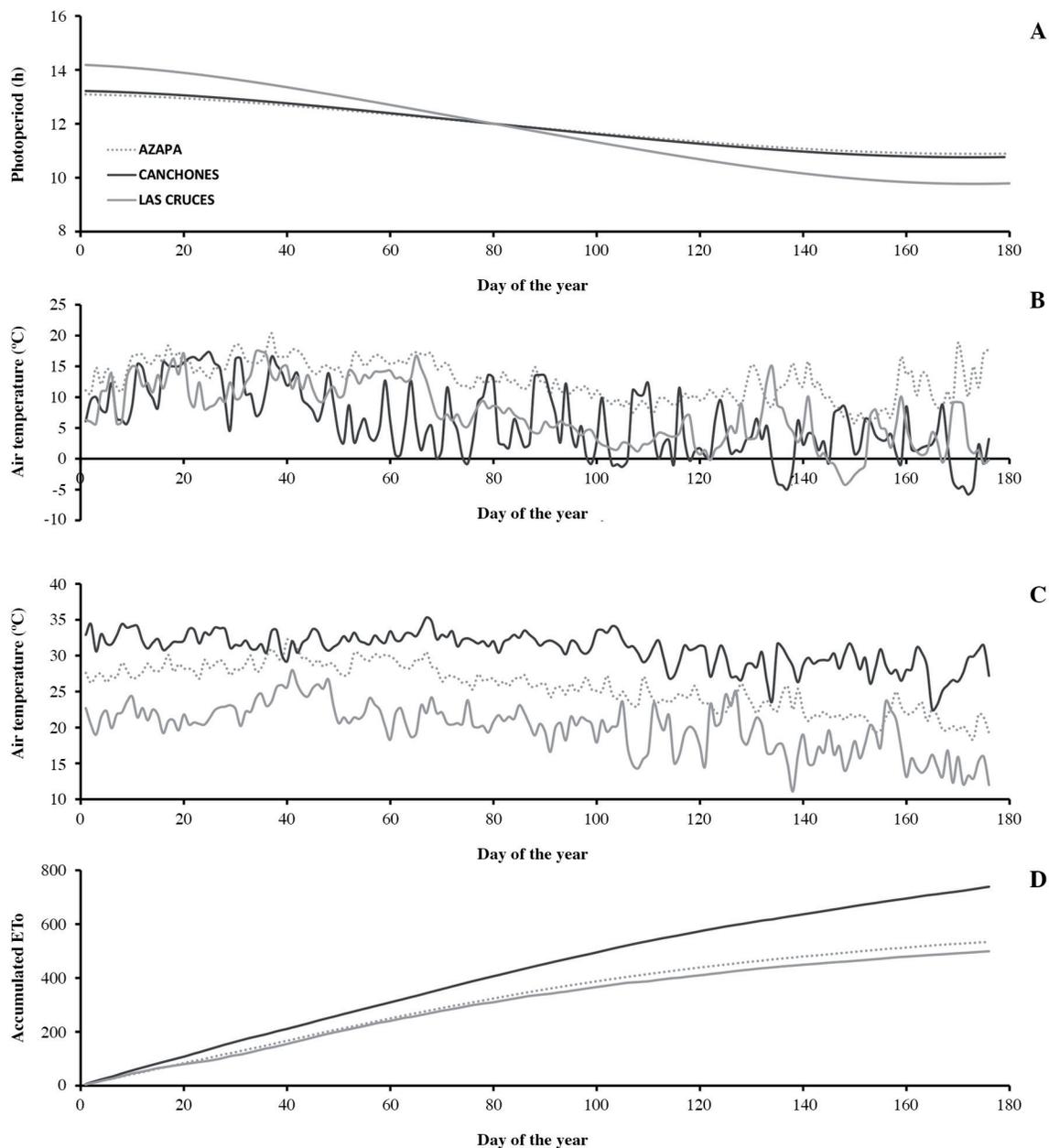

Figure 2. Days after sowing to flower initiation (A) and physiological maturity (B) of chia plants with different sowing dates (F1-F5) in three localities. The missing data of physiological maturity for Canchones and Las Cruces resulted from plant death due to the effect of frost and/or low temperature.
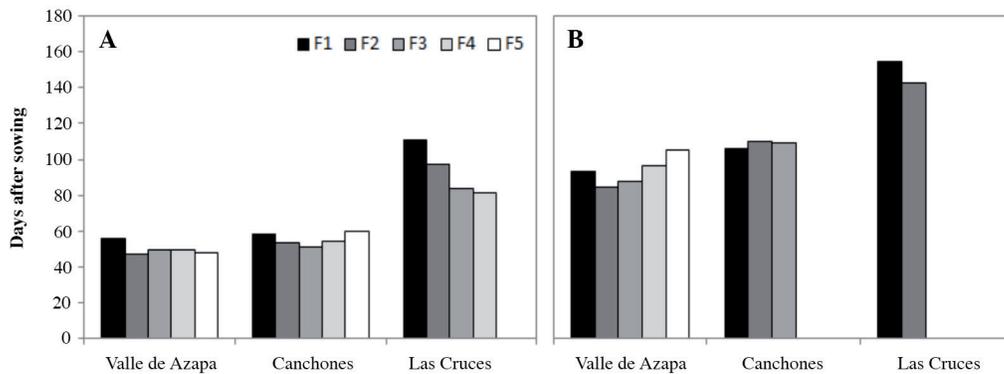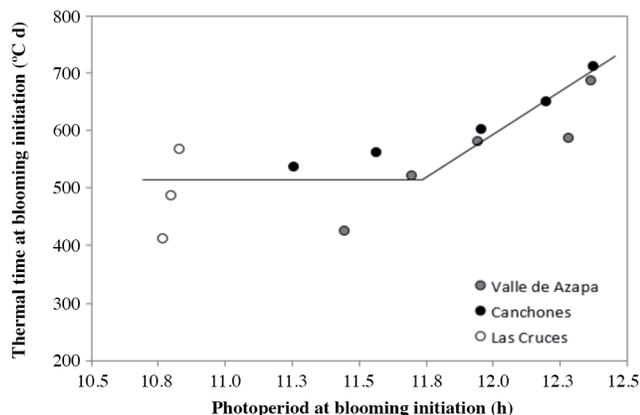
Figure 3. Relation between accumulated growing degree days according to Equation [1] and base temperature of 10 °C and day length according to Equation [2], and flowering initiation of chia in different localities and sowing dates.

Growth parameters and crop yield

The behavior of biomass production, yield, and harvest index did not present the same tendency in the different localities (Table 4). Only VA had a significant interaction between sowing date and phenotype ($P < 0.05$), thus in this locality the effect of the individual factors (sowing date and accession) are not clear (Table 3); however, in all experimental sites the effect of sowing date was significant ($P < 0.01$), thus the tendency in VA was a decrease in biomass production with later sowing and increase in yield and harvest index. This is explained by the fact that in the latest sowing dates there were lower temperatures, which did not reach the level of plant damage, which prolong the period of grain filling and thus increase yield and harvest index (Tables 3 and 4). Biomass production increased in CH with later sowing, but yield and harvest index decreased (Table 3). In this case there was less thermal oscillation, which implies high temperature accumulation, however at the end of summer there were periods of cold during the day and freezes in autumn (Figure 1B, 1C). Due to these conditions, length of grain fill period was relatively constant between sowing dates, but the later sowings had less metabolic activity during grain filling due to cold stress and later damage from freezes. This resulted in lower yield in the later sowing dates (Table 4). In LC, the three parameters mentioned above decreased with later sowing dates. The difference between types of chia was significant for biomass and harvest index variables only in LC; dark phenotype produced greater values. The lowest yields were observed in LC (70 to 129 kg ha^{-1}). The presumed reason was that low temperatures experienced during plant reproductive stage, which affected grain filling during sowing dates F1, F2, and F3, resulted in most plants not reaching necessary physiological maturity, some did not even start flowering in period F5. Similarly, CH plants were damaged during flowering by low temperature for the last two sowing dates (F4 and F5).

The combined ANOVA indicated a significant interaction between locality and sowing date. The greatest yields were achieved during periods F5 and F1 in VA and CH, with yields of 2500 and 1900 kg ha^{-1}, respectively, while the lowest yields were obtained in LC for all sowing dates, with a mean of 105 kg ha^{-1} (Figure 4). Valle de Azapa produced high and stable yields independent of sowing date, in contrast to CH where F3 produced a significant reduction in yield with delayed planting date (49% reduction between F1 and F3).

Components of yield and association among variables

Delaying sowing date tended to generate larger inflorescences in all localities (from 13.6 to 34.1 cm). However, in CH there was a significant interaction between sowing date and accession ($P \leq 0.05$). Here the white phenotype, planted at the earliest date generated relatively small inflorescences (Table 5). The number of inflorescences increased with later sowing dates in all localities except CH, where the incidence of frost altered this tendency without significant differences ($P > 0.05$) between

Table 3. Planting date, days after planting and accumulated growing degree days in chia plants in different development stages for each locality.

Locality	Planting date	Flowering beginning			Physiological maturity			Harvest maturity		
		DAS	Date	GDDi	DAS	Date	GDDi	DAS	Date	GDDi
Valle de Azapa										
F1	4 Jan	56	1 March	649.1	93	7 April	1001.4	136	20 May	1.271.6
F2	18 Jan	47	6 March	558.0	84	12 April	1067.2	136	4 June	1.186.1
F3	4 Feb	49	25 March	551.4	87	2 May	811.6	134	18 June	1.084.7
F4	18 Feb	49	8 April	489.0	96	25 May	782.9	123	21 July	1.115.3
F5	6 March	48	23 April	392.6	105	19 June	728.7	148	1 Aug	927.7
Canchones										
F1	4 Jan	58	3 March	709.3	106	20 April	1165.3	139	23 May	1.393.0
F2	18 Jan	53	11 March	643.5	110	8 May	1110.4	137	5 June	1.282.8
F3	1 Feb	51	24 April	606.9	109	21 May	1048.4	130	11 June	1.159.4
F4	18 Feb	54	13 April	568.2	-	-	-	-	-	-
F5	1 March	60	30 April	547.6	-	-	-	-	-	-
Las Cruces										
F1	4 Jan	111	25 April	519.1	-	-	-	170	23 June	585.1
F2	18 Jan	97	25 April	436.5	-	-	-	158	26 June	502.6
F3	2 Feb	84	27 April	333.4	-	-	-	144	26 June	396.8
F4	18 Feb	81	10 May	238.1	-	-	-	-	-	-
F5	4 March	-	-	-	-	-	-	-	-	-

DAS: Days after sowing; GDDi: accumulated growing degree days (> 10 °C).

Table 4. Biomass, yield and harvest index for chia grown in different localities and with different sowing dates (SD).

Factor	Biomass			Yield			Harvest index		
	VA	CH	LC	VA	CH	LC	VA	CH	LC
				kg ha^{-1}					
SD									
F1	7.300a	7.594b	5.152a	1.479b	1.912a	127a	0.22d	0.27a	0.04a
F2	4.151c	9.291b	3.066b	1.375b	1.622b	114a	0.34c	0.21a	0.04a
F3	3.529c	12.330a	2.051c	1.337b	941c	75b	0.38c	0.09b	0.03b
F4	3.710c			2.285a			0.56a		
F5	5.611b			2.468a			0.44b		
	**	**	**	**	**	**	**	**	**
Accession									
White	4.856a	9.817a	2.931b	1.669b	1.460a	104a	0.38a	0.19a	0.03b
Dark	4.864a	9.660a	3.915a	1.908a	1.524a	107a	0.40a	0.19a	0.04a
	ns	ns	**	**	ns	ns	ns	ns	**
SD × Accession									
F1×White	8.971a	6.504a	4.281a	1.324d	1.823a	129a	0.15e	0.28a	0.04a
F1×Dark	5.629bc	8.684a	6.024a	1.634d	2.002a	125a	0.29d	0.25a	0.04a
F2×White	3.635d	10.703a	2.660a	1.222d	1.808a	113a	0.34cd	0.20a	0.03a
F2×Dark	4.667cd	7.879a	3.473a	1.527d	1.437a	115a	0.33cd	0.22a	0.04a
F3×White	3.498d	12.245a	1.854a	1.280d	941c	70a	0.37bcd	0.08a	0.02a
F3×Dark	3.559d	12.415a	2.248a	1.393d	941c	81a	0.39bc	0.10a	0.03a
F4×White	3.559d			2.484b			0.58a		
F4×Dark	3.860d			2.085c			0.54a		
F5×White	4.618cd			2.032c			0.44b		
F5×Dark	6.604bc			2.903a			0.44b		
	**	ns	ns	**	ns	ns	*	ns	ns

*, **Significant at the 0.05 and 0.01 probability levels, respectively.
HI: Harvest index; VA: Valle de Azapa; CH: Canchones; LC: Las Cruces; ns: nonsignificant.

Figure 4. Effect of five sowing dates on chia grain yield established in three locations (Valle de Azapa, Canchones, and Las Cruces).
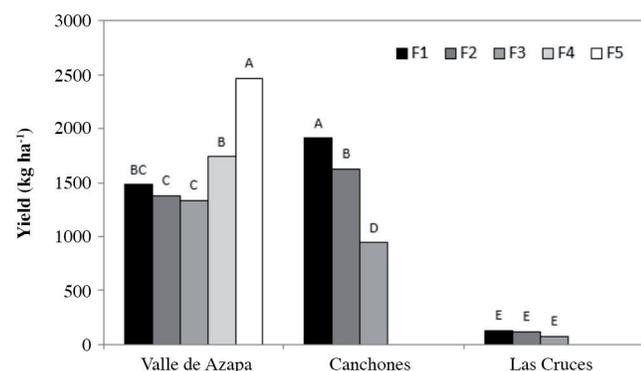
Different letters on bars indicate significant differences between sowing dates and localities, according to Duncan test (P < 0.05). n = 12.

sowing dates (Table 5). There was interaction between sowing date and phenotype only in LC; white seeds sown earlier (F1) produced a mean of two inflorescences less than the rest of treatments. It should be noted that there were nonsignificant differences between the phenotypes for any of these variables.

The results of the principal components analysis are represented in Figure 5. There was a significant association among growth variables with sowing dates in VA and CH. Las Cruces was excluded from this analysis due to the lack of thermal accumulation (Tables 2 and 3 and Figure 3) that generated different situations. Components 1 and 2 explained 76% of the observed variance; seed yield was positively and significantly associated with inflorescence length (r = 0.48, P ≤ 0.05), harvest index (r = 0.85, P ≤ 0.001), and with the number of inflorescences per plant (r = 0.85, P ≤ 0.001) and thus with important components in determining yield. Yield

Table 5. The length of central axis inflorescence and number of inflorescences per plant of chia in different localities and different sowing dates (SD).

Factor	Central axis inflorescence length			Nr inflorescences per plant		
	VA	CH	LC	VA	CH	LC
		cm				
SD						
F1	13.6d	14.6c	15.1c	11.2b	8.6a	5.4b
F2	14.0d	18.2b	16.1b	10.3b	11.3a	6.8a
F3	17.3c	20.4a	18.6a	9.8b	8.2a	6.5a
F4	19.6b			10.1b		
F5	34.1a			13.8 a		
	**	**	**	**	ns	**
Accession						
White	19.3a	17.7a	16.5a	10.8a	10.0a	6.1a
Dark	20.1a	17.8a	16.7a	11.2a	8.7a	6.3a
	ns	ns	ns	ns	ns	ns
SD × Accession						
F1×White	13.3a	13.5d	15.4a	11.5a	8.0a	4.5b
F1×Dark	14.0a	15.8c	14.9a	10.8a	9.2a	6.2a
F2×White	13.4a	19.0ab	16.1a	9.3a	13.6a	6.9a
F2×Dark	14.5a	17.5bc	16.2a	11.2a	9.0a	6.6a
F3×White	17.5a	20.5a	18.1a	9.7a	8.3a	7.0a
F3×Dark	17.0a	20.2a	19.1a	9.8a	8.1a	6.1a
F4×White	17.0a			9.8a		
F4×Dark	22.2a			10.3a		
F5×White	35.3a			13.7a		
F5×Dark	32.9a			14.0a		
	ns	*	ns	ns	ns	**

*, **Significant at the 0.05 and 0.01 probability levels, respectively.
VA: Valle de Azapa; CH: Canchones; LC: Las Cruces; ns: nonsignificant.

and length of the main inflorescence were negatively and significantly associated with day length at the beginning of flowering. The lowest yields observed in CH and sowing dates F1 and F2 in VA were associated with the longest day lengths. Photoperiod was positively and significantly associated with plant height and biomass at flowering.

Figure 5. Biplot of principal components for yield and growth variables: Biomass at flowering and harvest, length of central axis inflorescence, number of inflorescences per plant, plant height, and harvest index.
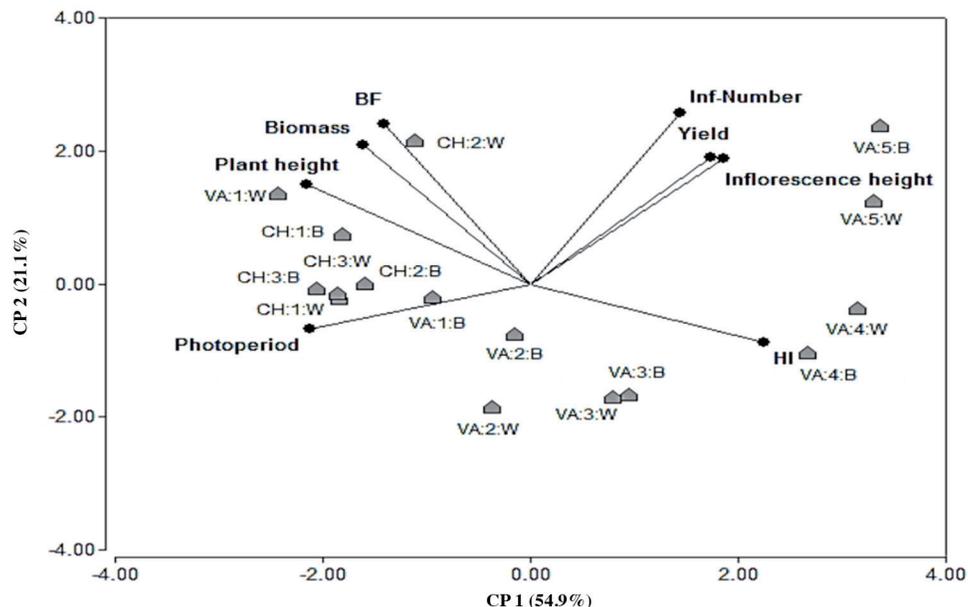
HI: Harvest index; Photoperiod: day length at the beginning of flowering; BF: biomass at the beginning of flowering. Pentagons represent different sowing dates in localities of Valle de Azapa (VA) and Canchones (CH). B: dark chia accession; W: white chia phenotype; 1, 2, 3, 4 and 5: sowing dates F1, F2, F3, F4, and F5, respectively.

Total oil, –linolenic (omega 3) and linoleic acid content in seeds

The total seed oil content and the content of α-linolenic and linoleic acids obtained in VA and CH are shown in Table 6. These parameters were not determined for LC due to the scarce seed production and the notorious damage effect observed in them as a result of the low temperatures during grain filling. The results show that there were nonsignificant differences between phenotypes for total oil content; however, α-linolenic and linoleic acid content tended to be higher in VA. There was significant interaction between sowing date and phenotype in all localities except CH, where there was no interaction with total oil content and no difference between sowing dates (Table 6). The highest values of total oil content, α-linolenic and linoleic acid content were found in the latest sowing dates; F4 (February 18) and F5 (March 6) showed significantly greater values in white and dark phenotypes, respectively (Table 6). There was no clear tendency in CH with respect to sowing date; phenotype was more relevant in this case, as indicated above.

DISCUSSION

Flower induction by short days is common in tropical crops such as chia and this helps the plant to synchronize development with the rainy season, which occurs in the warmest time of year (Jamboonsri et al., 2012; Busilacchi et al., 2013). The geographic location (between 18° and 33° S lat) and the climatic conditions of the sites (VA - coastal desert, CH - normal desert, and LC - Mediterranean) caused flower induction to occur at the beginning of autumn, when

temperatures were decreasing (Figure 1). The result of this was that in CH and LC, for the later sowings, flowering occurred in the presence of low temperatures and even frosts. The short-day character of chia was reflected by the timing of flowering, which was stable in VA and CH independent

Table 6. Total oil, alpha-linolenic (omega 3) and linoleic acid content in seeds in two localities (Valle de Azapa and Canchones) established in different sowing dates (SD).

Factor	Total oil		Omega 3		Linoleic acid	
	VA	CH	VA	CH	VA	CH
			L ha⁻¹			
SD						
F1	343.2c	249.1a	188.5bc	194.3a	56.3c	70.1a
F2	324.9c	312.5a	157.3c	147.2b	45.4d	50.9a
F3	321.3c	330.7a	192.2bc	169.2ab	53.5cd	55.5a
F4	446.5b		267.3a	–	74.7b	–
F5	508.1a		295.6a	–	84.8a	–
	***	ns	***	***	***	***
Phenotype						
White	396.4a	291.2a	234.0a	150.7b	65.7a	53.2b
Dark	381.2a	303.7a	206.4a	189.8a	60.2a	64.4a
	ns	ns	ns	***	**	***
SD × Phenotype						
F1×White	324.8c	238.4b	181.3cd	185.4b	53.8c	67.8ab
F1×Dark	361.7bc	259.7ab	195.8cd	203.2ab	58.7c	72.4a
F2×White	325.0c	353.1ab	152.6d	167.2b	43.2d	58.6b
F2×Dark	324.7c	272.0ab	162.1cd	127.3c	47.5cd	43.1c
F3×White	321.0c	282.1ab	201.2c	99.6c	54.7cd	33.1c
F3×Dark	321.5c	379.4a	183.2cd	238.8c	52.3cd	77.8a
F4×White	583.9a	–	376.5a	–	104.1a	–
F4×Dark	309.0c	–	158.1cd	–	45.3cd	–
F5×White	427.3bc	–	258.6b	–	72.5b	–
F5×Dark	588.9a	–	332.7a	–	97.0a	–
	***	ns	***	***	***	***

*, **Significant at the 0.05 and 0.01 probability levels, respectively.
VA: Valle de Azapa; CH: Canchones; LC: Las Cruces; ns: nonsignificant.

of sowing date (Figure 3) and where mean temperature and day length did not show large variation during the growing season (Figure 1). By contrast, in LC, where variation in day length was greater, there was a reduction in days to flower initiation as the day length decreased. Similar behavior has been observed in other species that are sensitive to photoperiod (Christiansen et al., 2010). We found a photoperiod threshold of 11.8 h for flower initiation, similar to that indicated by Jamboonsri et al. (2012), who reported 12 h for domesticated chia germplasm, while Busilacchi et al. (2013) indicate values of 12-13 h. Above this threshold, thermal requirements of the crop increased linearly, with a difference of up to 200 °C d between day lengths above and below the threshold (Figure 3). One result of this was that chia thermal requirements in LC were lower than in VA and CH, since in LC the plants always experienced a day length below this threshold. However, life cycles were longer in LC (Table 2) due to a slower development rate as a product of lower thermal accumulation, which did not allow grain filling. This situation is characteristic of some short-day species such as sorghum (Craufurd and Qi, 2001), *Miscanthus sacchariflorus* (Jensen et al., 2013) and soybean (Jiang et al., 2011) under these conditions.

Together, these results suggest that day length sensitivity of chia is quantitative; when exposed to decreasing day lengths floral initiation is accelerated. However, if plants do not experience a sufficiently long day length, flowering will occur when a certain quantity of day degrees is accumulated. In this study the quantity of day degrees was between 600 and 700 °C d, with a base development temperature of 10 °C. These results concur with studies in other quantitative short-day species from tropical climates such as *Miscanthus*, which requires 600 °C d to flowering (Jensen et al., 2013) and sorghum which requires 870 °C d (Ritchie and Alagarswamy, 1989). The study of Lobo et al. (2011) in Tucumán, Argentina, showed that January sowing generated plants with a development cycle of approximately 160 d, which is very similar to that observed in LC, which is at a similar latitude (Table 3). These authors indicated that sowing at a later date could be risky because of the possibility of frost during the reproductive stages. This sensitivity to frost confines the development of chia to zones with few or no freezing events or to areas with temperatures that are not lower than 5 °C during flowering (Lukatkin et al., 2012).

It has been observed that many tropical plants suffer important frost damage when they are exposed to temperatures slightly below 0 °C and cold damage has been sometimes been reported at temperatures close to 5 °C (Lukatkin et al., 2012). Critical periods for flower initiation and for flower development have also been observed in various species of genus *Salvia*. For example, *Salvia leucantha* Cav. requires 12 h light for floral induction and 10 h light to continue flower development (Armitage and Laushman, 1989). Further studies are needed to verify if chia behaves similarly.

Our observed yields were all superior to those that have been reported in the literature with the exception of LC, where low temperatures during flowering and grain-filling periods significantly reduced yields (Figure 4). This is similar to studies performed at relatively high latitudes, such as in Choele-Choel (39°11' S lat), Argentina, and Tucson (31°14' S lat; Arizona, USA), where chia plants died due to freezing before flowering. It is not surprising that low temperatures affect growth and yield of chia, considering that the species is adapted to temperatures that fluctuate between 11 and 36 °C (Ayerza and Coates, 2009).

Studies performed in Argentina yielded from 606 to 1400 kg ha^{-1} (Lobo et al., 2011). In Paraguay yields of 1600 kg ha^{-1} were reported (Bochicchio et al., 2015), while in the state of Jalisco, Mexico, the main productive zone of chia in this country, mean yields of 1200 kg ha^{-1} were obtained. In our study, the yields of sowing dates F1 and F2 (between 4 January and 4 February) in CH were systematically and significantly superior to those of VA (Figure 4). In spite of being at similar latitudes, these localities have important climate differences, given that VA has coastal influence, with higher minimum and lower maximum temperatures than CH, and an atmospheric demand (reference evapotranspiration accumulated to Julian day 100) of 387.7 mm, compared to 495 mm in CH. Although the meteorological conditions were similar at the beginning of the season, from February on the minimum temperatures in CH were substantially less than in VA, which may explain low yield in third sowing (F3) in the latter locality. The high yields obtained from F4 and F5 in VA were associated with favorable day-length conditions, lower mean temperatures and the high levels of radiation that characterize these latitudes. The lower temperatures in these two sowing dates in VA would have generated higher levels of unsaturated fatty acids such as α-linolenic and linoleic (Table 6). This was reported by Ayerza (2011), who found changes in chia oil composition (higher level of unsaturation of fatty acids) in higher altitude ecosystems in which temperature was lower than in localities at lower altitudes. This would also explain the lack of significance in oil contents in the first three sowing dates in VA and CH, since temperatures observed in these three dates were similar in both localities (Figure 1).

The dark phenotype tended to have higher yield, although this was only significant in VA (Table 4). Something similar occurred in the fatty acid profile, where the dark phenotype showed higher levels of α-linolenic and linoleic acids, although the differences were only significant in CH. The similarity between phenotypes may be due to the fact that both came from the same area (Santa Cruz de la Sierra in Bolivia) and that both were growing within their adaptation limits, as has been observed for other species of arid zones such as jojoba (Ayerza and Zeaser, 1987). The dark phenotype only had a greater level in CH (in the three sowing dates in which seeds were obtained), coinciding with the report of Ayerza and Coates (2009), who indicated that this phenotype probably has greater capacity to adapt to the environmental conditions in which it was growing. However, other studies of Ayerza (2011; 2013) found nonsignificant difference in oil content and fatty acids profile between spotted-black seeds and white seed of chia grown in different ecosystems.

The studies indicate that the larger differences found in oil content and fatty acid composition are due to location (because of environmental differences) rather than chia seed coat color (Ayerza, 2010).

Plant height generally decreased with later sowing and thus shorter day length; in VA heights did not surpass 60 cm when plants were sown in the middle of February (F4, data not shown). Similar results were obtained by Busilacchi et al. (2013) in chia as well as in other short day plants such as *Amaranthus* (Al Hakimi, 2005) and soybean (Jiang et al., 2011). However, plant height was greater in the last sowing in VA (beginning of March, F5); plant height reached almost 90 cm (data not shown) due to the large size of the inflorescence of the central axis, which averaged more than 30 cm in length (Table 5). Larger inflorescence found in later sowings would be associated with better distribution of assimilates, as has been observed in soybean (Han et al., 2006).

Plant height was not positively associated with yield in chia, in contrast with species such as wheat, in which introduction of dwarfing genes in varieties produced superior yields relative to those obtained in taller plants by increasing the harvest index (Zapata et al., 2004). Greater plant height in chia was linked to a lower harvest index (Figure 5), consistent with the results of Berti et al. (2011) in camelina (*Camelina sativa* [L.] Crantz). Plants had greater height under long days (data not shown), along with shorter inflorescences and smaller number of inflorescences (Table 5), all of which indicate less assimilates used for grain production and thus lower harvest index (Table 4). This concurs with the report of Han et al. (2006) in short-day soybean plants, in which shorter days in autumn promoted the partition of dry material to the seeds.

Inflorescence height was negatively associated with the number of vegetative nodes, and number of vegetative nodes was positively associated with day length (data not shown). Thus flowering dynamics in chia is almost completely determined by the interaction of day length and photoperiod (data not shown), and not by the number of vegetative nodes as in *Chrysanthemum* (Mattson and Erwin, 2005), a short day plant in which flowering is also determined by a critical number of vegetative phytomers (Jensen et al., 2013).

The greatest yields observed in this study were associated with greater biomass accumulation, which was mainly generated by producing large inflorescences on shorter plants. These were plants that had better assignation of their resources and thus had higher harvest indices. Additionally, the highest yields were also associated with latitudes with longer day lengths. These results have important implications for the management of chia, since shorter, higher yielding plants can also be more efficiently harvested using mechanized harvesting methods.

CONCLUSIONS

The conditions of coastal desert and normal desert climates in Valle de Azapa (VA) and Canchones (CH), respectively, provide the best conditions for chia production under the conditions of this study. However, the coastal desert climate, with less thermal oscillation, less extreme temperatures and without freezes during the entire season, make VA even more appropriate for the production of this species. Thus in the VA sowing between middle of February and middle of March may produce yields greater than 2000 kg ha^{-1}, oil content above 550 L ha^{-1} and α-linolenic and linoleic acid content above 350 and 90 L ha^{-1}, respectively.

Chia cultivation is not advisable in LC, due mainly to the low thermal accumulation in a dry Mediterranean climate with marine influence, along with temperatures below 5 °C in April, the period in which the reproductive stage begins. Although there are low temperatures and freezes in CH beginning in April, there is high thermal accumulation that allows plants established in January to complete their phenological development adequately. Thus in CH, sowing should ideally be confined to the beginning of January. Here we determined a day length threshold of 11.8 h for floral initiation. When plants are exposed to shorter days flower initiation is more precocious, but when the day length is not adequate plants only begin to flower when they have accumulated 600-700 °C d. This suggests that chia has a quantitative type day length sensitivity; however, further studies are required to corroborate this hypothesis.

ACKNOWLEDGEMENTS

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