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The Seed's Protein and Oil Content, Fatty Acid Composition, and Growing Cycle Length of a Single Genotype of Chia (*Salvia hispanica* L.) as Affected by Environmental Factors

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Abstract: as a botanical source, variability in chia seed composition could be expected between growing locations, and between years within a location, due to genotype and environment effects as well genetic x environment's interactions. The objective of the present study was to determine the location effect on the growing cycle length, and seed's protein content, lipid content, and fatty acid profiles, of a single chia genotype. Seeds of chia genotype Tzotzol grown on eight sites in five different ecosystems were tested. One site was in Argentina, in the Semi-Arid Chaco ecosystem (T_5); one was in Bolivia, in the Sub-Humid Chaco ecosystem (T_4); and six in Ecuador, one in the Coastal Desert (T_3), two on the Tropical Rain Forest (T_2), and three in the Inter-Andean Dry Valley ecosystem (T_1). Seeds from plants grown in T_4 and in T_3 contained significantly (*P*<0.05) more protein percentage than did seeds from the other three ecosystems. No significant (*P*<0.05) differences in protein content were found between T_3 and T_4, and between T_1, T_2, and T_5. Seeds from T_1 and T_5 ecosystems, with 33.5 and 32.2%, respectively, were the numerically highest oil content producers, but their results were only significantly (*P*<0.05) higher when compared with the T_2 seeds. Significant (*P*<0.05) differences in palmitic, stearic, oleic, linoleic and α-linolenic fatty acids between oils from seeds grown in different ecosystems were detected, however. Oil of seeds grown in the T_3 ecosystem had the palmitic, stearic and oleic fatty acids' highest contents. Palmitic and oleic fatty acid levels were significantly (*P*<0.05) higher when were compared to that of seeds grown in the T_1 ecosystem, and stearic when was compared to that of seeds grown in the T_5 ecosystem; ω-6 linoleic fatty acid content was significantly (*P*<0.05) lower in oils of seeds produced in T_1, and T_2 than in those produced in T_3, T_4, and T_5 ecosystems; ω-3 α-linolenic fatty acid content was significantly (*P*<0.05) higher in seeds produced in T_1, than in those produced in T_3, T_4, and T_5, but not in those produced in T_2.

Key words: α-linolenic acid, *Salvia hispanica* L., fatty acids, protein, omega-3, South America, seed production

1 INTRODUCTION

Changes in human diet and lifestyle have been closely related to the growing epidemic of chronic disease affecting both developed and undeveloped regions of the world[1]. Cardiovascular heart disease (CHD), a chronic disease, remains the leading cause of both death and disability in the Western industrialized world and is growing rapidly in the unindustrialized countries, also[1,2].

Many studies have demonstrated that increased intake of lipids, in particular saturated (SAT), trans-fatty acids, and polyunsaturated ω-6 fatty acids, are closely related with the incidence of CHD[3-5]. In addition, a number of medical and epidemiological studies have shown that consuming lipids rich in ω-3 fatty acids reduce the risk of suffering a CHD[6-8].

Chia, along with corn, beans, and amaranth, was a core component in the diet of many pre-Columbian civilizations in America, including the Mayan and Aztec populations[9]. Chia contains the richest botanical oil source of α-linolenic acid known. A number of papers reported that chia seed was used successfully to increase the ω-3 fatty acid content of animal products such eggs, poultry meat, pork

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meat, rabbit meat, and caw's milk[10-17], and added as an ingredient of the final foods like bread and other bakery products, also[18,19]. In addition, the benefit effects of feeding chia seed and oil on rat plasma CHOL, LDL, HDL, TG contents and fatty acid composition were reported recently by two different controlled studies[20,21].

As a botanical source, variability in chia seed composition could be expected between growing locations, and between years within a location, due to genotype and environment effects as well genetic x environment's interactions. Chia is cultivated by its special seeds biochemical composition, then genotype variability related with crop growing ecosystems needs to be explored. Although, the ecosystem effect on chia seeds biochemical composition has been early reported[22-25], all of these studies were performed just at the level of a species. The objective of the present study was to determine the location effect on the growing cycle length, and seed's protein content, lipid content, and fatty acid profiles, of a single chia genotype.

2 EXPERIMENTAL

2.1 Seed samples

Seeds of chia genotype Tzotzol grown on eight sites in five different ecosystems were tested (Table 1). The seeds were collected at each growing field. In the case of seeds from Inter-Andean Dry Valley growing cycle length, Semi-Arid Chaco, and Tropical Costal Desert ecosystems, original data were utilized separately as part of other different studies by the author. One site was in Argentina, in the Semi-Arid Chaco ecosystem (T_5); one was in Bolivia, in the Sub-Humid Chaco ecosystem (T_4); and six in Ecuador, one in the Coastal Desert (T_3), two on the Tropical Rain Forest (T_2), and three in the Inter-Andean Dry Valley ecosystem (T_1). Data from different sites within an ecosystem were analyzed as replication. Within each region where the chia was grown, representative fields were selected for sampling, with a total of 20 samples harvested. After the samples were collected, they were cleaned by hand and sent to the laboratory for chemical analysis. In the case of T_1 and T_3 the seeds were harvested by hand, while for T_2, T_4 and T_5 they were collected from the combine after mechanical harvesting. The sites in the Semi-Arid Chaco and Inter-Andean Dry Valley growing cycle length ecosystems were sown in 2006, and the sites in the other three ecosystems in 2007. Irrigation was used only at the Inter-Andean Dry Valley growing cycle length, and Coastal Desert, the driest ecosystems.

The Tzotzol genotype seeds were originally collected in the area where descendants of the Nahuas still cultivate the crop, and then were multiplied for a number of years in experimental plots[9]. This genotype had previously been classified as having been domesticated, based on the presence of human selected-traits, having a higher seed mass, closed calyces that prevent seed shattering and dispersal, and determinacy of flowering and seed set described by Cahill & Provance (2002)[26] and Cahill (2004)[27].

2.2 Laboratory analysis

Crude nitrogen of the chia seed samples was determined by a standard micro-Kjeldahl method and was converted to protein content using a 5.71 conversion factor.

Lipids were extracted and converted into fatty acid methyl esters using the IRAM 5-560II method[28]. Fatty acid methyl esters were separated and quantified by automated gas chromatography (Model 6890, GC; Hewlett Packard Co., Wilmington, DE 20006) equipped with flame ionization detectors and a 30 m × 530 μm i.d. capillary column (Model HP-FFAP; Hewlett Packard Co., Wilmington, DE, USA).

2.3 Statistical analysis

A one-way analysis of variance (ANOVA) was performed for protein content, oil content, and individual fatty acid

Table 1 Locations Where Chia Tzotzol-genotype Was Grown.

Origen	Ecosystem	Temp. Mean/year (°C)	Rainfall Mean/year (mm)	Elevation (m)	Latitude	Soil type
T_1	Inter-Andean Dry Valley[2]	15-17	300	1600-2200	00°29′47″N	Cambisols[45]
T_2	Tropical Rain Forest[1]	25	>3000	300	00°45′00″N	Regosol lateritico[45]
T_3	Tropical Costal Desert[2]	25	407	48	02°18′00″S	Entisols[47]
T_4	Sub-Humid Chaco[1]	24	1157	265	17°17′00″S	Mollic planosols[45]
T_5	Semi-Arid Chaco[1]	17	560	1156	25°07′48″S	Calcaric rhegosols[46]

[1] without irrigation
[2] with irrigation

content. When the F-value was significant ($P < 0.05$), means were separated using Student-Newman-Keuls Test. Additionally correlation and regression analyses were undertaken to develop the relationships between ecosystem elevation and growing cycle length, protein and oil contents, and between α-linolenic and oleic and linoleic fatty acids[29].

3 RESULTS

3.1 Growing cycle length, seed protein content, and oil content

The growing cycle length, protein and oil content, and fatty acid compositions are presented in Table 2. The growing cycle length ranged from 100-days at the Rain Forest ecosystem to 150-days at the Semiarid Chaco ecosystem. Growing cycle length of a chia crop in each ecosystem was 150, 130, 120, 105, 100 days in T_5, T_1, T_4, T_3, and T_2, respectively. The regression coefficient (R^2), presented in Fig. 1, showed a positive relation of growing cycle length with ecosystem elevation (R^2=0.99, P<0.0001).

The seed oil and protein contents were affected by the growing environment. Protein content tended to decrease as altitude of the ecosystem where the seed was grown increased. However, the results were not always significantly (P<0.05) different. Seeds from plants grown in the Sub-Humid Chaco and the Coastal Desert ecosystem contained significantly (P<0.05) more protein percentage than did seeds from the other three ecosystems. No significant (P<0.05) differences in protein content were found between T_3 and T_4, and between T_1, T_2, and T_5.

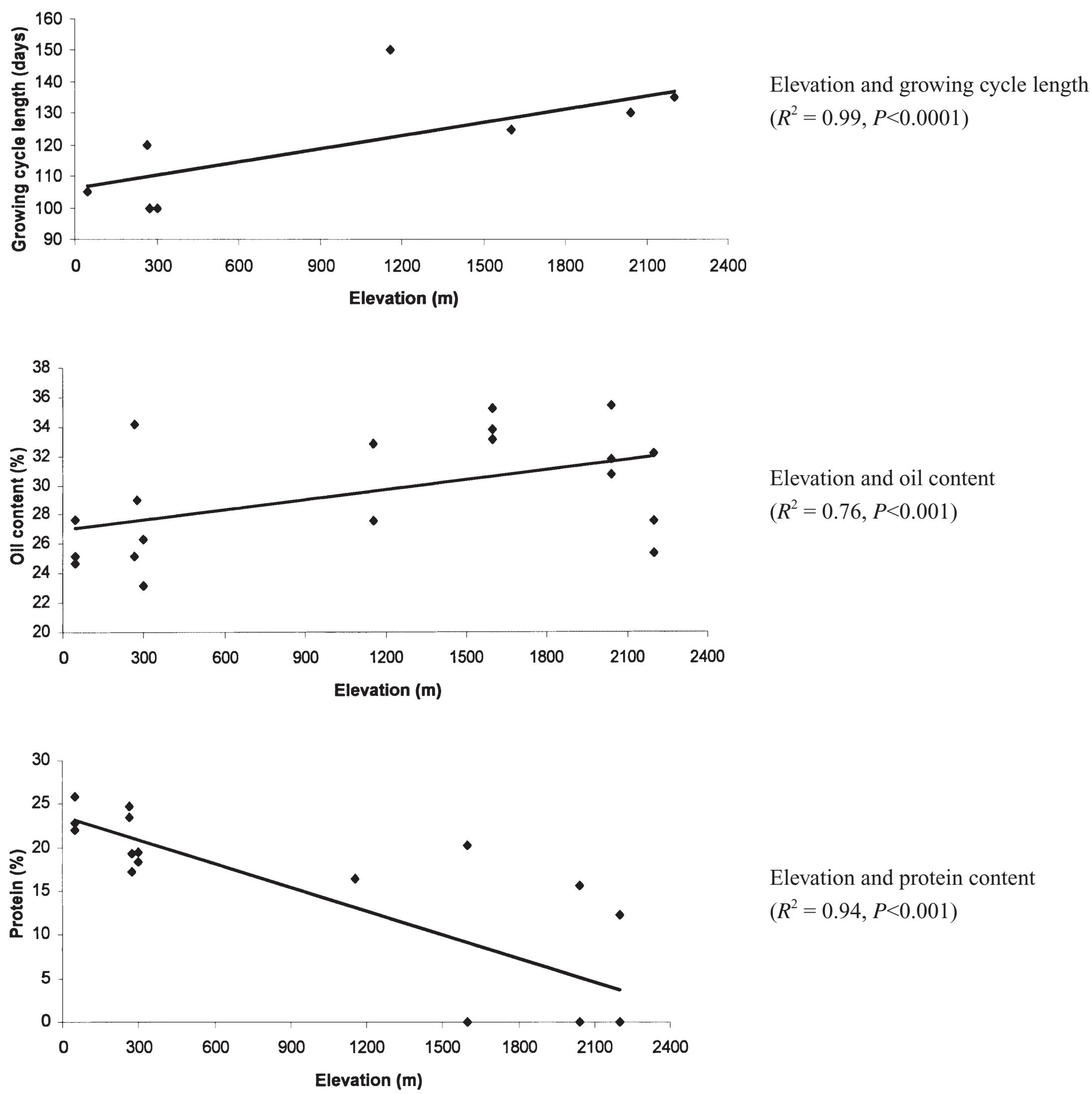

Fig. 1 Relationship between Elevation and Growing Cycle Length (GCL), Protein Content, and Oil Content.

Table 2 Seed's Protein Content and Oil Content, and Growing Cycle Length of *Salvia hispanica* L. Genotype Tzotzol Grown at Five Different Environments.

Origen	Protein	Lipids	Growing period length
	(%)		(days)
T_1	16.03[b1]	32.20[a]	130
T_2	18.58[b]	25.93[b]	100
T_3	23.60[a]	26.67[ab]	105
T_4	24.15[a]	26.40[ab]	120
T_5	16.45[b]	33.50[a]	150
LSD[2]	4.97	5.69	—

[1] in a column within a group, means with the same latter are not statistically different (P<0.05)

[2] least significant difference for P<0.05

In opposition to protein content, in general, the seeds' oil content tended to increase as altitudes of the ecosystem were the seed grown increased. However, as in the protein content, the oil content results' differences were not always statistically significant (P<0.05). Seeds from T_1 and T_5 ecosystems, with 33.5 and 32.2%, respectively, were the numerically highest oil content producers, but their results were only significantly (P<0.05) higher when compared with the T_2 seeds.

Regression analyses were performed for elevation vs. protein and oil contents. The regression coefficient (R^2) and significance (P) levels are presented in Fig. 1. The land elevation was negatively related with protein content (R^2=0.94, P<0.001), and positively related with oil content (R^2=0.76, P<0.001).

3.2 Seed oil fatty acid composition

Gas chromatography analysis of the oil composition showed the presence of α-linolenic, linoleic, oleic, stearic and palmitic fatty acids in the seeds from all ecosystems (Table 3). In addition, six more fatty acids were identified in all analyzed seed samples, myristic, arachidic, gadoleic, behenic, eracic, and lignoceric. However, as all of them were present just in traces, those fatty acids were omitted.

Significant (P<0.05) differences in palmitic, stearic, oleic, linoleic and α-linolenic fatty acids between oils from seeds grown in different ecosystems were detected, however. Oil of seeds grown in the T_3 ecosystem had the palmitic, stearic and oleic fatty acids' highest contents. Saturated palmitic and monounsaturated oleic fatty acid levels were significantly (P<0.05) higher when were compared to that of seeds grown in the T_1 ecosystem, and saturated stearic when was compared to that of seeds grown in the T_5 ecosystem.

Polyunsaturated ω-6 linoleic fatty acid content was significantly (P<0.05) lower in oils of seeds produced in T_1, and T_1 than in those produced in T_3, T_4, and T_5 ecosystems. Polyunsaturated ω-3 α-linolenic fatty acid content was significantly (P<0.05) higher in seeds produced in T_1, than in those produced in T_3, T_4, and T_5, but not in those produced in T_2; no significant (P<0.05) differences in α-linolenic fatty acid contents between seeds from all other ecosystems were detected.

The ω-6:ω-3 ratio was significantly (P<0.05) lower in oils from seeds grown in T_1 and T_2 compared to that in oils from seeds grown in all other ecosystems. Oil of seeds from T_2 ecosystems had a SAT:PUFA ratio significantly (P<0.05) lower than that found in oils from seeds grown in T_1, T_4, and T_5 ecosystems.

Regression analysis was done to explore the trend of associations between fatty acids, and its coefficient (R^2)

Table 3 Fatty Acid Composition of *Salvia hispanica* L. Genotype Tzotzol Seeds Grown at Five Different Environments.

Origen	16:0	18:0	18:1	18:2	18:3	SAT	PUFA	ω-6:ω-3	PUFA:SAT
				(%)				(rate)	
T^1	6.39[bl]	3.54[ab]	6.51[b]	19.97[b]	64.71[a]	9.93[a]	81.68[a]	0.26[b]	8.25[a]
T^2	8.13[ab]	3.44[ab]	6.84[ab]	17.65[b]	64.08[ab]	11.57[a]	81.73[a]	0.28[b]	7.09[ab]
T^3	9.66[a]	4.34[a]	9.29[a]	20.30[a]	60.05[b]	13.99[a]	76.00[a]	0.37[a]	5.84[b]
T^4	7.11[ab]	3.44[ab]	8.32[ab]	20.45[a]	60.10[b]	10.13[a]	80.55[a]	0.34[a]	7.96[a]
T^5	6.89[ab]	2.36[b]	6.73[ab]	22.50[a]	60.35[b]	9.26[a]	82.85[a]	0.37[a]	9.01[a]
LSD[2]	2.45	1.35	2.23	2.10	4.02	3.62	5.80	0.07	1.86

[1] in a column within a group, means with the same latter are not statistically different (P<0.05)

[2] least significant difference for P<0.05, n: 20

[3] Percentage of total fatty acids

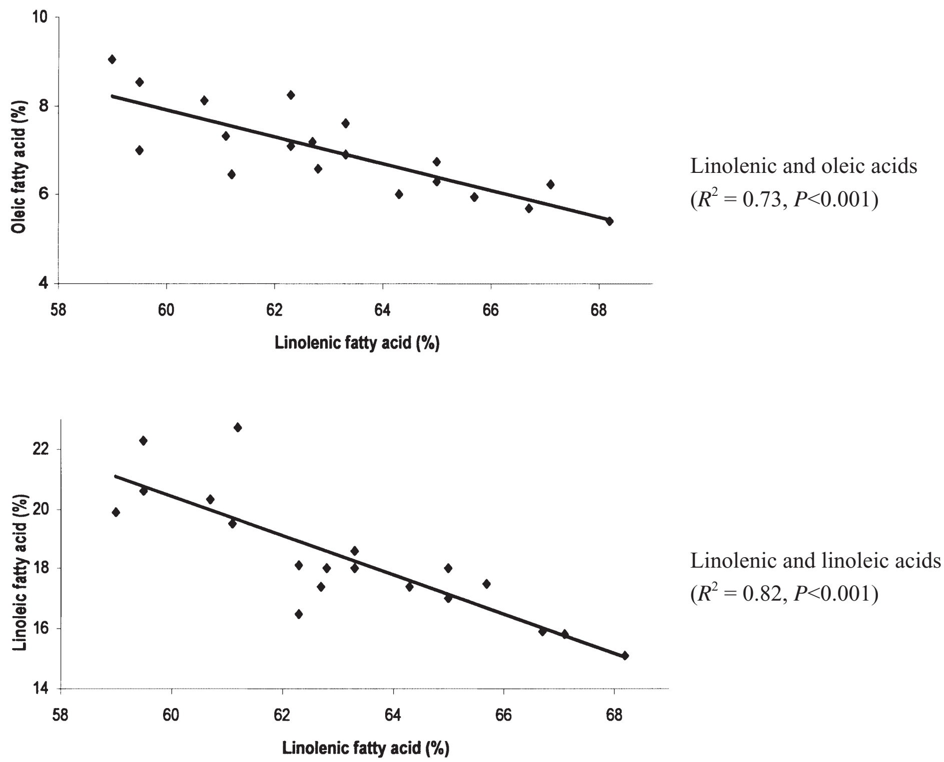

Fig. 2 Relationship between α-Linolenic and Oleic, and Linoleic Fatty Acid Contents.

and significance (P) levels are presented in Fig. 2. The α-linolenic fatty acid percentage was negatively related with oleic fatty acid percentage (R^2=0.73, P<0.001), and with linoleic percentage (R^2=0.82, P<0.001).

4 DISCUSSION

In general, increases in land elevation are corresponding by decreases in environment temperature, and a strong relation between growing season temperature and a number of basic physiological metabolic process of plants was demonstrated[30,31]. Thus, the significant (P<0.05) positive relation between growing cycle length, and the growing land elevation found herein for the chia crop, could be related to that[22,23].

The seed composition differences among ecosystems are due to effects of one or more environmental factors. The effects of temperature, light, soil type, and nutrition can affect seed protein, and oil quantity and quality[32,33]. The protein decrease and the oil content increase effect related to the seed developing process temperature, were reported for chia and for other crops as well[23,34,35]. In general, as altitude decreased, with a subsequent temperature increase, protein content of other crops such as sorghum and soybeans tended to increase [36,37]. However, as a direct relationship between soil pH and seed composition was reported for other oilseed crops, this effect may have contributed, together with other factors to the results found herein[38].

Crude protein values found in this study were all out of the 18.7-23.1% range of the *Salvia hispanica* L. specie seeds commercially produced in 2004 in nine sites of Argentina, Bolivia, Colombia, and Peru, early reported by Ayerza and Coates (2004)[23].

From the oil content results observed in this study, only two origins are in the range of 32.2-36.8%, 30-32%, or 28.8-32.7% of chia commercially produced in 1998 in nine sites of the Semi-Arid Chaco, in 1996 in three experimental plots in the Semi-Arid Chaco ecosystem, or in seeds commercially produced in 2004 in four countries of South America, respectively[23-25], and no one up the 35.6-38.6% of seeds grew commercially in 1993 in five sites of the Arid and Semi-Arid Chaco ecosystems[22]. However, the oil content variation in the seeds studied herein was larger than that observed in the seeds reported above, by Ayerza and Coates (2004)[23], Ayerza (1995)[22], and Coates and Ayerza (1996, 1998)[24,25].

Seed composition differences between reports could be explained by environmental differences between sites of this study and those earlier reported, including less environmental variation between those sites than among the five ecosystems where the chia seeds were grown in this study. As just one genotype was utilized herein, but unknown ones in the earlier works, there may also be genetic or genetic × environment interaction influences in seed composition variation between studies.

Although there was an overall significant relationship (R^2) between the crop growing site elevation for crops' growing length, crude protein and lipid content percentages, it was not true for all individual fatty acids profiles (data not show). Previous multi location chia trials showed that not always temperature effect had the same variance for the three C-18 fatty acids (Ayerza and Coates, unpublished), suggesting additional influence of other environmental factors, as was reported for other crops[39].

Cool temperatures generally increased the level of unsaturation of chia fatty acids as for other oilseed crops[22,40]. However, the variation in α-linolenic fatty acid content found herein, cannot be fully explained by the negative relationship between land elevation and temperature. This general relation could be affected by one or more environmental factors, such, rains and light[41], then, land elevation-temperature relation could be altered and cannot allow to relate seeds' fatty acid unsaturation level and land elevation.

All of the α-linolenic fatty acid contents measured herein were higher than the 52% contained in seeds collected in Argentina in 1994[22]. The comparative highest α-linolenic fatty acid content showed by the oil extracted from seed grown in T_1 and T_2 ecosystems were similar to the highest one of the 54.2- 64.2% range of seeds collected in various sites of Argentina, Bolivia, Colombia and Peru[23].

The significant negative relationship of α-linolenic fatty acid contents with the two more saturated 18-C fatty acids, oleic ($P<0.05$) and linoleic ($P<0.01$), are in agreement with previous observations reported for number of crops, as almonds[42], chestnuts[43], soybeans[34] and flaxseed which is a rich source of α-linolenic fatty acid[44]. This strong inverse association found herein is supported by the fact that the biosynthesis of α-linolenic fatty acid through the process of desaturation of oleic fatty acid, via linoleic fatty acid by the action of specific desaturase enzymes[32].

Dietary ω-6 and ω-3 fatty acid relation has been identified as a risk factor of suffering a CHD, and a way of lowering the risk is to keep dietary ω-6:ω-3 fatty acid ratio as low as possible, with 1:1 being ideal[3]. Western diets do not provide these ratios, mainly due to their high ω-6 fatty acid content. As a ω-3 source, chia is consumed either as an oil or as whole/ground seed, the significant ($P<0.05$) lower ω-6:ω-3 rate (up −30%), showed by the oil from seeds grown in T_1 and T_2 ecosystems compared with the other ones, could indicate an added health benefit for its seeds.

5 CONCLUSIONS

In summary, the results of the study showed there were differences among ecosystems for all measured traits, crops' growing length, crude protein and lipid content percentages, and fatty acid profiles, in seeds of *Salvia hispanica* L. genotype Tzotzol. Additional multi location and multi year trials are required to confirm the results from this study and to assess fully the adaptability and stability of the Tzotzol genotype.

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