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Effects of Seed Color and Growing Locations on Fatty Acid Content and Composition of Two Chia (*Salvia hispanica* L.) Genotypes

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Abstract The objective of this study was to investigate the effect of chia (*Salvia hispanica* L.) seed coat color on oil content and fatty acid composition, as well as the effect of different growing areas on chemical variation. This study was carried out using white and black-spotted chia seeds grown together at five locations of Ecuador. Oil content was not significantly ($P < 0.05$) different for any of the comparative analyses performed between white and black-spotted seeds at all, although significant differences in oil content among locations were detected. The seeds from the San Pablo location showed the highest oil concentration (34.5%). No significant differences among fatty acids at any of the location were detected between white and black-spotted seeds; however, significant differences in fatty acids composition between sites were found. Overall, significant ($P < 0.05$) differences in palmitic, oleic, linoleic, and α-linolenic fatty acid compositions among oils from seeds grown in different locations were detected. In conclusion, this paper shows that the larger differences found in oil content and fatty acid composition are due to location (because of the environmental differences) rather than chia seed coat color.

Keywords *Salvia hispanica* L. · Chia · α-Linolenic · Omega-3 · Fatty acids · Lipids · Oilseed

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Introduction

Chia (*Salvia hispanica* L.) is an annual herb of the Lamiaceae family. In pre-Columbian times, its seeds were one of the basic foods of Central American civilizations [1]. Corn, beans, chia, and amaranth were the most important food for more than 11 million people when Columbus arrived in America. Chia seed was also part of holy ceremonies as an offering to the Nahua gods [2].

Chia oil contains one of the highest known concentrations of α-linolenic fatty acid, up to 67.8% [3]. Recently, chia seed has become important for human health and nutrition because its ω-3 fatty acid content promotes beneficial health effects [4, 5].

It is theorized that the chia seeds commercialized today were selected by Nahua botanists, but came into the twenty-first century as a mixed population. This mixture continues to be grown by the descendants of the Nahua and Maya nations living in the mountains of southern Mexico, northern Guatemala, and Nicaragua [1]. Seed coat color in chia ranges from black and black-spotted to white. However, chia commercialized today is mainly black-spotted, followed by a low but increasing percentage of white seeds [1]. As was demonstrated by Cahill and Provance [6], single genes govern seed coat color and stem pigmentation; a single recessive gene, designated scc, was found to govern the white seed characteristic.

Chia is cultivated as a rich source of ω-3 fatty acids and for its high content of α-linolenic acid. Thus, any possibility of genotype variability needs to be explored. The objective of this study was to investigate the effect of chia (*Salvia hispanica* L.) seed coat color on oil content and fatty acid composition, as well as the effect of different growing areas on chemical variation.

Materials and Methods

This study was carried out using white and black-spotted chia seeds grown together at five locations of Ecuador (Table 1). The black-spotted and the white seeds belong to the the Tzotzol and Iztac genotypes, respectively, as was reported by Ayerza and Coates [7].

The experimental design was a randomized complete block with six replications in the case of Salinas, Patate and Guayllabamba locations, and with three replications in the case of Santa Elena and San Pablo locations. Each plot consisted of three 8- to 10-m-long rows, spaced 0.75 m apart. The plots were maintained using the same cultural and irrigation practices at all locations (Table 1), with all field work carried out by hand. An exception was the San Pablo location where plots were not irrigated. All the seed from each plot was hand harvested and cleaned. A subsample from each treatment and each replication was obtained to conduct laboratory analyses.

The 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 [1]. Both genotypes have been classified previously as domesticated, based on the presence of human-selected traits: higher seed mass, closed calyces that prevent seed shattering and dispersal, and determinacy of flowering and seed set described by Cahill and Provance and Hernandez-Gomez et al. [6, 8, 9].

Determination of Fatty Acid Composition

Lipids were extracted from the samples according to the method described by Folch et al. [10]. Total lipids were then converted into fatty acid methyl esters using the IRAM 5-560II method [11], which is equivalent to ISO 5509-1978 item 6 [12]. Fatty acid methyl esters were separated and quantified by an automated gas chromatograph (Model 6890, GC; Hewlett Packard Co., Wilmington, DE) equipped with flame ionization detectors and a 30 m × 530-μm i.d. capillary column (Model HP-FFAP-Free fatty acid phase; Hewlett Packard Co., Wilmington, DE). The temperatures of the oven, injector, and detector were set at 180, 290 and 330 °C, respectively. The fatty acid composition of each sample was determined by integrating the recorded peaks using Hewlett-Packard ChemStation Software. Results were expressed as percentage of total fatty acids.

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed for oil and individual fatty acid content. When the F value was significant ($P < 0.05$), in the case of two treatments, means were separated using least significant difference test (LSD). For more than two treatments, the Student Newman-Keuls test was used. Additionally correlation analysis was undertaken to develop the relationship between α-linolenic and oleic and linoleic fatty acids [13].

Results

Oil content as a percentage of seed weight was not significantly different for any of the comparative analyses performed between white and black-spotted seeds at any of the locations tested (Table 2). As well, overall the black-spotted and white seeds exhibited no significant differences in total lipid content (Table 2).

Gas chromatography analysis of the oil composition showed the presence of palmitic, stearic, oleic, linoleic and α-linolenic fatty acids in both white and black-spotted color seeds from all locations. In addition, six more fatty acids were identified in all analyses: myristic, arachidic, gadoleic, behenic, eracic, and lignoceric. However, as all of them were present just in traces, those fatty acids were omitted in this paper. No significant differences between fatty acids and ω-6:ω-3 rate were detected at any location (Table 2) between white and spotted seeds.

Over the five sites, significant differences in oil content average among locations were detected (Table 3). San Pablo seeds showed the highest oil content. However, they were significantly different from that from Santa Elena and Guayllabamba, but not to seeds from Patate and Salinas locations.

Table 1 Locations where chia was grown

Location	Ecosystem	Latitude	Elevation (m)	Soil type [26]	Irrigation	Sowing (Day/month)	Harvest (Day/month)
Santa Elena	Costal desert	2°18′00″S	48	Entisols	Yes	15/01	30/04
Salinas	Low inter-Andean valley	00°47′90″N	1,621	Cambisols	Yes	15/06	15/12
San Pablo	High inter-Andean valley	01°47′90″N	2,010	Cambisols	No	17/04	17/10
Patate	High inter-Andean valley	01°18′50″S	2,042	Cambisols	Yes	15/06	15/12
Guayllabamba	High inter-Andean valley	00°03′26″S	2,200	Cambisols	Yes	15/06	15/12

Table 2 Fatty acid composition of *Salvia hispanica* L. black-spotted and white coat seed colors

Location	Genotype	Oil %[A]	Percentage of total fatty acids					ω-6:ω-3 rate	Linolenic[C] g/kg of seed
			Palmitic	Staric	Oleic	Linoleic	Linolenic		
Santa Elena	White	25.8[a]	7.53[a]	3.33[a]	7.56[a]	19.23[a]	61.73[a]	0.31[a]	159.27[a]
	Spotted	26.15[a]	7.94[a]	3.51[a]	8.19[a]	19.7[a]	60.05[a]	0.33[a]	156.88[a]
	SD[B]	3.77	1.96	0.80	3.49	0.95	6.23	0.05	21.19
Salinas	White	32.07[a]	6.33[a]	4.78[a]	6.35[a]	15.7[a]	66.17[a]	0.24[a]	212.06[a]
	Spotted	33.23[a]	6.3[a]	3.4[a]	5.78[a]	15.6[a]	67.33[a]	0.23[a]	223.77[a]
	SD	7.11	0.44	3.37	0.78	0.72	1.53	0.02	45.55
San Pablo	White	34.43[a]	5.9[a]	3.1[a]	8.23[a]	18.18[a]	63.63[a]	0.29[a]	219.13[a]
	Spotted	35[a]	6[a]	3.5[a]	9.4[a]	19.3[a]	60.85[a]	0.32[a]	212.99[a]
	SD	3.18	1.51	0.68	1.66	2.38	3.76	0.05	30.29
Patate	White	32.77[a]	4.43[a]	3.5[a]	6.83[a]	17.43[a]	64.53[a]	0.27[a]	211.41[a]
	Spotted	31.17[a]	6.48[a]	3.86[a]	7.92[a]	17.3[a]	63.33[a]	0.27[a]	196.96[a]
	SD	8.65	0.82	1.06	0.44	1.46	2.46	0.02	48.80
Guayllabamba	White	29.33[a]	6.47[a]	3.55[a]	6.8[a]	17.93[a]	63.67[a]	0.28[a]	186.64[a]
	Spotted	30.23[a]	6.38[a]	3.36[a]	6.73[a]	18[a]	63.47[a]	0.28[a]	191.99[a]
	SD	6.94	0.64	0.71	1.33	1.51	3.16	0.03	43.28
Overall	White	31.05[a]	6.56[a]	3.61[a]	7.16[a]	17.63[a]	64.03[a]	0.28[a]	199.08[a]
	Spotted	31.25[a]	6.56[a]	3.53[a]	7.21[a]	17.75[a]	63.4[a]	0.28[a]	198.28[a]
	SD	2.91	0.54	0.63	0.86	1.12	3.04	0.02	21.50

Means in a column within a group with the same letter are not statistically different ($P < 0.05$)

[A] Percentage of dry matter

[B] Least significant difference for $P < 0.05$

[C] Kilograms of seed by oil percentage by linolenic percentage

Table 3 Overall chia seeds oil content and fatty acid composition

Genotype	Location	Oil %[A]	Percentage of total fatty acids					ω-6:ω-3 rate	Linolenic[C] g/kg of seed
			Palmitic	Stearic	Oleic	Linoleic	Linolenic		
Overall	Santa Elena	25.94[c]	7.69[a]	3.40[a]	7.81[a]	19.42[a]	61.06[c]	0.32[a]	158.31[c]
	Salinas	32.65[ab]	6.32[b]	4.09[a]	6.07[b]	15.65[d]	66.75[a]	0.23[c]	217.91[a]
	San Pablo	34.48[a]	6.12[b]	3.22[a]	8.47[a]	18.28[b]	62.98[b]	0.29[b]	217.18[a]
	Patate	31.98[ab]	6.45[b]	3.68[a]	6.93[b]	17.37[c]	63.93[b]	0.27[b]	204.18[ab]
	Guayllabamba	29.78[b]	6.43[b]	3.46[a]	6.77[b]	17.97[bc]	63.57[b]	0.28[b]	189.27[b]
	SD[B]	3.31	0.54	0.99	0.85	0.80	1.90	0.02	20.88

Means in a column within a group with the same letter are not statistically different ($P < 0.05$)

[A] Percentage of dry matter

[B] Least significant difference for $P < 0.05$

[C] kg of seed by oil percentage by linolenic percentage

Overall, as in the oil content case, significant differences of individual fatty acids among locations were found (Table 3). Significant differences in palmitic, oleic, linoleic, and α-linolenic fatty acids between oils from seeds grown in different locations were detected. The significant highest α-linolenic fatty acid concentration (66.75%) was determined in oil of seeds from the Salinas location. Oil of seeds from Santa Elena location showed the highest significant concentration of linoleic (19.42%), oleic (7.81%), and palmitic (7.69%) fatty acids.

In addition, oil of seeds from the Santa Elena location had a significantly higher ω-6:ω-3 rate compared to that from oil of seeds harvested in all other locations (Table 3). Combining oil and α-linolenic fatty acid content, Salinas and San Pablo seed oils had significantly higher α-linolenic fatty acid content, expressed as g of fatty acid per kg of seed (Table 3), than those from Guayllabamba and Santa Elena oils, but not Patate seed oil.

Overall, α-linolenic fatty acid was negatively correlated with linoleic and oleic fatty acids content. Computed for these relationships, correlation coefficients were $r = 0.863$ ($P < 0.001$) and $r = 0.778$ ($P < 0.001$), respectively.

Discussion

The results of the analysis for oil and fatty acid content revealed a large variation in oil composition that was presumably affected by growth conditions and not for seed coat colors. The present investigation showed that the content and composition of chia oil were mainly determined by the effects of environmental factors such as elevation, soil and weather conditions.

Both black-spotted and white color coat seeds have been produced by domesticated chia plants since Pre-Columbian times [2]. Genetic studies suggested that there was a single origin for the actual commercial cultivars with reduced genetic variability [9, 14]. Then, a lack of significant difference in oil contents and fatty acid compositions between white and black-spotted color coat seed, at any of tested locations, could be explained by the reduced genetic variability between these two genotypes. The results found herein were in agreement with observations of chia oil yields and fatty acid composition reported recently [15]. Ayerza and Coates [7] also reported non-significant differences between the chia varieties Tzotzol and Iztac 1 on oil content and fatty acid composition; however, the three sites tested where located at the same ecosystem.

Significant differences in oil content and fatty acid composition of chia seeds among growing areas were consistent with other multi-location trials [16, 17]. Overall, seeds produced at the San Pablo location had a significantly higher oil concentration than seed produced at the Guayllabamba and Santa Elena locations, providing 15.8 and 24.8% more than Guayllabamba and Santa Elena locations, respectively. Similar chia yield variations among locations have been reported [15, 16, 18].

The temperature affecting the oil content variability during the seed developing process was reported for chia and for other crops, as well [15, 17, 19, 20]. Generally, as the altitude decreases, temperature increases [20, 21], oil content of a number of crops such as sorghum, soybean, chia, and others tended to decrease [15, 20, 22]. The Santa Elena location has the lowest elevation of all five locations tested. Thus, the significant lower oil content of seed from Santa Elena compared to seeds from all other locations could be related, at least partially, to the altitude differences between locations of production.

Warm temperatures generally increase the level of saturation of chia fatty acids, like for other oil seed crops [16, 19, 23, 24]. However, the variation in α-linolenic fatty acid, as was found herein, could not be fully explained by the negative relationship between land elevation and oil desaturation levels. This significant difference of α-linolenic fatty acid content between seeds grown in low and high inter-Andean mountain valleys suggested that a limit exists beyond which increasing growing land elevation will not increase chia oil unsaturation level. As a consequence, the seeds of Salinas had a significantly lower ω-6:ω-3 ratio as well. This observation has important health implications since a way to lower the risk of coronary heart disease is to keep dietary ω-6:ω-3 fatty acid ratios as low as possible [25].

The highly significant ($P < 0.01$) negative relationship of α-linolenic fatty acids with two more saturated 18-C fatty acids, linoleic and oleic (data not shown), were in agreement with results recently reported in other chia study [15]. This strong inverse relation is supported by the biosynthesis of α-linolenic fatty acid through the process of desaturation of oleic fatty acid via linoleic fatty acid by the action of desaturase enzymes [20, 23].

In conclusion, this paper showed that larger differences in oil content and fatty acid composition were produced by location (due to environmental differences) rather than genotypes and white and black-spotted color of chia seed coat. However, additional multi-location and multi-year trials are required to assess fully the complete chemical composition of both seed coat colors' genotypes.

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