



## Metabolomic analysis by UAE-GC MS and antioxidant activity of *Salvia hispanica* (L.) seeds grown under different irrigation regimes



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### ARTICLE INFO

#### Keywords:

Chia  
Polyphenols  
TPC  
Management  
Alpha-linolenic acid  
Quality

### ABSTRACT

Chia (*Salvia hispanica* L.) is an emerging crop with a high content of  $\alpha$ -linolenic acid and metabolites of industrial and pharmaceutical interest but information on metabolome variations in response to agricultural management is scarce. We investigated the yield and metabolic profile of the seeds of two chia populations, one commercial black (B) and one long-day flowering genotype (G8), in response to two irrigation levels: replacement of 100% ET<sub>0</sub> (I) or rainfed (NI). Seed yield was higher in irrigated plots in G8 only (0.255 kg m<sup>-2</sup> for I vs 0.184 kg m<sup>-2</sup> for NI) while it was very low regardless of irrigation in B due to late flowering. Ultrasound assisted extraction (UAE) of seeds followed by gas chromatography-mass spectrometry (GC/MS) analysis showed differences in fatty acids and the major classes of organic compounds due to both genotype and irrigation, especially in the non-polar phase where irrigated samples showed a higher content of  $\alpha$ -linolenic and other fatty acids and a lower oleic/linoleic ratio (47.4 in NI vs. 39.6 in I). The antioxidant activity, expressed as trolox equivalent antioxidant capacity (TEAC), ranged from 1.317 ± 0.027 to 2.174 ± 0.010 mmol TEAC/g of defatted chia seed after 2 and 40 min respectively, and was negatively affected by irrigation. The total polyphenolic content (TPC) measured with the Folin-Ciocalteu method, also decreased with irrigation. According to our results irrigation can affect chia yield, metabolome and antioxidant behavior but some of the effects are genotype-dependent.

### 1. Introduction

Chia (*Salvia hispanica* L.) is an ancient short-day flowering crop with center of origin between Mexico and Guatemala (Cahill, 2004). It was one of the staple crops in pre-Columbian Central America but its diffusion was drastically reduced after the Spanish domination. Chia was re-discovered in the 90s by researchers intending to propose alternative crops to farmers (Coates, 1996; Gentry et al., 1990), and has thereafter spread to several areas of the world, at first due to favorable market placement linked to its nutraceutical properties, and more recently as a source of compounds of cosmetic, medical and industrial interest (Hermoso-Diaz et al., 2014; Lu and Foo, 2002; Muñoz et al., 2013). The *Salvia* genus includes more than 900 species, and in the New World around 500 species belong to the subgenus *Calosphace* (Benth.) and may be grouped in complexes of species with common medicinal activity, mostly related to the content of terpenes in leaves. Jenks and Kim (2013) listed external application (antimicrobial, skin problems treatments and antirheumatic) and internal uses (gastro-intestinal, gynecological and neurological) in the *mirto* complex, primary uses for the respiratory system in the *Nucchu* complex, and primary uses for biliary

and kidney problem in the *Li'l + +*, *Cantueso* and *Manga-paqui* complexes. They also report that *S. divinorum* Epling and *Játiva-M.* are the most studied medicinal sage leaves within the *Calosphace* subgenus, due to their use as a hallucinogen by Mexican shamans and their selective kappa-opioid receptor agonist activity. The chia complex, including 18 species besides *S. hispanica* (2013), on the other hand, is the most renowned for the production of indehiscent dry fruits, commonly referred to as “seeds”, rich in oil.

Chia seeds are one of the richest natural sources of omega 3 fatty acids (Ayerza and Coates, 2011; Ayerza, 1995) and show a high content of protein with a balanced composition in essential amino acids (Ayerza, 2013) and fiber (Capitani et al., 2012). A part of the fiber is located in the outer cells of the fruit and is extruded at the fruit surface upon hydration (Muñoz et al., 2012), forming a mucilaginous capsule with rheological properties that make it promising for industrial and medical uses: it is highly hygroscopic, viscous and adhesive (Švec et al., 2016). Many antioxidants have been identified in chia seeds, extracted oil and mucilage, especially phenolic acids and flavonoids, besides polyunsaturated fatty acids (Amato et al., 2015; da Silva Marineli et al., 2014). Chia seeds or their products are therefore increasingly proposed

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not only as food but also as a component for biodegradable film (Capitani et al., 2016; Muñoz et al., 2012), thickening agents (Coelho and de las Mercedes Salas-Mellado, 2015; Felisberto et al., 2015; Iglesias-Puig and Haros, 2013; Menga et al., 2017), anti-corrosive agents (Hermoso-Díaz et al., 2014), cosmetics (Muñoz et al., 2013) and medicaments (Vuksan et al., 2010). A strong variation in chia seeds' composition has been reported: for instance, oil content ranges from little over 20% to over 36% (Ayerza and Coates, 2004; Ayerza, 1995; Coelho and de las Mercedes Salas-Mellado, 2015; da Silva Marineli et al., 2014; Ixtaina et al., 2011). Variability has mainly been researched in relation to genotype and environment: Ayerza (2009) reports a range in total fat content from 25.93% to 33.50% for the same genotype of chia grown in five different environments. The fatty acids profile, especially the content of  $\alpha$ -linolenic acid, is also affected by elevation in seeds of this species, even within the same genotype (Ayerza and Coates, 2011; Martínez-Cruz and Paredes-López, 2014). This is probably largely due to thermal effects of elevation, as reported by Ayerza and Coates (2004) who found a correlation between temperature and chia oil fatty acids measured across different environments; they concluded that levels of fatty acids' unsaturation in chia increase at cooler temperatures, as observed for other oil seed crops due to saturase-desaturase dynamics. In an experiment conducted across different countries in America, Ayerza (2009) reports a direct relationship between elevation and oil content and an inverse relationship between elevation and the content of proteins. Several environmental variables might be involved besides temperature, including soil properties and a negative correlation between oil and protein as found in other crops. Ayerza and Coates (2011) suggest that the relationships of oil and protein content and oil saturation with elevation are strong enough that they could be used to trace the growing environment of chia. Ayerza (2009) also found differences in protein content for the same genotypes grown in different environments, but could not prove differences among genotypes within a site, except for one variety at one site. In a further study, Ayerza (2013) could not find significant differences between two genotypes of different seed coat color for protein, oil, fiber, amino acids, and antioxidant content. (Silva et al., 2016) found that a white and a black seed crop with the same seed yield produced different amounts of unsaturated fatty acids: the white seed genotype yielded more linoleic and  $\alpha$ -linolenic acids (6.0 and 17.0 kg ha<sup>-1</sup> respectively) than the black seed one (4.4 and 16.7 kg ha<sup>-1</sup> respectively). de Falco et al. (2017b) studied the metabolic profile of the seeds of seven chia populations, including commercial and early flowering mutant genotypes, and showed significant differences in the metabolic fingerprinting of the different populations using nuclear magnetic resonance (NMR) and chemometrics. An investigation of the metabolome with gas chromatography-mass spectrometry has not yet been performed on chia seeds after agronomic management.

Very little information is available about the variation in chia seeds composition with agronomic management. Amato et al. (2015) compared nitrogen fertilization regimes on chia seed composition and found a higher *p*-anisidine value, content of phenols and oxidative stability in plots fertilized with organic nitrogen only, whereas the addition of mineral nitrogen in topdressing increased free acidity, chlorophyll and carotenoids content. de Falco et al. (2017b) reported that the effect of mineral nitrogen supply on chia positively affects the content of aliphatic free amino acids, and negatively that of the main carbohydrates and flavonoids. Heuer et al. (2002) found that salinity of irrigation water decreases the oil content of chia seeds and increased their content of palmitic and linoleic acids.

Irrigation is one of the major agronomical factors conditioning crop yield and composition, and namely that of oilseeds (Flagella et al., 2002). Silva et al. (2016) did not find a significant effect of irrigation on chia seed yield and content of linoleic and  $\alpha$ -linolenic acids. However authors point out that their experiment was conducted using short-day flowering genotypes at a latitude higher than optimal; due to

photoperiod sensitivity flowering was delayed and temperatures during seed maturation were too low to allow complete grain filling. In this condition, temperature and not water was the limiting factor and even fully irrigated plots yielded poorly. More information is therefore needed on the response of chia to irrigation taking photoperiod sensitivity into account. The objective of this research was to study the response of chia to irrigation with the hypothesis that irrigation affects the yield of chia seeds, the fatty acid profile and the production of secondary metabolites found in the polar and non-polar extracts. The hypothesis that responses to irrigation are different in short-day and long-day flowering genotypes at high latitudes was also tested by using a short-day commercial chia seed source and a long-day flowering mutant.

## 2. Materials and methods

### 2.1. Plant material

Black chia (*Salvia hispanica* L.) seeds (B) were obtained from a commercial retailer (Eichenhain-Hofgeismar-DE) and seeds of one long-day flowering mutant genotype (G8) were obtained as described in Jamboonsri et al. (2012) and were kindly supplied through an agreement between the University of Basilicata and the University of Kentucky (US).

### 2.2. Growth conditions

Plants were grown in Basilicata (Southern Italy-Lat. N 40° 51' 37,59" Lon. E 15° 38' 49,43") on a Luvi-vertic Phaeozem (Luss, 2007), loam soil (43.6% of sand, 34.2% silt and 22.1% clay) in the period June-December 2014. Soil water content was 0.279 g g<sup>-1</sup> at -0.03 MPa and 0.137 g g<sup>-1</sup> at -1.5 MPa. A field factorial randomized block design with three replications was established to test the two genotypes B and G8 with two levels of irrigation:

NI = no irrigation

I = nonlimiting water supply. We provided 100% of ET<sub>0</sub> corresponding to the evaporative demand of the atmosphere (Allen et al., 1998) measured with a TE-ETG atmometer (Tecnoel, Rm Italy).

The irrigation system was drip with pre-installed emitter lines with drippers at 200 mm distance and maximum flow rate 6.6 × 10<sup>-5</sup> m<sup>3</sup> s<sup>-1</sup> at 1 × 10<sup>5</sup> Pa. Plot size was 8 × 5 m with a density of 20 plants m<sup>-2</sup>. The crop was sown on June 26, 2014. Precipitation and irrigation amounts are shown in Fig. 1; total precipitation was 197 mm during the experiment and all plots received the same initial regime with 52 mm of irrigation to help crop establishment. Treatments were differentiated at 51 DAS (day after sowing) when the I treatment was left in rainfed conditions and the NI treatment was irrigated receiving 172 mm of further irrigation. Evaporation was read daily and irrigation was performed when a set amount was reached. In order to cover the crop's needs for root establishment and full deployment of deep rooting potential, irrigation schedule and amount ranged from irrigation with around 4.5 mm every 4 days up to 15 DAS, to an irrigation amount of around 14 mm on average per irrigation thereafter. This corresponded to a number and timing of irrigations varying according to evaporation and the timing of precipitation: 5 irrigations in July (with a maximum of 10 days without irrigation due to precipitation), 10 in August (every 2-4 days), 2 in September (with a maximum of 26 days without irrigation due to precipitation) and October (with a maximum of 21 days without irrigation due to precipitation). Seeds of G8 and B were harvested at 132 and 173 DAS respectively, on one sample of 20 plants per replication. A trench dug after harvest showed roots up to 2 m and a total soil depth exceeding 3 m.

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