



Effect of water availability on growth, water use efficiency and omega 3 (ALA) content in two phenotypes of chia (*Salvia hispanica* L.) established in the arid Mediterranean zone of Chile



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ABSTRACT

Chia (*Salvia hispanica* L.), has achieved economic importance due to the products which are obtained from its leaves with antioxidant capacity and especially its seeds, because they contain omega 3. However, there is a lack of information on optimal agronomic management practices and especially the influence of water availability on its establishment and production. The objective of this study was to evaluate the effect of different irrigation rates on growth and water use efficiency (WUE) in the production of leaf biomass, seeds and omega 3 in two phenotypes of chia, black and white. We applied three irrigation treatments calculated to be 100, 70 and 40% of the mean evaporative demand (ET₀) calculated weekly, designated as T1, T3, T5 black phenotype and T2, T4 and T6 white phenotype; T1 and T2 irrigated to 100%; T3 and T4 irrigated to 70% and T5–T6 irrigated to 40% ET₀. There was no difference between phenotypes, however, water treatment affected the gas exchange parameters photosynthesis, stomatal conductance and transpiration, which ranged from 26 to 4 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 4.5 to 1.5 $\text{mmol m}^{-2} \text{s}^{-1}$ and 0.6 to 0.1 $\text{mol m}^{-2} \text{s}^{-1}$ from treatment (54 days after sowing) to harvest, respectively. A significant effect of water availability on WUE was observed in biomass production and yield, with 0.87 and 0.11 kg m^{-3} , respectively. Biomass production and yield were higher in plants irrigated at 100% ET₀. Finally, reduced availability of water increased oil yield by 27% and the WUE for ALA from 3.4 to 8.6 mg L^{-1} . These findings demonstrate that WUE for biomass and yield is a constant value; that chia is highly sensitive to water deficit but adopts adaptive strategies that maintains its yield and increases the percentage of lipids and omega3.

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1. Introduction

Chia is native to Central America. It is one of the plant species with highest concentration of the fatty acid Omega 3, which may be produced in a variety of climates from tropical to desert (Coates and Ayerza, 1998); it has great productive potential and industrial use for the oil obtained from the seeds. Its characteristics make it an alternative to deal with problems of food security and climate change. It is thus urgent to increase agricultural areas and productivity per unit area using new species, varieties and technology.

This is complicated by the increasing worldwide scarcity of water (FAO, 2002, 2013; Fereres et al., 2011; Medrano et al., 2015). It is estimated that in 2020 nearly a third of the world population will live in arid zones (De Almeida et al., 2012) and that there will be an increase in the frequency and severity of droughts in some regions of the world that will decrease water security (Steduto and Hsiao, 2007; Medrano et al., 2015). In Chile it is anticipated that there will be an intensification of aridity in the northern zone, an advance of the desert towards the south and a reduction in water in the central zone by 2040 (Meza et al., 2010).

Predicted climatic changes suggest that in the future it may be necessary to select species with low water requirements (Corell et al., 2009) that efficiently produce large amounts of biomass, a desirable quality in species resistant to water stress (De Almeida

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et al., 2012; Medrano et al., 2015). *Salvia hispanica* L. (chia) is an interesting option, which has been described as being drought resistant while maintaining high growth under reduced water availability. There are two types of chia which differ by the color of the epicarpio or outer covering of the seed (Rovati et al., 2012; Ayerza, 2013). Black seeds, which are really greyish-brown with dark brown blotches, are more frequent than white seeds (Di Sapio et al., 2012). There are also seeds of a uniform maroon color, which are empty seeds caused by low temperature (Rovati et al., 2012), since the species does not tolerate frost during development (Bendaña, 2012). Chia seeds are considered to be a functional food because of their protein content, soluble and insoluble fiber, antioxidants, phytosterols and minerals, and especially because of the high amount of ALA or omega3 in their oil (Ayerza, 1995; Ayerza and Coates, 2006, 2009; Ciftci et al., 2012; Jiménez et al., 2013) and angiotensin converter enzyme, suggesting that these peptides may promote cardiovascular health as hypotensive agents (Segura-Campos et al., 2013). Chia grains have a high content of essential fatty acids; 25–39% of their weight is oil, and up to 68% of this may be omega3 (Ayerza, 2011). The protein content of chia seed is greater than that of traditional seeds such as wheat, maize and rice, which have 29, 10, 11 and 8% respectively (Coates and Ayerza, 1996). There have been no studies on the effects of water deficit in chia.

It is known that plants produce a wide range of primary and secondary metabolites during vegetative growth and reproduction. Primary metabolites have essential roles in photosynthesis, respiration, growth and development, while secondary metabolites are natural products not essential for basic growth, but which frequently participate in adaptation to biotic and abiotic environmental stresses. Because these secondary metabolites are expendable for growth and development, their components may be modified continuously and thus adapt more readily to the exigencies of a changing environment (Hartmann, 2007; Sánchez-Rodríguez et al., 2012). This plasticity is reflected in their attributes, which are diverse, often unique to a particular species and generally have an adaptive function (Hartmann, 2007; Krol et al., 2014). Some secondary metabolites are produced continuously in plants, while others are only produced in response to stress signals and may increase and/or decrease in concentration due to lower water availability.

This study evaluated the effect of water availability on growth and water use efficiency (WUE) for biomass production, yield in grains, oil and omega3 in two chia phenotypes established in a Mediterranean arid zone of Chile. The specific objective was to determine the minimum amount of water necessary to produce the greatest yield in grains and omega 3.

2. Materials and methods

2.1. Study area

The trial was performed from January to June, 2014 in the Experimental Station Pan de Azúcar (30°02'16"S, 70°41'48"W) which belongs to INIA (Instituto de Investigaciones Agropecuarias), located in the Región de Coquimbo in northern Chile. The climate is a warm desert, with mean maximum temperature of 28 °C in January and mean minimum of 5 °C in July; the locality has a yearly water deficit of 800–2100 mm and 75 mm mean annual precipitation (Uribe et al., 2012). The soil is in the sandy loam texture class, with 54.8, 28.2 and 17% sand, silt and clay, respectively, an apparent density of 1.23 g cm⁻³, effective depth of 80 cm, well-drained and with low (1.2%) organic material content. The field capacity and wilting point values are 0.148 g g⁻¹ and 0.620 g g⁻¹ respectively.

2.2. Plant material and growth conditions

Two phenotypes of chia (*Salvia hispanica*) were used in the trial, identified as black and white; these were from Santa Cruz de La Sierra, Bolivia and were provided by the Benexia Company. Seeds were sown on 23 January 2014 in a continuous stream manually at 1 cm depth, with a seed dose of 5 kg ha⁻¹. Plants were thinned to 67 plants m⁻² twenty days after sowing (DAS).

Soil analysis indicated nitrogen, phosphorous and potassium content of 25, 27 and 120 mg kg⁻¹, respectively. Using the information obtained from the analysis of water and soil and based on the usual commercial fertilizer dosage, 30 kg ha⁻¹ N and 37 kg ha⁻¹ P, we used fertirrigation to apply partial doses equivalent to 20 kg monoammonium phosphate and 18 kg potassium sulfate (Sadzawka et al., 2006). Weeds were controlled manually up to approximately 60 DAS, when the crop reached maximum cover. The physical soil constants were determined using a pressure chamber to determinate water content at field capacity (-33 kPa) and the permanent wilting point (-1500 kPa).

2.3. Treatments, experimental design and irrigation

The trial consisted of 6 treatments combining a phenotype factor with "white" and "black" levels and an irrigation factor with 100%, 70% and 40% of the reference evapotranspiration demand (ET_o) calculated daily by the method of Penman Monteith (FAO, 2006) and summarized weekly (Table 1). The experimental design was split plots in six completely randomized blocks. The main plot was irrigation and the subplots were phenotypes. The experimental unit was defined as a plot of 6 rows of 5 m length, with 0.6 m between rows. The distance between drips in a line of tape was 0.2 m. Irrigation treatments started at 53 DAS, at the state of the fourth pair of leaves. Drip irrigation consisted of a line of tape with a flow of 2 L h⁻¹, controlled using a Rain Bird programmer (Rain Bird Corporation, Tucson USA) and maintaining independent tubing for each water treatment; each had a solenoid and flow meter to record the total water applied in m³. Plants were irrigated when there was a loss of between 5–10 mm from ET_o.

ET_o was calculated using data from the monitoring and ecological modeling station "Pan de Azúcar" of the Instituto Nacional de Investigaciones Agropecuarias de Chile (INIA), located at 30.06° S, 71.233 W at an elevation of 135 m above sea level and 100 m from the experimental site, adjusted to the FAO 56 protocol (Method FAO Penman-Monteith, 1990). To apply variable amounts of water in each treatment we used manual irrigation programming, but with automatic operation to calibrate the system. To do this, before the trial began and with the irrigation network installed in the field we applied irrigation times of 1, 2, 3, 4 and 5 min using a Rain Bird ESP-LX programmer to each experimental sector, repeating the operation 6 times, and all volumes were quantified using a domiciliary flow meter (precision 1 L) in each irrigation subsector. This allowed us to associate irrigation times of the programmer to the actual number of liters discharged by the emitters in each experimental subsector (CV = 0.05 and singular losses of irrigation subsectors less than 2%). We used as irrigation criterion the weekly accumulation of ET_o, replenishing 100, 70 and 40% of the evapotranspiration over the period. Although we expected that irrigation at 100% ET_o would not generate water stress, the production of dry matter was lower in this treatment, which may have been due to application of excess water or root anoxia. Silva et al. (2010) showed that excessive irrigation of a CAM plant produced a decrease in aerial biomass, and it is known that in many crops (for example grapes), irrigation at 100% ET_o decreases yield.

Soil humidity content was monitored with EC1 and DS3 probes (Decagon Devices) with two objectives, to detect the accumulation

Table 1
Meteorological condition and irrigation accumulated from sowing to harvest in chia established in Intihuasi, IV region.

Month	Mean Temperature	VPD (KPa)	ET _o (mm month ⁻¹)	Mean RH (%)	Irrigation (m ³ ha ⁻¹)		
	°C				40%	70%	100%
January	18.4	0.69	151.8	72.8	266.2	253.5	240.3
February	17.5	0.61	117.4	75.8	1127.4	1119.6	1123.2
March	15.1	0.44	91.6	81.7	1558.9	1574.8	1557.5
April	13.2	0.42	61.79	81.1	1889.9	2046.2	2717.2
May	11.9	0.41	52.79	83.7	2106.3	2486.7	3833.2
June	10.3	0.36	37.20	82.3	2146.1	2591.6	4102.9

VPD: vapour pressure deficit; ET_o: potential evapotranspiration; and RH: relative humidity.

of salts that could affect crop growth and to observe changes in volumetric humidity (m³ m⁻³) in the experiment.

2.4. Gas exchange and water relation measurements

We measured stomatal conductance (mol H₂O m⁻² s⁻¹), photosynthesis (μmol CO₂ m⁻² s⁻¹) and transpiration (mmol H₂O m⁻² s⁻¹) simultaneously with an infrared gas analyzer (ADC, model LC-Pro) every 15 days in four plants per experimental unit, around 14:00 h. Instantaneous intrinsic water use efficiency (iWUE; μmol CO₂ mmol H₂O⁻¹) was estimated as the quotient between CO₂ assimilation and stomatal conductance. Leaf water potential (Ψ ; MPa) was measured every 15 days about 14:00 h using a Scholander pressure chamber (Scholander et al., 1965). The same leaf samples used to measure Ψ were also used to calculate the value of the solute potential (Ψ_s , MPa) and estimate osmotic adjustment, according to the methodology of Babu et al. (1999) and Silva et al. (2007). This was done using a vapor pressure osmometer, which measures the concentration of leaf fluid. This value was transformed to Ψ_s using the Van't Hoff equation (Taiz and Zeiger, 2006).

2.5. Biomass production measurements and yield; water use efficiency estimation

Crop aerial biomass was measured every 15 days after establishment by harvesting 0.25 m² per experimental unit and drying in an oven at 70 °C until constant weight. In the same sample the leaf area index (LAI) was also measured every 15 days with an electronic planimeter (LiCor 3100). We measured yield, biomass and yield components (kernels per square meter and weight of 1000 kernels) at physiological maturity by harvesting 2 m² per experimental unit. All growth variables were expressed in terms of dry matter (0% humidity). We estimated WUE based on biomass (WUE_b; kg m⁻³ ha⁻¹) and yield (WUE_y; kg m⁻³ ha⁻¹), using the amount of water applied to each treatment.

2.6. Oil and ALA (omega 3) content in seeds and WUE

To quantify the effect of water treatments and/or phenotypes, after harvest we sampled 500 g of seeds per treatment and genotype; we used the modified cold method of Folch et al. (1957) to extract the oil and determine the lipid profile of each oil sample. To each 100 g of chia seeds ground in a processor we added 500 mL of a 2:1 V:V mixture of chloroform and methanol and mixed for 5 min in a processor. Mixtures were then filtered twice; the filtrate obtained 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 the 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; the chia oil remaining was 25%–39% of total seed weight.

2.7. Lipid profile

Oil samples of each treatment were homogenized and then total fatty materials were extracted using the method of Bligh and Dyer (1959) in methanol: chloroform 2:1 with Mg₂Cl (0.5 N). Total fatty material and chloroform were recovered; the latter was evaporated using N₂, thus obtaining the total lipid fraction available in the seeds. To determine the fatty acid profile of the fatty material extracted it was first methylated with boron trifluoride (BF₃) in 12% methanol according to Morrison and Smith (1964) and then with NaOH in methanol to obtain free methylated esters of fatty acids. These were suspended in hexane in order to be injected in a gas chromatograph. We calculated the efficiency of conversion of grains to oil (kg L⁻¹), WUE_L and WUE_{ALA} in units of mg L⁻¹, as functions of the results of the oil obtained from the grains, the fatty acids measured in the oil and the total quantity of water applied per treatment.

2.8. Statistical analysis

Statistical analyses were performed using the InfoStat statistical software (Di Rienzo et al., 2011) and/or R version 3.2.1 (R Core Team, 2015). The significance level was set at 0.05 for all comparisons. Differences in photosynthesis, transpiration, stomatal conductance, WUE_i, water potential and solute potential were analyzed using general linear and mixed models for repeated measures, with DAS, irrigation treatment, phenotype and interactions as fixed factors, and block, main plot nested in the block and subplot nested in the main plot as random effects. Model selection in each case was based on fulfillment of the assumptions of the statistical analysis and using AIC index values (Akaike information criterion; Bozdogan, 1987). Yield, biomass, yield components and seasonal WUE were evaluated using general mixed models; the fixed factors were irrigation, genotype and their interaction, while the random factors were blocks and the main plot nested in the blocks. In all cases post-hoc DGC analyses were used when appropriate.

3. Results

3.1. Crop phenology and growing environment

The emergence of the crop occurred at 4 DAS; first true leaf at 12 DAS, branching initiation (beginning of irrigation) at 53 DAS, initiation of inflorescences at 68 DAS, flowering at 98 DAS and harvest at 153 DAS. Table 1 shows that during the development of the crop there was a normal transition from summer to autumn with a decrease in air temperature and increase in humidity, with a consequent decrease in the atmosphere pressure deficit and reference evapotranspiration. The total amount of water applied per treatment was 4102, 2591 and 2146 m³ ha⁻¹ for 100%, 70% and 40% ET_o, respectively.

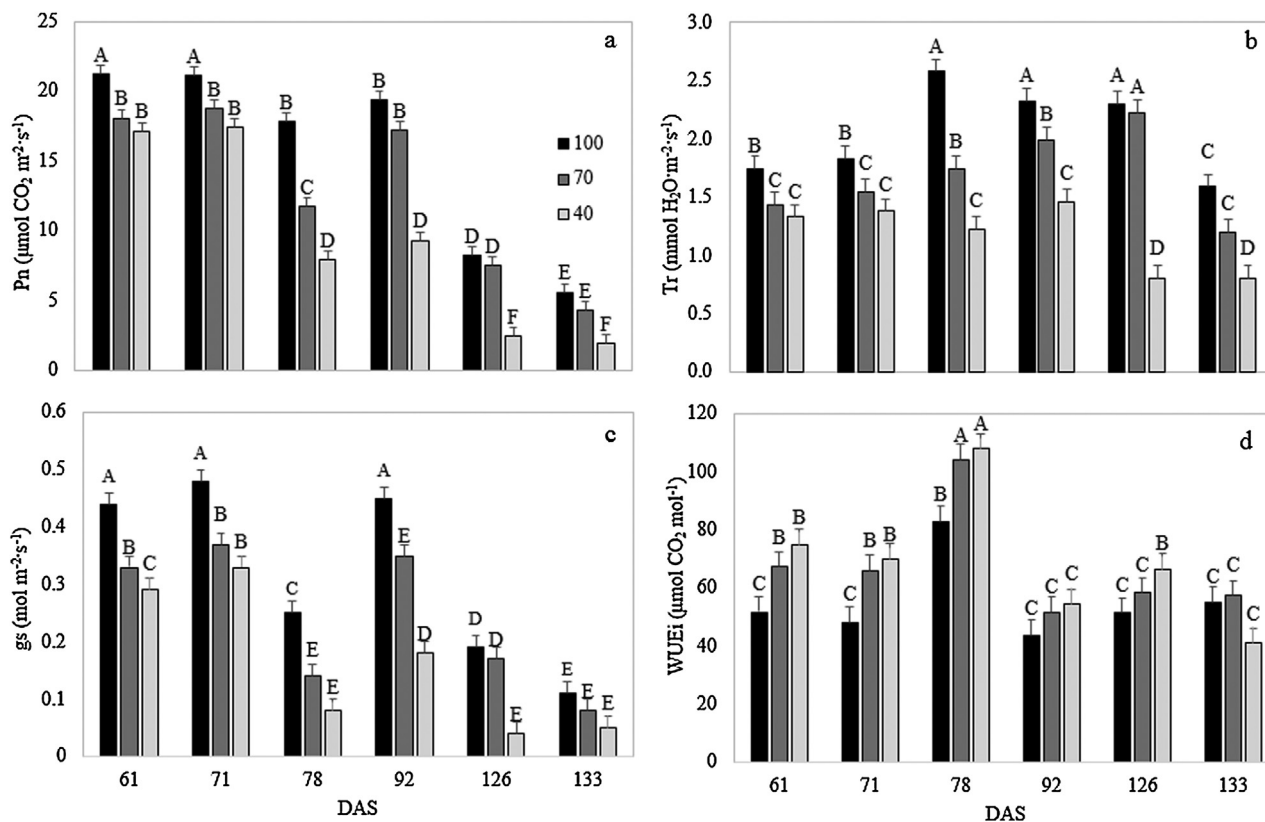


Fig. 1. Evolution of net photosynthesis (a), transpiration (b), stomatal conductance (c) and intrinsic water use (d). Values are means \pm 1 S.E. ($n = 8$) in chia under three irrigation treatments: 100, 70 and 40% of ET_0 . Different letters indicate significant differences according to DGC post-hoc analysis.

3.2. Gas exchange and intrinsic iWUE

In the parameters of gas exchange, photosynthesis rate (P_n ; Fig. 1a), transpiration rate (T_r ; Fig. 1b) and stomatal conductance (g_s ; Fig. 1c) (Fig. 1) there was significant interaction between DAS and irrigation ($p < 0.05$ in all cases) but no effect of phenotype or interaction of phenotype with other factors. Independent of measurement date (DAS), the 100% treatment showed greater P_n , T_r and g_s with respect to the 40% ET_0 treatment. A phenological effect was observed over time in the P_n and g_s variables, which decreased as the crop cycle advanced; this did not occur in T_r . Similar behavior was observed iWUE (Fig. 1d), with greater values in the 40% and 70% treatments than with 100% ET_0 . However, the differences were much less than in the other gas exchange variables; large differences were not observed during the crop cycle except at 78 DAS, when iWUE increased in all treatments associated with low values of g_s .

3.3. Leaf water potential and osmotic adjustment

The water relations in terms of Ψ and Ψ_s showed a significant interaction between DAS and irrigation (Fig. 2a and b; $p < 0.05$ in both cases) and effect of phenotype ($p < 0.05$ in both cases), but no interaction of phenotype with other factors. The leaf water potential of the black phenotype was -0.97 MPa, greater than the -0.99 MPa of the white phenotype. This tendency was reversed in terms of solute potential, in which the black phenotype was inferior to the white, with values of -1.16 and -1.12 MPa, respectively.

Water stress had a significant effect on Ψ , which was more negative when less water was available independent of measurement date; the changes over time in the different irrigation treatments were discrete. The differences in osmotic adjustment between treatments in terms of Ψ_s were more pronounced, with an impor-

tant effect in the 40% treatment compared to that observed in Ψ . Between the first and last osmotic adjustment measurements of the season we found a value of -0.49 MPa for 40% ET_0 , a similar value of -0.43 for 70% ET_0 , but only -0.25 MPa for 100% ET_0 (Fig. 2B).

3.4. Seasonal crop growth and water use efficiency

Seasonal production of dry biomass (Fig. 3a) and water use efficiency (Fig. 3c) showed a significant interaction between DAS and irrigation ($p < 0.05$ in all cases) but no effect of phenotype or interaction of phenotype with other factors, following a sigmoid tendency with biomass production at end of the cycle of 8000 and 5000 $kg\ ha^{-1}$, respectively (Fig. 3a). In terms of water use efficiency the 70% and 100% treatments were superior, due to the strong effect of water stress in biomass production. It is important to note that the results shown are from a chia crop in which leaf abscission was not yet evident, thus the biomass values are greater than those observed at physiological maturity. This difference was found beginning at 80 DAS, thus at flower initiation stressed plants had produced 44% less biomass compared to those with 100% ET_0 . These results were coherent with the maximum LAI recorded at 89 DAS, which was about 3.0 in the 40% ET_0 treatment; this is a reduction of close to 30% compared to the LAI obtained with 100% ET_0 irrigation (Fig. 3B).

It may be seen that at the beginning of flowering (vertical arrows in Fig. 3a and c) there was a decrease in biomass per surface area in the 40% and 70% treatments and a decrease in the growth of the 100% treatment associated with leaf abscission that began with the appearance of blossoms, which was not compensated by the growth in the reproductive structures.

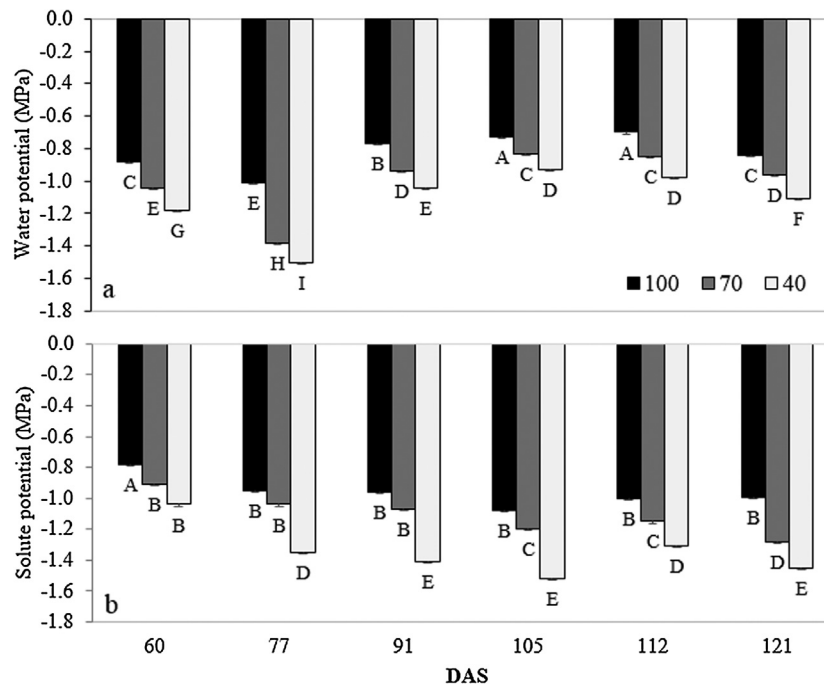


Fig. 2. Seasonal courses of midday leaf water potential (a) and leaf solute potential (b) measured in chia under three irrigation treatments: 100, 70 and 40% of ET_0 . Values are means \pm 1 S.E. ($n=8$). Different letters indicate significant differences according to DGC post-hoc analysis.

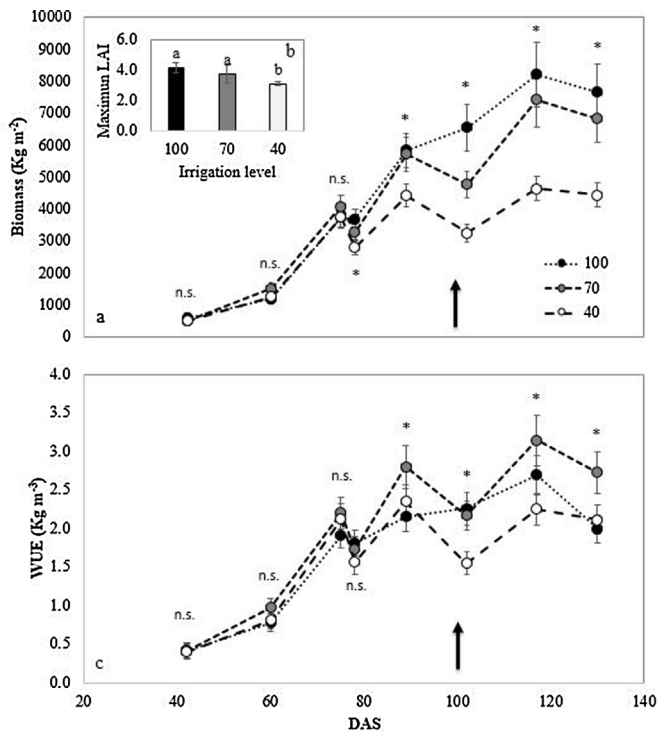


Fig. 3. Evolution of aerial biomass (a), maximum leaf area index (b) and biomass water use efficiency (c) in chia under three irrigation treatments: 100, 70 and 40% of ET_0 . Values are means \pm 1 S.E. ($n=8$). The arrow indicates the beginning of flowering. The symbols "n.s." and "*" represent the non-significance and significance of treatment factor at each measurement time.

3.5. Biomass, yield, yield components and oil production at harvest

There were significant differences in dry matter production at harvest (153 DAS; Table 2) both among phenotypes and irrigation

levels, which were present during the entire growth cycle (Fig. 3a). Treatments 40% of ET_0 had the least accumulation of dry mass, 1798 kg ha⁻¹, which was 48.5% less than that produced by 100%. At harvest, black chia had more accumulated dry mass, 2733 kg ha⁻¹, which was 18% greater than that produced by white chia.

Water stress did not affect grain yield, there were no significant differences in this parameter with reduction of applied water; the mean grain yield was 308 kg ha⁻¹ (Table 2). However, white chia produced 21% more grain yield than black chia; interaction between the factors was not significant. In terms of yield components, plants irrigated with 40% ET_0 showed significantly greater 1000 grain weight, which was almost 1.1 gr. However, this treatment produced the fewest number of grains per area, reaching only 235,025 gr m⁻², while the treatments with 70% and 100% ET_0 yielded a mean of about 535,000 gr m⁻², showing a clear compensatory effect of these components under water stress (Table 2).

No significant differences in oil production (Kg ha⁻¹) was found for the factors irrigation and phenotype or in their interaction, the mean was 27 kg ha⁻¹. The omega 3 composition of the oil was not affected by the irrigation regime; the mean was 15.4 kg ha⁻¹. There was a significant difference between the phenotypes; the white phenotype produced 17.0 kg ha⁻¹ of omega 3 while the black phenotype produced 13.7 kg ha⁻¹. The interaction among factors was not significant. The same tendency was observed for linoleic acid; the white phenotype produced more (6.0 kg ha⁻¹) than the black phenotype (4.4 kg ha⁻¹), and neither irrigation nor the interaction was significant. The overall mean of linoleic acid production was 5.2 kg ha⁻¹ (Table 2).

Principal component analysis (Fig. 4) showed a significant association between yield and grain weight ($r=-0.8$; $p=0.05$), grain number ($r=0.93$; $p=0.0069$), production of omega 3 ($r=0.98$; $p=0.0006$) and linoleic acid ($r=0.88$; $p=0.022$).

The weight of the grains was associated negatively and significantly ($r=0.85$; $p=0.0324$) with the number of grains per square meter, and production of omega-3 was associated positively and significantly ($r=0.92$; $p=0.0099$) to the production of linoleic acid

Table 2
Yield, biomass, yield components, total oils production, omega 3 and linoleic acid by irrigation treatment and phenotype in chia. Intihuasi, 2014.

	Biomass		Yield		HI		KN	KW		Oil		Omega 3	Linoleic acid			
	Kg ha ⁻¹		Kg ha ⁻¹		–		m ⁻²	G		Kg ha ⁻¹		Kg ha ⁻¹	Kg ha ⁻¹			
Irrigation																
100	3136.2	A	358.2	A	0.1	A	562160	A	0.6	B	30.63	A	17.4	A	5.6	A
70	2625.2	A	327	A	0.1	A	507771	A	0.7	B	23.25	A	16.3	A	5.7	A
40	1797.6	B	238.2	A	0.2	A	235025	B	1.1	A	29.07	A	12.4	A	4.2	A
Phenotype																
W	2306.7	B	337	A	0.2	A	468779	A	0.8	A	28.61	A	17.0	A	6	A
B	2732.6	A	278.6	B	0.1	B	401192	A	0.8	A	26.68	A	13.7	B	4.4	B
Interaction																
100W	2781.1	B	381.5	A	0.2	A	583937	A	0.7	C	33.93	A	13.08	B	6.7	A
100B	3491.3	A	334.9	A	0.1	B	540383	A	0.6	C	27.32	B	11.63	B	4.48	B
70W	2373.6	C	370.8	A	0.2	A	537220	A	0.7	C	25.83	B	19.18	A	6.55	A
70B	2876.9	B	283.1	B	0.1	B	478323	A	0.7	C	20.67	B	13.45	B	4.8	B
40W	1765.4	C	258.7	B	0.2	A	285180	B	1.2	A	26.07	B	18.8	A	4.62	B
40B	1829.7	C	217.7	B	0.1	B	184869	C	0.9	B	32.07	A	16	B	3.87	B
Irrigation	***		n.s.		n.s.		***		***		n.s.		n.s.		n.s.	
Phenotype	***		*		***		n.s.		n.s.		n.s.		**		**	
Interaction	n.s.		n.s.		n.s.		n.s.		*		n.s.		n.s.		n.s.	

HI: harvest index; KN: number of kernels per square meter; and KW: kernel weight. The symbols “*”, “***”, “*****” and “n.s.” represent significance level at 0.05, 0.01, 0.001 and non-significant respectively.

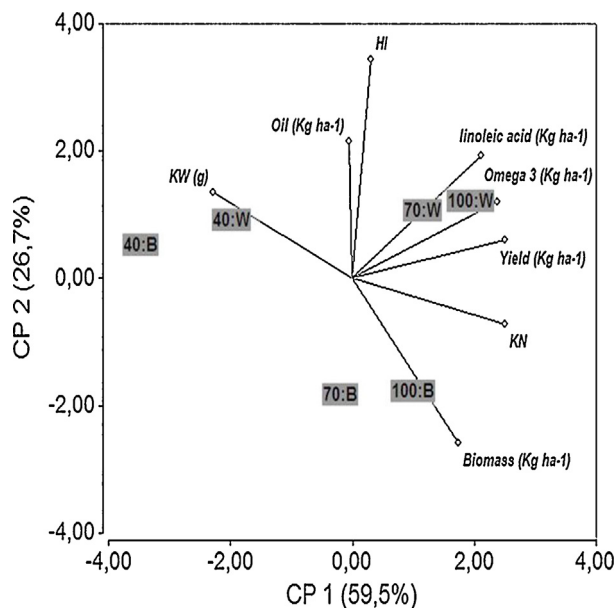


Fig. 4. Biplot of the first and the second principal components for biomass, harvest index (HI), yield, oil, number of grains (KN), kernel weight (KW), total oil production (Oil), linoleic acid and omega 3 production of two chia phenotypes (White: W and Black: B) grown under irrigation of 100, 70 and 40% ET₀. Variables are represented by vectors and factors (phenotype and irrigation) are represented by gray squares.

3.6. Efficiency of water use in the production of biomass, yield and oil

WUE for biomass production was different between phenotypes; black chia had WUE_b of 0.94 kg m⁻³, which was 16% greater than that obtained in white chia (Table 2), which was associated with the observed differences in harvest biomass. However, there was no effect of water treatment; the mean WUE_b was 0.8 kg m⁻³ and there was no interaction between the factors. WUE_b did show temporal differences, at 117 DAS plants irrigated with 70% ET₀ showed the highest value, 3.2 kg m⁻³, although this was not significantly different from the control, while the maximum value for plants irrigated at 40% ET₀ was 2.2 kg m⁻³ (Fig. 4).

There were no significant differences in WUE_Y between water treatments or phenotypes or interaction between these factors, the overall mean was 0.11 kg m⁻³. There appeared to be a tendency to greater values in plants irrigated at 70% ET₀ (Table 2).

There was a significant effect of irrigation regime in the efficiency of water use in oil production; the 40% treatment yielded 0.014 kg oil m⁻³, compared to the 0.009 and 0.007 kg oil m⁻³ in the 70% and 100% treatments, respectively (Table 3).

The efficiency of water use in omega 3 production was not affected by irrigation, phenotype or interaction between these; the mean value was 0.0055 kg omega 3 m⁻³. The mean efficiency for linoleic production (0.0017 kg m⁻³) was not affected by the irrigation regime, however the white phenotype was more efficient than the black phenotype, with values of 0.002 and 0.0016 kg m⁻³, respectively. The interaction was not significant (Table 3).

4. Discussion

The diurnal and seasonal tendencies of the parameters of gas exchange in chia obey the typical model described for C3 species (Farquhar and Von Caemmerer, 1982; Medrano et al., 2002), with a maximum at midday and declining at sunset. However, stomatal conductance is controlled not only by water availability, but by a complex interaction of factors internal and external to the leaf (Flexas and Medrano, 2002). The P_n, E and g_s in chia responded strictly to the level of irrigation received with significant differences, but with no difference between the phenotypes. From the beginning of the water treatment all responses showed the same percentage decrease, about 25%. However, water deficit affected transpiration (30%) more than photosynthesis (22%). A close relation between tolerance to water deficit and levels of gas exchange has been found in many species. In some cases the most resistant plants may show greater gas exchange than others at the same water potential (Lavini et al., 2008). They also have the capacity to maintain gas exchange levels when water stress becomes more severe, as in beans (Aguirre-Medina and Kohashi-Shibata, 2002) and cotton (Ullah et al., 2008). This did not happen in chia; this species is highly sensitive to lack of water in terms of gas exchange. At a daily scale stomatal conductance in chia is closely tied to soil water availability and atmospheric evaporative demand (AED). Thus at a maximum daily AED of 1.2 KPa we found significant differences in stomatal conductance; 50% less in plants irrigated at

Table 3

Water use efficiency for biomass production, yield of total oils, omega 3 and linoleic acid by irrigation treatment and phenotype in chia. Intihuasi, 2014.

	WUE Biomass		WUE yield		WUE oil		WUE omega3		WUE Linoleic acid	
	Kg m ⁻³		Kg m ⁻³		Kg m ⁻³		Kg m ⁻³		Kg m ⁻³	
Irrigation										
100	0.76	A	0.09	A	0.007	B	0.004	A	0.001	A
70	1.01	A	0.13	A	0.009	B	0.006	A	0.002	A
40	0.84	A	0.11	A	0.014	A	0.006	A	0.002	A
Phenotype										
W	0.81	B	0.12	A	0.01	A	0.006	A	0.002	A
B	0.94	A	0.1	B	0.01	A	0.005	A	0.0016	B
Interaction										
100 W	0.68	C	0.09	A	0.008	B	0.005	B	0.0017	A
100 B	0.85	B	0.08	B	0.007	B	0.004	B	0.0012	B
70 W	0.92	A	0.14	A	0.01	B	0.007	A	0.0023	A
70 B	1.11	A	0.11	A	0.008	B	0.005	B	0.0018	A
40 W	0.82	B	0.12	A	0.012	A	0.006	B	0.0020	A
40 B	0.85	B	0.1	A	0.015	A	0.006	B	0.0018	A
Irrigation	n.s.		n.s.		***		n.s.		n.s.	
Phenotype	***		**		n.s.		n.s.		**	
Interaction	n.s.		n.s.		n.s.		n.s.		n.s.	

Where WUE is the water use efficiency for production of biomass, yield, oil, omega 3 and linoleic acid per hectare. The symbols **, ***, ****, ***** and "n.s." represent significance level at 0.05, 0.01, 0.001 and non-significant respectively.

40% ET₀ and 25% less at 70% ET₀ compared to plants irrigated at 100% ET₀.

The values found for gas exchange parameters are reflected exactly in those of instantaneous WUE (Pn Tr⁻¹), which reached maximum values that may be associated with the maximum rate of relative growth, thus the measurement at this scale of WUE would be a constant for the species (values not shown). Thus transpiration decreased in stressed plants compared to non-stressed plants, and assimilation also decreased due to the reduction of stomatal conductance. However, the transpiration rate was relatively constant from 78 to 126 DAS in stressed plants, probably due to osmotic adjustment (Fig. 2). Irrigation at 40% ET₀ produced greater intrinsic WUE for the same reason, since although CO₂ assimilation decreased, stomatal conductance decreased even more, producing greater intrinsic WUE. According to Cardona et al. (2013), the reduction in transpiration produced by stomatal closure allows iWUE to increase; this efficiency is a measure of carbon gain per unit stomatal conductance. The difference between these two parameters lies in that instantaneous efficiency depends upon the plant and the environmental conditions, while intrinsic WUE measures differences related to the capacity of the leaf to regulate photosynthesis and stomatal conductance (Medrano et al., 2007). In a parallel scanning electron microscopic study of leaf anatomy we observed the presence of mucilaginous plugs of the stoma in stressed plants; these plugs appear to serve to maintain the leaf water balance and contribute to greater tolerance to water deficit.

Since there was no effect of phenotype on gas exchange, we conclude that probably black and white chia have similar drought tolerance; this makes sense considering that they did not show differences in parameters of water relations. However, the irrigation treatments produced significant effects on Ψ and Ψ_s , as has been shown in a number of studies (Lisar et al., 2012); both potential values are involved with low water availability. Given that the measurement of Ψ_s is performed in fluid extracted from the same plant in which Ψ was measured, we suggest that chia is capable of osmotic adjustment as a response to water deficit. The results in a parallel study of pressure-volume curves also demonstrated the osmotic adjustment in stressed chia plants. Chia is probably not a plant resistant to water stress, if we understand the term resistance to mean the capacity to produce under stress conditions, but rather it attempts to make several adjustments to tolerate water stress, which are coherent with the results for gas exchange and

its capacity for osmotic adjustment. Thus chia is capable of osmotic adjustment to both slight (70%) and strong (40%) water deficits. In these conditions it maintains the pressure potential that facilitates the processes that depend on turgor.

The total production of dry aerial mass is a function of water availability, thus when the water applied was reduced less dry material was produced. This occurs because leaf growth is sensitive to even a slight water deficit, which may inhibit their growth without affecting photosynthesis (Hsiao, 1993; Hsiao and Xu, 2000). The aerial dry mass produced in this study was 3136 kg ha⁻¹ (Table 2); considering that 21% of this was leaf biomass we would have 658.6 kg ha⁻¹, and if the LDW only represents 7% it would be possible to produce 9400 kg ha⁻¹ of fresh leaf biomass with irrigation at 100% ET₀ and 5000 kg ha⁻¹ with 40% ET₀. A study of leaf development demonstrated that extracts obtained from the leaves of chia plants have antioxidant potential; the polyphenol profile has also been characterized (Ayerza, 2013), constituting a new line of research towards the integral exploitation of chia plants. This will make it a double-purpose species, with production of quality seeds in the northern zone, while towards the central zone it could be used to produce leaf mass.

Certain crops under slight water deficit restrict excessive vegetative growth, which may increase yield efficiency, commonly called the harvest index (Steduto and Hsiao, 2007). The lower leaf area index as a result of the reduction in water availability (Fig. 3A) would be explained by inhibition of leaf growth (Hsiao and Xu, 2000), or because a slight water stress over time may induce the senescence of the oldest leaves, which becomes more evident when the crop is near maturity. It should be emphasized that defoliation in chia is normal, since in Mexico it has been observed that the plant loses all its leaves when it reaches anthesis (Orozco, 1993), which is associated with the beginning of flowering.

There was no significant difference among water treatments in grain yield, although there appeared to be a tendency to lower yield with water reduction, probably caused by the reduction in water availability (irrigation to 40% ET₀) significantly reducing the grain density per surface area (Table 2), which is concordant with other studies that indicate that water stress reduces the number of grains per plant. A study in soya showed that plants under water stress reduced the number of seeds, but the plants compensated the yield by producing heavier seeds (Eck et al., 1987). This is similar to our results with chia, given that the treatments with least water avail-

able (irrigated to 40% ET₀) showed the greatest weight of 1000 seeds (Table 2).

The mean yield obtained in this study was 308 kg ha⁻¹ (Table 2), inferior to commercial yields obtained in Argentina (600 kg ha⁻¹, Coates and Ayerza, 1998); yields of up to 1.200 kg ha⁻¹ have been obtained in irrigated crops (Pérez et al., 2012). A study performed in Chile to analyze the effect of sowing date on yield in a latitudinal transect from 34° to 18° S Lat. obtained yields that fluctuated between 300 and 2900 kg ha⁻¹ (personal communication). The yield we obtained probably demonstrates the effect of climate, due to its geographic location (30° S) associated with low temperatures, as well as loss due to wind and birds. During the trial there were temperatures below 10 °C at the beginning of grain filling, which may explain the low yield. However, a trial performed by our group in 2015 (data not shown) in the same experimental site produced yields of up to 1200 kg ha⁻¹; more favorable temperatures may have influenced this yield. Ayerza and Coates (2006, 2009) indicated that the crop is sensitive to photoperiod since it is a short-day plant, thus its period of growth and fruiting depend on the latitude at which it is grown; also it does not tolerate freezing in any development state and grows with temperatures from 12 °C to 32–33 °C (Bendaña, 2012).

White chia had less accumulation of dry material and greater grain yield than black chia (Table 2), which indicates that it has better efficiency in the partition of assimilates in the plant, favoring the formation of grains over the rest of the biomass (Evans, 1993). However, Di Sapio et al. (2012) argued that black seeds are present in a greater proportion than the white seeds, and it has been reported that grain yield of black chia was 14% greater than that obtained with white chia. Grains of a uniform maroon color are also present; these weigh less since they are empty seeds with little or no development of seed structures and are produced by low temperatures (Rovati et al., 2012).

Differences between black and white seeds have been found in protein content and fatty acid composition (Ixtaina et al., 2010). Black seeds have greater protein content, while white seeds have more α-linolenic acid (Ayerza and Coates, 2006, 2009). The proportion of white seeds in seed mixtures has decreased during the process of domestication of the species, possibly due to selection in favor of black seeds, since the Aztecs understood the greater yield of these seeds and the better quality of the oil used for painting (Ixtaina et al., 2010).

According to our results, those responses as a function of discrete and specific measures do not manage to show the genotype differences that are expressed in the long term. It is worth mentioning that most studies have concentrated on short-term stress. However, the response mechanism to a long, continuous stress is different, due to the formation of new metabolites that will produce changes in gene expression (Krol et al., 2014).

Water deficit in chia produces an increase in oil production and percentage of lipids extracted. Finally, omega 3 also shows a tendency to increase with less water availability. These results must be corroborated with other trials, which are currently underway. WUE evaluated at different time scales and in different organs indicate that this parameter of functional and adaptive integration is a constant in chia, with no significant influence of phenotype.

5. Conclusions

There were no significant differences between chia phenotypes based on discrete or instantaneous measurements such as gas exchange and water relations. A continuous decrease in stomatal conductance suggests that chia has efficient adaptive control over water loss by transpiration, maintaining relatively high rate of assimilation. Thus its photosynthetic water use efficiency was con-

served among irrigation treatments. However, there were clearer differences in the long-term responses evaluated. Decreased water availability increases oil and fatty acid production, and water use efficiency of biomass (WUE). In spite of the large effect of water availability on a number of parameters evaluated, from gas exchange to biomass yield, reflected in a decrease of up to 50%, this effect was not significant in yield of chia or in the efficiency of water use for yield production. This may probably be explained by a strategy of assimilate distribution, for example in stomatal control of water loss and in osmotic adjustment. Thus chia is sensitive to the lack of water, but adopts adaptive strategies that maintains its yield.

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