



Root morphology and shoot growth in seedlings of chia (*Salvia hispanica* L.)

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Abstract The use of chia (*Salvia hispanica* L.) seeds as functional food is increasing worldwide and research on plant traits is important as the crop spreads. The seedling stage has been identified as crucial for establishment, survival, and competition with weeds but information on chia at this stage is scanty, especially regarding root traits. In this study, 8 genotypes of chia: 4 populations (Australia, Mexico, Peru, Basilicata) and 4 breeding lines (G3, G8, G17, WS) were evaluated under controlled conditions at two early stages (six leaves, S-6; ten leaves, S-10) with the aim to assess growth characteristics and variability in root morphological traits. The shoot height and dry matter were measured together with the following root traits: dry matter and architectural parameters characterized through image analysis of root scans: length, surface, average diameter, tips, forks and crossings. Plants showed a greater phenotypic variation at S-6

than at S-10 (average CV = 40% and 22%, respectively). A high variability was recorded for root biomass, root volume, number of forks and crossings (average CV = 54, 40, 44 and 48%, respectively), while root diameter showed lower variability (average CV = 10%). Four principal components explained more than 97% of the total variance and root length, surface area, volume and average diameter contributed mainly to the variability. Cluster analysis showed that genotypes were very different for morphological root traits. Australia and G8 had the most developed root system with longer and coarser roots. Our results could be used in a breeding program for new cultivars with a more vigorous seedling.

Keywords Genotypes · Root morphology · *Salvia hispanica* L. · Seedling growth

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Introduction

Health awareness is a leading factor of dietary choices and this has led to an increase in the demand for foods rich in nutraceuticals (Souza and Chaves 2017). As a consequence traditional crops have been rediscovered and proposed to an ever increasing world market. This is the case of chia (*Salvia hispanica* L.), an annual herbaceous plant native to Mesoamerica, belonging to the mint family (*Lamiaceae*).

Interest in chia is mainly due to its nutritional and nutraceutical properties: leaves show a high level of antioxidants and antimicrobials (Amato et al. 2015; Elshafie et al. 2018) and seeds are rich in fiber, proteins, essential fatty acids such as omega-3 and -6, vitamins and antioxidants (Muñoz et al. 2013). Growth and development of chia, originally a short-day flowering species, is driven by day length and temperature as it is not frost-tolerant. Therefore, although the cultivation of chia has extended beyond its native tropical habitat (Jamboonsri et al. 2012), this is limited to a restricted range of latitudes. In order to extend the range of this crop to higher latitudes and other temperate areas and regions, new genotypes were obtained by using different techniques including mutation (Jamboonsri et al. 2012). Recent research indicates that chia could be grown for biomass under Mediterranean climatic conditions (Bochicchio et al. 2015). Amato et al. (2015) found that the quality of seeds of *S. hispanica* produced in Southern Italy was not significantly different in terms of human nutrition than that of commercially available seeds from traditional and new growing areas. These results indicate the possibility of introducing chia cultivation in the Mediterranean region, provided that successful establishment and plant growth of the crop are ensured. The optimal conditions for chia growth are a well distributed rainfall during early growth and development, coupled with dry conditions during seed maturation and harvesting (Wilson and Hildebrand 2010). Grimes et al. (2018) found that environmental conditions like precipitation or temperature, are more important than agronomic practices in determining the potential yield and seed quality of chia. In particular, chia seeds need moisture for seedling establishment and this could be a limiting factor to the diffusion in Mediterranean environments where sowing takes place in the spring. The unreliability of spring water supply in semi-arid climates poses problems of crop establishment.

Germination and seedling establishment are critical stages in the plant life cycle. In crop production, early stand establishment determines plant density, uniformity and competitive ability towards weeds especially for soil nutrients and water (Thelen 2006). Indeed, a rapid seedling development is a major breeding target in many crop species as seedling vigour is closely associated with crop growth and yield (Finch-Savage and Bassel 2015). The ability to compete with weeds

early is of utmost significance for chia given its initial slow growth, and especially crucial since chemical control protocols have not been proved satisfactory yet (Coates 2011). Plant growth is a biological process highly affected by the coordination of above-ground shoots and below-ground roots that operates through energy production in leaves in response to the availability of water and nutrients (Paul and Foyer 2001). Shoot size is important because the large shoot system of vigorous seedlings has more green tissue exposed to intercept solar energy, which is converted in carbohydrates to supply growth needs of the plant. According to Mielke and Schaffer (2010), the growth characteristics can indicate seedling adaptability and tolerance to environmental conditions. Survival and establishment of seedlings is largely dependent on their ability to rapidly initiate new roots and on their root morphology. This is especially important in small-seeded species with limited potential allocation of biomass to roots (Moles and Westoby 2004). The most common approach for assessing seedling quality is the study of root morphology and the relationship between shoot and root development (Mašková and Herben 2018).

Roots ensure plant stability, explore the soil volume, provide water and nutrients and represent the dynamic interface between plant and soil. Due to difficulties in root phenotyping, total root mass has frequently been used to compare root systems. Nevertheless, root biomass alone cannot adequately describe many root characteristics involved in plant–soil relationships. Indeed, root morphology traits including total root length, diameter, surface area, and branching have been shown to be relevant for root systems' functions (Hanslin et al. 2019). Root architectural traits vary both among species and within genotypes of crop species and are potential selection criteria in breeding of many crops (Tracy et al. 2019). Overlapping quantitative trait loci (QTLs) of seedling root traits and crop yield suggest that early stage phenotyping should have some predictive value towards mature plants. (Tuberosa et al. 2002; Wasson et al. 2012; Iannucci et al. 2017). Identification of important root morphological characteristics may be useful in the breeding and improvement of species still little explored such as chia. Indeed, despite the economic and dietary importance of this species, little is understood on the root system development at seedling stage.

In dicots root systems consist of a main root and several orders of basal roots with their respective laterals. Even at the seedling stage therefore root systems are a heterogeneous assembly of fine and coarse roots with a wide variety of diameters. The mature root system of dicots can be considered an extension of these structures with increasing axes length and branching order (de Graaff et al. 2013; Zhao et al. 2017). Phenotype expression of root architecture arises from both plant genetics and soil conditions (Correa et al. 2019). Plant roots respond dynamically to soil bio-physico-chemical properties which in turn vary in time and space, therefore they can represent a strong source of variation. However, Moreau et al. (2017) suggest that root traits depend much more on genetics than on plant growth stages, and variability due to soil conditions does not result from major changes in the rules governing primary root emergence, elongation and branching. This makes research on seedling root systems under non-limiting water and nutrient supply a precious tool to identify root traits relevant for plant behavior. Root architecture has high structural complexity, but poor accessibility and visibility. Recently methods based on scanner- image analysis have become available therefore measuring roots is easier and faster (Judd et al. 2015).

The present study aims to characterize the plant growth of chia at early stage of development, to identify the traits that could determine a successful establishment of the crop. The objectives of the present study are therefore to: (1) characterize eight chia genotypes in terms of root and shoot traits; (2) investigate the relationships between these plant traits at early stage of growth and (3) identify the root morphological characteristics that determine genotypic diversification.

Material and methods

Plant materials

Eight chia seed sources, here referred to as genotypes, were used in this study: 3 commercial sources corresponding to populations commonly grown in different environments (Australia, Mexico, Peru), 1 black chia population (Basilicata) obtained from a commercial source (Eichenhain–Hofgeismar–DE)

and grown in Southern Italy (Lat. N 40°51'37,59" Lon. E 15°38'49,43"), and 4 mutants (G3, G8, G17, WS). The materials were kindly provided by the University of Kentucky (U.S.A.) through a memorandum of understanding with the University of Basilicata (Italy).

Plant growth and sampling

Rhizotron experiments were conducted at the Research Centre for Cereal and Industrial Crops (CREA-CI) at Foggia, Italy. Before sowing undamaged seeds were surface sterilized by soaking them in 2% sodium hypochlorite for 5 min, and then rinsed several times with distilled water. The seeds were put into Petri dishes on a sheet of filter paper (Whatman No.1) which was moistened with 5 mL deionized water, and were kept in a dark incubator at constant temperature of 20 °C for 48 h. The experiments were performed using a completely randomized design, with six replicates. For each replication, three germinated seeds (roots, < 5 mm) of a given genotype were transferred in a plastic pot (diameter, 7 cm; height, 26 cm) containing 1.3 kg of a soil mixture (soil: sand, 60:50; v/v). Soil characteristics are reported in Iannucci et al. (2017); then 40 kg/ha NH₄NO₃ (26% elemental nitrogen) was applied. The pots were lined with filter paper (Whatman 3 MM) to avoid soil loss. Immediately after sowing, 200 mL deionized water were added to each pot. The pots were placed in a growth chamber with a 16-h/8-h light/dark period at 20 °C/16 °C, with a light intensity of 1000 μmol photons/m²/s photosynthetically active radiation at the leaf surface. Plants were regularly watered with deionized water at 3-day intervals to 70% of field capacity. After the seedlings produced their first foliar leaf, pots were thinned by leaving the largest seedling only. Two destructive measurements were performed at both the S-6 (six leaves) and S-10 (ten leaves) plant developmental stages (Brandán et al. 2019) for four genotypes: Australia, G3, G8 and Peru. For some genotypes, plants were sampled at only S-6 (Basilicata, and WS) or S-10 (G17, Mexico) because low germinability resulted in a reduction of viable replications.

At each sampling date, the days after sowing and maximum plant height (cm) were recorded and plants were gently pulled out of the pots. Any residual plant material was manually removed from pots. Plants

were cutting just above the root crown and roots and shoots were immediately washed. Roots were stored at 4 °C in ethanol solution (50% v/v) until morphological characterization. After analysis, the above- and below-ground biomass was oven dried for 72 h at 70 °C, and weighed, to obtain the shoot dry weight (g/plant) and root dry weight (g/plant).

Seedling growth measurements

Root samples were placed in a transparent tray (20 cm × 30 cm) with a 4-mm to 5-mm deep layer of water and scanned with a high resolution scanner at 200 dpi (Perfection V700/V750 2.80A; Epson). Root measurements were conducted using WinRhizo image analysis software (version 4.0b; Regent Instruments Inc., Quebec, Canada). The following traits were measured: total length (cm), surface area (cm²), mean diameter (mm), volume (cm³), and number of tips, forks and crossings. All parameters were assessed individually, and roots were then classified into five different diameter classes (0.0–0.5 mm, 0.5–1.0 mm, 1.0–1.5 mm, 1.5–2.0 mm, > 2.0 mm).

We also calculated root to shoot biomass ratio (RSR), total root length density (root length per unit of soil volume; RLD, cm cm⁻³), specific root length (root length per unit of root biomass; SRL, m g⁻¹) and root length to shoot biomass ratio (RLSR, m g⁻¹). For the four genotypes examined in both S-6 and S-10 stages of growth, plant growth indices were calculated according to Hunt et al. (2002): relative growth rate for shoot (RGR_S, g g⁻¹ d⁻¹), relative growth rate for root (RGR_R, g g⁻¹ d⁻¹), relative growth rate for total plant (RGR_P, g g⁻¹ d⁻¹), shoot elongation rate (SER, mm d⁻¹) and root elongation rate (RER, m d⁻¹). Logarithmically transformed root and shoot biomass were used for RGR calculations.

Statistical analysis

For each stage of plant development one-way ANOVA was performed to test the significance of differences in all traits among the chia genotypes. Multiple comparison of means was carried out using the Fisher's protected least-significant difference (LSD) at $P < 0.05$. Correlation analysis between all trait pairs was calculated by using Pearson's correlation coefficients. Multivariate analysis with principal component analysis (PCA) based on correlations, was

applied to the whole dataset. Prior to calculations all data were standardized by Z-score transformation to a mean of zero and variance of one to overcome differences in size during recording (Sneath and Sokal 1973). Components representing original variables (traits) were also extracted, and only those with eigenvalues ≥ 1.0 were considered as representing a major contribution to the total variation (Hossain et al. 2016). The first and second principal component axes scores were plotted to aid visualization of differences between genotypes. Genotype scores (eigenvectors) of the first four principal components, which explained 98% of total variation, were used in cluster analysis, to measure hierarchical similarity among genotypes. From these values a Euclidean distance matrix was established (Flores et al. 1997) so as to obtain a relative dendrogram. Entries were clustered using Ward's minimum-variance method. Grouping of genotypes was performed through automatic truncation available as part of the software. Results of this clustering were combined with results of the PCA analysis as a visual aid in discerning clusters and a pattern (color) map was constructed to represent the variations in root morphology. Redundancy of correlated variables was reduced by excluding from calculations for PCA and cluster analyses number of forks and crossings, number of tips in 0.0–0.5 diameter class and RLD, because they were strongly correlated ($r > 0.98^{**}$) with at least three other variables.

Correlation analysis and ANOVA were performed with the STATISTICA software (StatSoft version 7.1; StatSoft, Inc., Tulsa, OK, USA), while multivariate analysis was performed with JMP (version 10.0; SAS Institute Inc., Cary, NC, USA).

Results

Plant height, shoot dry weight and all root morphological traits were significantly influenced by the genotype (Tables 1, 2, 3). Treatment S-6 always showed greater phenotypic variation than S-10 (CV = 40% and 22% on average, respectively). A high variability was recorded for root biomass, root volume, number of forks and crossings (CV = 54, 40, 44 and 48% on average, respectively). Genotype G3 had the highest plants (21 cm, on average) but low shoot and root biomass whereas the highest root dry weight and total plant biomass were found for

Table 1 Means and mean separation results for shoot and root traits of 4 genotypes of chia harvested at two developmental stages of growth

Genotype	Plant height (cm)		Shoot dry weight (g)		Root dry weight (g)		Total dry weight (g)	
	S-6 ^a	S-10	S-6	S-10	S-6	S-10	S-6	S-10
Australia	16.3b	18.6b	0.180ab	0.486a	0.067a	0.161ab	0.247a	0.647a
Basilicata	15.8b	–	0.143a–c	–	0.045ab	–	0.188ab	–
G3	19.9a	21.9a	0.134bc	0.437b	0.029b	0.096b	0.163b	0.533b
G8	17.7ab	18.8b	0.188a	0.481ab	0.063ab	0.118ab	0.251a	0.599ab
G17	–	19.9ab	–	0.467ab	–	0.107b	–	0.574ab
Mexico	–	18.5b	–	0.489a	–	0.137ab	–	0.626ab
Peru	16.0b	19.4ab	0.162a–c	0.466ab	0.051ab	0.183a	0.213ab	0.650a
WS	17.4b	–	0.122c	–	0.038ab	–	0.159b	–
Mean	17.1	19.5	0.149	0.471	0.051	0.134	0.212	0.605
CV (%)	13.4	13.2	29.1	12.8	58.3	49.5	32.2	17.0
LSD ($P \leq 0.05$)	2.3	2.9	0.051	0.046	0.034	0.074	0.073	0.098

Values with different letters within each column are significantly different at the Fisher's protected least-significant difference (LSD) ($P < 0.05$). NS, not significant

^aS-6, 6th leaf developmental stage; S-10, 10th leaf developmental stage

Table 2 Means and mean separation results for root length, surface area, average diameter and root volume of 4 genotypes of chia harvested at two developmental stages of growth

Genotype	Root length (m)		Surface area (cm ²)		Average diameter (mm)		Root volume (cm ³)	
	S-6 ^a	S-10	S-6	S-10	S-6	S-10	S-6	S-10
Australia	18.7a	33.5a	159.7a	393.6a	0.27ab	0.37a	1.09a	3.70a
Basilicata	13.5b	–	105.4b	–	0.25ab	–	0.66b	–
G3	10.1b	25.0c	82.3b	271.6c	0.26ab	0.34b	0.53b	2.36b
G8	13.7b	28.5bc	105.3b	313.1bc	0.28a	0.35ab	0.75ab	2.75b
G17	–	25.4c	–	261.6c	–	0.32b	–	2.15b
Mexico	–	32.7ab	–	340.6ab	–	0.33b	–	2.84b
Peru	13.0b	30.7ab	100.5b	324.7bc	0.24b	0.34b	0.62b	2.78b
WS	11.3b	–	93.9b	–	0.26ab	–	0.62b	–
Mean	13.7	29.3	110.6	317.5	0.26	0.34	0.73	2.76
CV (%)	33.8	16.5	42.8	21.4	11.5	9.1	50.7	28.2
LSD ($P \leq 0.05$)	4.8	4.4	51.2	64.9	0.03	0.02	0.41	0.77

Values with different letters within each column are significantly different at the Fisher's protected least-significant difference (LSD) ($P < 0.05$). NS, not significant

^aS-6, 6th leaf developmental stage; S-10, 10th leaf developmental stage

Australia (S-6) and Peru (S-10) (Table 1). Although plants were harvested at the same stage of development and were kept under the same conditions, there was considerable variance in their root systems in

terms of total length (range 10.1–18.7 m and 25.0–33.5 m for S-6 and S-10, respectively) (Table 2). Root length was highest for Australia, although at S-10 values were not significantly different from those of

Table 3 Means and mean separation results for number of tips, forks and crossings of 4 genotypes of chia harvested at two developmental stages of growth

Genotype	Tips (no.)		Forks (no.)		Crossings (no.)	
	S-6 ^a	S-10	S-6	S-10	S-6	S-10
Australia	4550.5a	6376.2a	9428.2a	25,084.5a	4436.2a	12,188.0a
Basilicata	3297.2ab	–	5416.7b	–	2492.5b	–
G3	2142.8b	4857.0bc	3459.8b	15,082.8 cd	1587.5b	6665.0c
G8	3013.7b	5734.7ab	5437.3b	18,186.7b–d	2470.0b	8190.7c
G17	–	4538.7c	–	14,331.8d	–	6819.3c
Mexico	–	5905.8ab	–	22,500.3ab	–	11,575.8ab
Peru	3198.0ab	5603.5ab	5250.2b	19,468.5bc	2461.3b	9165.3bc
WS	2687.3b	–	4084.3b	–	1758.7b	–
Mean	3240.4	5502.6	5798.4	19,109.1	2689.5	9100.7
CV (%)	43.7	19.0	60.8	27.7	63.0	32.4
LSD ($P \leq 0.05$)	1533.7	1055.3	3756.1	4590.8	1794.1	2534.4

Values with different letters within each column are significantly different at the Fisher's protected least-significant difference (LSD) ($P < 0.05$). NS, not significant

^aS-6, 6th leaf developmental stage; S-10, 10th leaf developmental stage

Mexico and Peru. Average root diameter values showed a low variability in both S-6 and S-10 (CV = 10%, on average), and Australia had the significantly thickest roots under S-10 (0.37 mm), although not significantly different from that of G8. Australia showed also greater root area surface, root volume and more tips, forks and crossings (Table 3), although differences were not always significant. In order to better understand how specific root structures differed among genotypes, we measured the relative abundance of roots (on a root length basis) in three distinct diameter size classes: 0.0–0.5 mm, 0.5–1.0 mm and > 1.0 mm. Roots < 1.0 mm represent about 94% of total length (Supplementary Table 1). Length of each diameter class was expressed as a percentage of total length. The fraction of root length relative to total length in the > 1.0 mm size class differed significantly among genotypes: Australia and G8 showed the highest percentage in S-6 (8.6 and 10.6%, respectively) and Australia scored highest in S-10 (4.8%). Conversely, in the 0–0.5 mm diameter size class, relative root lengths ranged from 59% (G8) to 68% (Peru) in S-6 and from 79% (Australia) to 85% (G17) under S-10.

Root growth indexes help understand the partitioning of photosynthates and to define the extent to which plants explore soil (Table 4). The RSR is a measure of

the allocation of resources between roots and shoots. The RSR of plants at S-6 was higher than at S-10 and significant differences were detected among chia genotypes with G3 showing the lowest values although at S-10 the difference was significant with Peru only. Regarding structural root parameters, root length density (RLD) was highest for Australia although at S-10 differences with Mexico and Peru were not significant. Specific root length was higher in S-6 than in S-10 (+ 32%, on average), and this indicates a finer root system at earlier stages. Specifically, G3 showed a trend towards a high SRL (i.e., thin root systems) but differences were significant only with Australia (i.e., coarser root systems) at S-10. RLSR decreased from S-6 to S-10 (of 29%, on average) and showed significantly higher values in Australia, than in G3 and G8.

Growth indexes also describe time dynamics of plant size. RGR indicates a rate of change in size, i.e. an increment in plant weight per unit plant weight in time. The G8 genotype showed significantly highest RGR values for whole-plant and shoot growth, and higher than Australia and G3 for roots (Table 5). SER was lowest for Australia and RER for Australia and G3.

Correlation analysis shows that there are strong correlations for 68% of the traits, ($r \geq 0.66$, $P < 0.01$,

Table 4 Means and mean separation results for shoot and root traits of 4 genotypes of chia harvested at two developmental stages of growth

Genotype	RSR ^a		RLD (cm cm ⁻³)		SRL (m g ⁻¹)		RLSR (m g ⁻¹)	
	S-6 ^b	S-10	S-6	S-10	S-6	S-10	S-6	S-10
Australia	0.36a	0.33ab	1.87a	3.35a	316.1ab	213.8c	107.3a	69.5a
Basilicata	0.31a	–	1.35b	–	316.1ab	–	94.7ab	–
G3	0.22b	0.22b	1.01b	2.50c	337.6a	262.2a	74.4b	57.1c
G8	0.33a	0.25ab	1.37b	2.85bc	272.3b	243.0a–c	73.1b	59.9bc
G17	–	0.23b	–	2.54c	–	248.2a–c	–	54.8c
Mexico	–	0.28ab	–	3.27ab	–	243.6a–c	–	66.6ab
Peru	0.33a	0.39a	1.29b	3.07ab	326.2ab	219.6bc	87.7ab	66.4ab
WS	0.30a	–	1.13b	–	307.9ab	–	92.4ab	–
Mean	0.31	0.28	1.38	2.93	313.7	238.4	87.4	62.4
CV (%)	45.2	47.7	33.8	16.5	28.3	18.5	27.0	13.9
LSD ($P \leq 0.05$)	0.08	0.15	0.48	0.44	62.8	33.2	25.9	8.5

Values with different letters within each column are significantly different at the Fisher's protected least-significant difference (LSD) ($P < 0.05$). NS, not significant

^aRSR, ratio of root DW to shoot DW; RLD, total root length density; SRL, specific root length; RLSR, ratio of root length to shoot DW

^bS-6, 6th leaf developmental stage; S-10, 10th leaf developmental stage

Table 5 Means and mean separation results for five growth indexes in 4 genotypes of chia harvested at two developmental stages of growth (6th leaf and 10th leaf)

Genotype	RGR _S (g g ⁻¹ d ⁻¹) ^a	RGR _R (g g ⁻¹ d ⁻¹)	RGR _P (g g ⁻¹ d ⁻¹)	SER (mm d ⁻¹)	RER (m d ⁻¹)
Australia	0.16c	0.15c	0.15c	0.03b	2.33b
G3	0.28c	0.26c	0.28c	0.09a	3.35b
G8	0.98a	0.87a	0.94a	0.09a	6.48a
Peru	0.56b	0.57ab	0.56b	0.10a	6.57a
Mean	0.50	0.46	0.48	0.08	4.68
CV (%)	68.6	96.4	70.2	59.7	46.5
LSD ($P \leq 0.05$)	0.13	0.44	0.18	0.05	1.37

Values with different letters within each column are significantly different at the Fisher's protected least-significant difference (LSD) ($P < 0.05$). NS, not significant

^aRGR_S, relative growth rate for shoot; RGR_R, relative growth rate for root; RGR_P, relative growth rate for total plant; SER, shoot elongation rate; RER, root elongation rate

$n = 12$), while moderate correlations for 11% of the traits ($r \geq 0.51$, $P < 0.05$, $n = 12$) (Fig. 1). Differences in shoot and root dry weight were positively associated with corresponding differences in all morphological traits measured. Root surface area and diameter correlated positively with all of the morphometric parameters examined; root length was significantly correlated with all other parameters with the

exception of RSR. However, significant negative correlations were found between PH and RSR and RLSR. Finally, SRL was negatively correlated with most of the morphometric parameters examined, and positively correlated with RLSR.

The PCA was performed for each harvest time and four of the principal components had eigenvalues > 1 and accounted for more than 97% of the total variance

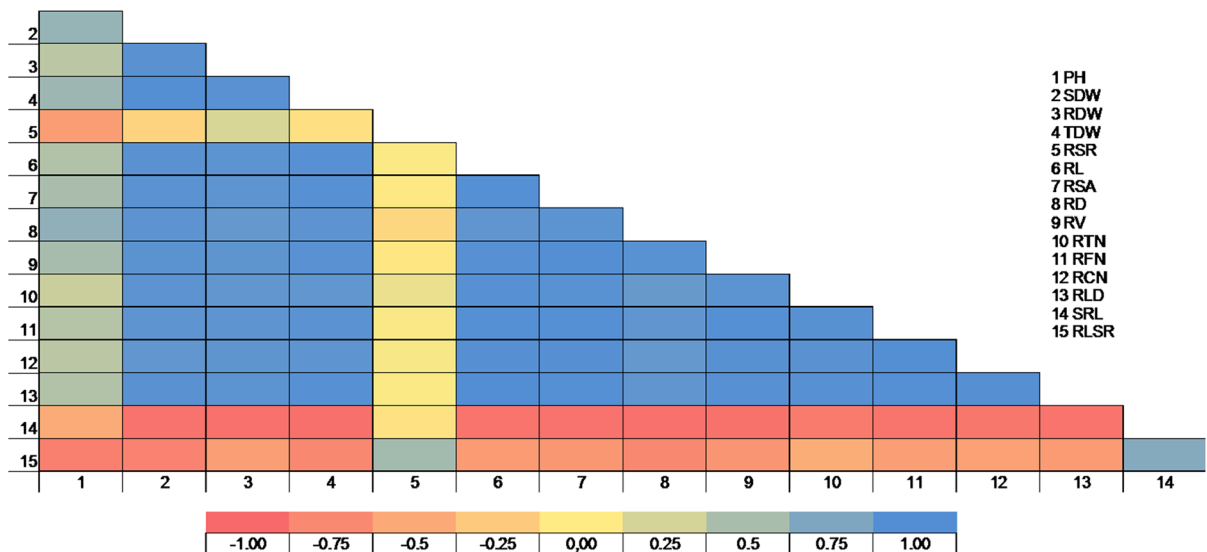


Fig. 1 Heat map of Pearson's correlation coefficients between plant traits. Plant height (PH, cm); shoot dry weight (SDW, mg plant⁻¹); root dry weight (RDW, mg plant⁻¹); total dry weight (TDW, mg plant⁻¹); ratio of root DW to shoot DW (RSR); root length (RL, cm), root surface area (RSA, cm²); root diameter (RD, mm); root volume (RV, cm³); root tip number (RTN); root

fork number (RFN); root crossing number (RCN); root length density (RLD, cm cm⁻³); specific root length (SRL, m mg⁻¹); ratio of root length to shoot DW (RLSR). Correlation coefficients were classified as weak (NS; $r < 0.50$, $n = 12$), moderate ($r \geq 0.51$, $P \leq 0.05$, $n = 12$) and strong ($r \geq 0.66$, $P \leq 0.01$, $n = 12$)

(Supplementary Table 2). At S-6, according to the loadings, PC1 was positively correlated with root length, surface, volume; PC2 was positively related to average diameter; number of tips in the diameter class 1.0–1.5 mm contributed mainly to PC3 and SRL was positively correlated to PC4. For S-10 harvest surface area, volume and both these traits in the diameter class 0.5–1.0 mm contributed to PC1; average diameter and root dry weight were the main traits in PC2; number of tips in the diameter class 1.0–1.5 mm and 1.5–2.0 mm contributed to the PC3 while the number of tips in diameter class > 2.0 contributed to PC4. Figure 2a, b reports the observation scores of the first two principal components for S-6 and S-10 separately, which accounted for more than 84% of variability and led to the differentiation of the chia genotypes. Genotypes appear clearly separated especially in the PC1 direction. Both plots showed that G8 and Australia are different from each other and differ from all other genotypes under study.

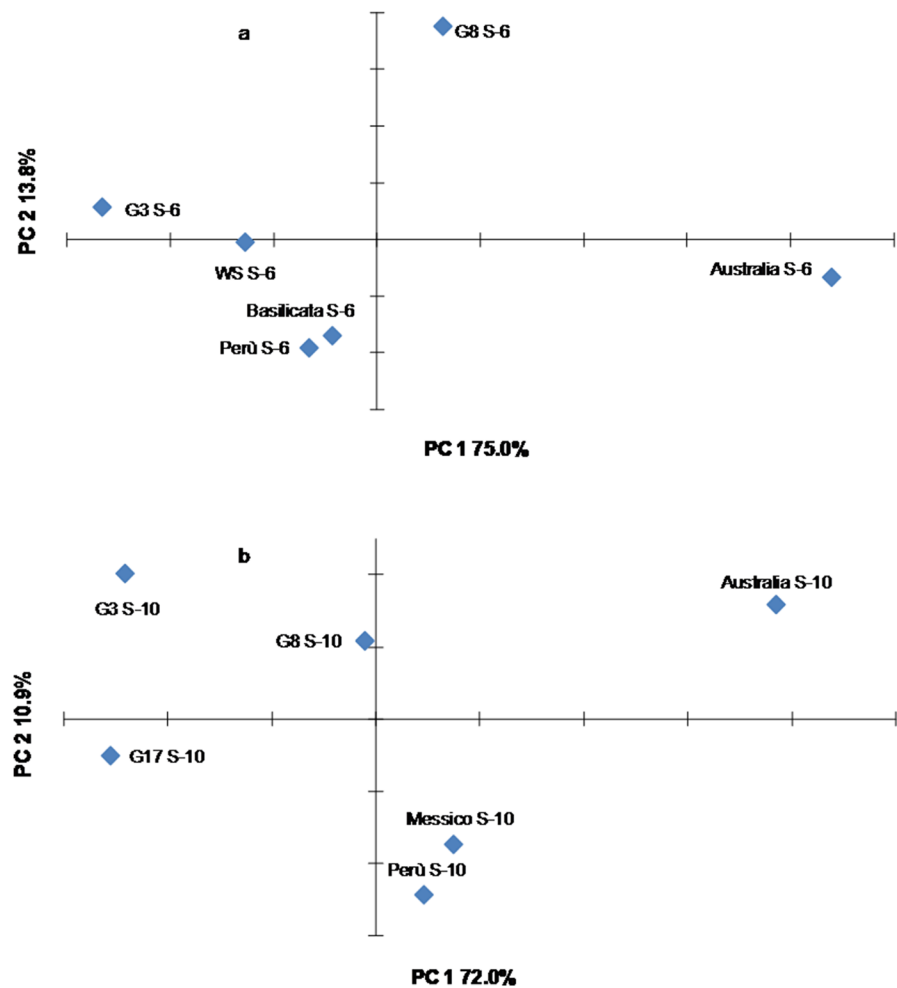
A dendrogram was constructed to visualize the relationships for shoot and root traits between the eight chia genotypes under two harvest times (S-6 and S-10) (Fig. 3). The pattern map of the values of all traits included in the figure helps indicate if a given

genotype is superior for one trait or is comparable to the others. The dendrogram divided the genotypes into 3 major groups and separated genotypes with more developed roots and a larger root diameter from those with shorter and thinner roots. Cluster 1 included Australia genotype with extensive roots characterized by greater average diameter, shoot and root biomass, root length, surface area and volume. Cluster 2 included four genotypes (Basilicata, G8, Mexico, Peru) with high values for both the dry matter accumulation (shoot and root), RSR and well-developed root system. Cluster 3 included the genotypes G3, G17 and WS with taller plants and very low values for all morphological traits and root density.

Discussion

Root growth strategies of seedlings are important for their survival and establishment, but the early development of leaves and roots is not sufficiently known in many crop plants, and especially so in *S. hispanica*. In this study, we supply new information on root traits related to seedling growth in a set of chia genotypes to identify the most significant characteristics for the

Fig. 2 Principal component analysis for plant and root morphological traits of 8 chia genotypes harvested at a) the sixth and b) tenth leaf development stage, respectively



development of vigorous seedlings which determine a successful establishment of the crop. These traits include morphology, architecture and biomass allocation patterns. The chia genotypes evaluated in this research exhibited genotypic variability for shoot and root dry weight and the extremes of the biomass accumulation range were Australia at the high-end of the range and G3 at the low-end. Measurements of root biomass and length are typically used to describe root extension in the soil profile, but root morphology and architecture provide information on the spatial configuration of root systems (Mašková and Weiser 2019). We found genetic variation for total root length, surface and volume. Similarly research on other crops at the seedling stage has shown genetic differences: variation in root architecture among and within temperate small grain cereals grown in controlled environment and in the field was reported by

Richards et al. (2007), who found an association between early root vigour measured in containers studies and deeper root systems in the field. As reported by Comas et al. (2013), the root system of herbaceous plants include coarse and fine roots: coarse roots have functions of anchorage and establish overall root system architecture, while fine roots are the most active portion in water and nutrients uptake. A deep, thick root system with large volume and surface area, such as that of Australia and to a lesser extent G8 in our data, should provide potential for colonizing a greater soil volume and allowing plants to extract soil moisture and nutrients in deep soil layers (Moreau et al. 2017). In our study, Peru showed higher root biomass and lower average diameter values particularly at the S-6 stage. This is confirmed by examination of the relative abundance of roots by diameter size classes. This feature allows to envisage a

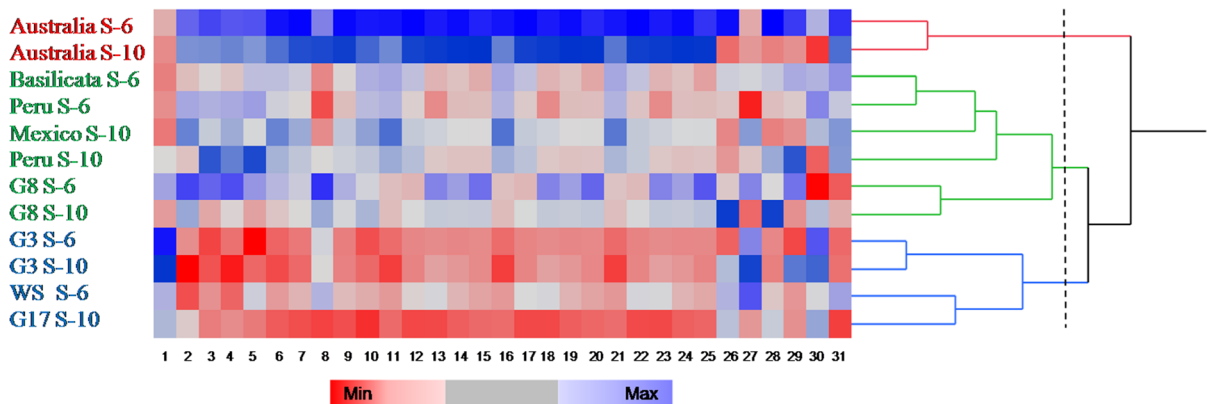


Fig. 3 Hierarchical cluster analysis dendrogram (Ward's method) and pattern map of the values of plant and root morphological traits of 8 chia genotypes. Legend: 1, plant height (cm); 2, shoot dry weight (g plant^{-1}); 3, root dry weight (g plant^{-1}); 4, total plant dry weight (g); 5, root/shoot ratio; 6, root length (RL, cm); 7, root surface area (RSA, cm^2); 8, root diameter (mm); 9, root volume (RV, cm^3); 10, root tip number (RTN, no.); 11, $0.0 < \text{RL} \leq 0.5$ (cm); 12, $0.5 < \text{RL} \leq 1.0$ (cm); 13, $1.0 < \text{RL} \leq 1.5$ (cm); 14, $1.5 < \text{RL} \leq 2.0$ (cm); 15,

RL > 2.0 (cm); 16, $0.0 < \text{RSA} \leq 0.5$ (cm^2); 17, $0.5 < \text{RSA} \leq 1.0$ (cm^2); 18, $1.0 < \text{RSA} \leq 1.5$ (cm^2); 19, $1.5 < \text{RSA} \leq 2.0$ (cm^2); 20, $\text{RSA} > 2.0$ (cm^2); 21, $0.0 < \text{RV} \leq 0.5$ (cm^3); 22, $0.5 < \text{RV} \leq 1.0$ (cm^3); 23, $1.0 < \text{RV} \leq 1.5$ (cm^3); 24, $1.5 < \text{RV} \leq 2.0$ (cm^3); 25, $\text{RV} > 2.0$ (cm^3); 26, $0.50 < \text{RTN} \leq 1.0$ (no.); 27, $1.0 < \text{RTN} \leq 1.5$ (no.); 28, $1.50 < \text{RTN} \leq 2.0$ (no.); 29, $\text{RTN} > 2.01$ (no.); 30, specific root length (m g^{-1}); 31, root length shoot dry weight $^{-1}$ (m g^{-1})

different role in soil-root interactions since fine roots play a primary role in exudation and ability to be colonized by soil microorganisms (Wasson et al. 2012; McCormack et al. 2015), rather than performing structural and transport functions. Branching and the number of root tips, the most active zones of growth, are crucial for the uptake of mobile resources (Canales et al. 2019). In our study tips were measured as the number of terminal portions of lateral and axial roots, while forks indicate the degree of lateral root branching. We found the highest and lowest values for both these traits in Australia and G3, respectively, indicating a very different potential for root uptake in the early stages of development.

In this study we found differences between genotypes in indices describing allometric relationships in chia. Root to shoot ratio decreased over time in all genotypes and was always < 1 indicating that this crop, under non-limiting water supply, invests most of its assimilates in aboveground biomass. Similar results are reported for other annual species (Thivierge et al. 2016). Mašková and Herben (2018) stated that RSR is an approximation of the competitive ability of the seedlings as faster development of shoots provides an advantage to their survival. Among chia genotypes, Peru showed the highest RSR and this could adversely affect the crop's ability to compete with weeds during

establishment. Conversely, as it represents a higher investment in the roots, it may provide an advantage in environments in which below-ground competition prevails.

Specific root length is an indirect measurement of root thickness as it depends on root diameter and/or tissue density. According to the analysis of Fitter (2002), our SRL values of root systems of young chia plants are relatively high and decrease through time as thickening of roots occur. The differences in SRL we found between genotypes correspond to a wide range of strategies linked to root architecture at early developmental stages, since investment in thicker or finer structures may lead to different levels of efficiency in soil volume exploration or nutrient acquisition (Comas et al. 2013). Higher SRL values (thinner roots), as we found in G3, indicates lower biomass-cost roots as confirmed by lower RSR values. Hernández et al. (2010) found that herbaceous plants adapted to dry conditions have smaller diameter (fine) roots with greater SRL, and this corresponds to high uptake rates of water and of relatively immobile nutrients. As plants grew SRL decreased whereas RLD increased. Root length density is an index of the occupation of space also related to water and nutrient uptake (Gregory 2006). In this study higher values of RLD and RLSR were recorded for Australia.

For four genotypes, we examined differences in root and shoot allocation and their change over time by means of growth indices. A high RGR allows the plant to rapidly increase in size and to occupy a larger space, both below and above ground, giving an advantage for the acquisition of resources. In our study, Australia showed lower values for all growth and elongation indices we calculated. Speed of root growth and elongation is crucial for initial establishments of plant species; however, plants cannot rely solely on these characteristics. The increase in root surface area, volume and branching may enable plants to acquire the necessary water and nutrients for survival and growth as well as successfully compete with neighboring plant species (Hanslin et al. 2019). The G8 genotype showed high values for root growth indices and a high average diameter; this suggests that G8 could produce roots of relatively large diameter with a high elongation rate.

The correlation analysis calculated over genotypes and stages of development showed that root length, was positively correlated with all morphological variables. Root length may be seen as the plant potential to explore below-ground space and values of length of the order of tens of meters indicate that chia seedlings at early stages of growth are characterized by an extensive root system and this can allow accessing a large soil volume. SRL was negatively correlated with all other root traits with the exception of RLSR. Wendling et al. (2016) found that root biomass and root density traits were highly negatively correlated to SRL in adult plants of chia. Furthermore, shoot biomass was positively related to root mass, root area and RLD.

Differences in root morphological traits of chia plants were further confirmed by PCA and cluster analyses which allow a clear visualization of genotypic variation and separation of genotypes with different rooting capacities during early seedling growth. Root traits associated with low diameter classes contributed little in the four components, which means that they were not good indicators for differences in performance during early development. Clustering identifies genotypes based on root morphology and seedling growth. According to Dunbabin et al. (2013) a good knowledge of the differences in root traits allows to hypothesize differences among genotypes in their ability to take up nutrients, also depending on environmental changes. Grouping of

genotypes by multivariate methods clearly separates Australia (with high root and shoot biomass, root length, surface area, volume, more tips, forks and crossings, thicker roots, high RLD, RLSR, and lower values for all growth and elongation indexes), G8 (with high root length thicker roots, higher values for RGR, SER and RER, lower SRL), from the other genotypes (with low biomass values, shorter and thinner roots, low root surface area, volume, tips, forks, RLD and RLSR). Moreau et al (2017) suggest that root traits depend much more on genetics than on plant growth stages and environment variability (i.e. soil-nitrogen availability or other factors). Thus, variability identified in this research for root traits could provide opportunities for selection and breeding as reported for other crops (Lilley and Kirkegaard 2011; Szoboszlay et al. 2015; Iannucci et al. 2017).

Conclusions

The analysis of root traits of 8 chia genotypes showed significant differences in architecture, relationship between shoot and root development and growth dynamics at the seedling stage. This suggests differences in chia genotypes' ability to occupy space, and to access or acquire soil-resources. Early root vigour and large root system are important traits that allow roots to explore soil and reach deep layers or exploit resources. These traits facilitate a successful plant establishment that is an essential requisite for crop production. According to our results two genotypes, Australia and G8, show massive root systems, and a higher proportion of thick, long roots. They could be good competitors in terms of ability to access water and nutrients in an extended soil volume and from the lower soil layers. These characteristics are considered beneficial for plant growth in a low nutrient soil and in dryland agricultural systems. Conversely, G3 exhibits a relatively low biomass and shows a higher partitioning to above-ground parts and to fine roots than other genotypes. This corresponds to a strategy of above-ground competition and to the high acquisition potential of water and poorly mobile nutrients in the top soil layers. However, such thin fine roots may not be able to penetrate compact soils. Given the importance of early root traits for plant establishment and competition, and for the behavior of plants at later stages, this study contributes to the knowledge of some

important aspects related to growth during early stages of development in chia, and the variability between chia genotypes may provides criteria for selection and breeding. Further research is needed to determine the relationship between seedling performance and successful establishment under specific environments and under the pressure of inter-and intra-specific competition.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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