



Root diversity in sesame (*Sesamum indicum* L.): insights into the morphological, anatomical and gene expression profiles

Ruqi Su^{1,2} · Rong Zhou¹ · Marie Ali Mmadi¹ · Donghua Li¹ · Lu Qin¹ · Aili Liu¹ · Jianqiang Wang¹ · Yuan Gao¹ · Mengyuan Wei¹ · Lisong Shi¹ · Ziming Wu² · Jun You¹ · Xiurong Zhang¹ · Komivi Dossa¹

Received: 21 May 2019 / Accepted: 15 July 2019 / Published online: 18 July 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Main conclusion Sesame harbors a large diversity in root morphological and anatomical traits and a high root biomass improves the plant aboveground biomass as well as the seed yield.

Sesame provides one of the most nutritious and healthy vegetable oils, sparking an increasing demand of its seeds. However, with the low yield and productivity of sesame, there is still a huge gap between the seed demand and supply. Improving the root system has a high potential to increase crop productivity, but information on the diversity of the sesame root systems is still lacking. In this study, 40 diverse sesame varieties were grown in soil and hydroponics systems and the diversity of the root system was investigated. The results showed that sesame holds a large root morphological and anatomical diversity, which can be harnessed in breeding programmes. Based on the clustering of the genotypes in hydroponics and soil culture systems, we found that similar genotypes were commonly clustered either in the small-root or in the big-root group, indicating that the hydroponics system can be employed for a large-scale root phenotyping. Our results further revealed that the root biomass positively contributes to increased seed yield in sesame, based on multi-environmental trials. By comparing the root transcriptome of two contrasting genotypes, 2897 differentially expressed genes were detected and they were enriched in phenylpropanoid biosynthesis, starch and sucrose metabolism, stilbenoid, diarylheptanoid and gingerol biosynthesis, flavonoid biosynthesis, suggesting that these pathways are crucial for sesame root growth and development. Overall, this study sheds light on the diversity of sesame root system and offers the basis for improving root traits and increasing sesame seed yield.

Keywords Candidate genes · Phenotyping · Root morphology · Sesame · Yield improvement

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00425-019-03242-y>) contains supplementary material, which is available to authorized users.

✉ Xiurong Zhang
zhangxr@oilcrops.cn

✉ Komivi Dossa
komiri.dossa@ucad.edu.sn

¹ Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, No. 2 Xudong 2nd Road, Wuhan 430062, China

² Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education/College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, China

Abbreviations

SDW	Shoot dry weight (g)
RDW	Root dry weight (g)
RSR	Root-shoot ratio (–)
RL	Root length (cm)
SA	Root surface area (cm ²)
RN	Root number (–)
RV	Root volume (cm ³)
MRL	Main root length (cm)
MRD	Main root diameter (cm)

Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oil crops and is known as the “queen of oil seeds” because of its high oil content and quality (Yang and Huang 2009; Gebregergis et al. 2018). Sesame is an indispensable material for many

traditional foods in many Asian countries such as China, Japan and Korea with extremely high nutritional value and a wide range of usages (Xu et al. 2018a, b). Furthermore, it has an important application value in medicine (Gharby et al. 2017). For example, sesame oil has been reported to possess anti-inflammatory and immunomodulatory functions (Hsu et al. 2005) and its lignan sesamin/sesamol has anti-hypertensive and neuroprotective effects (Cheng et al. 2006; Nakano et al. 2010). In addition, sesame oil can also be used in other industrial sectors to make a synergist for pyrethrum insecticides, and cosmetics (Anilakumar et al. 2010).

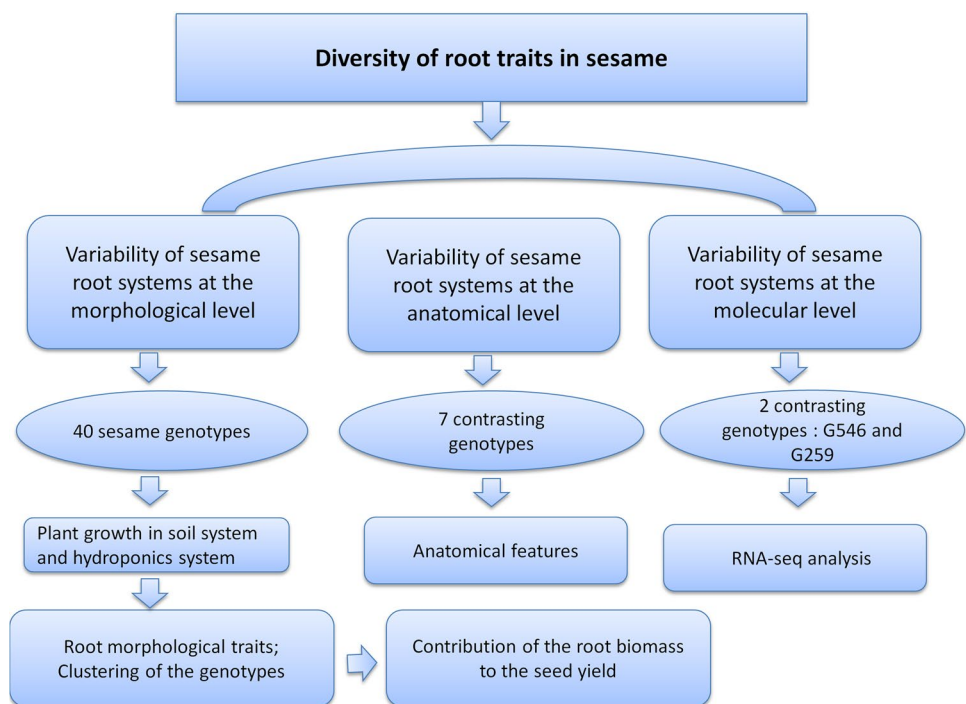
Sesame production is mainly concentrated in Asia and Africa and the annual demand of sesame seeds is experiencing a steep increase (Dossa et al. 2017). Although the global sesame planting area is extending particularly in Africa, the productivity and yield are still very low, resulting in a huge gap between seed demand and supply (Sarkar et al. 2016). In fact, sesame is cultivated in harsh environments and its growth and development are greatly affected by the adverse conditions (Witcombe et al. 2008). In addition to its own genetic factors, abiotic or biotic stresses such as diseases, insects, drought, waterlogging are important factors leading to the low yield of sesame (Li et al. 2012; Dossa et al. 2019).

Root morphology and physiological characteristics are closely related to yield. Svacina et al. (2014) demonstrated that varieties with a larger root system showed a higher yield and better grain quality in barley. Similarly, Wang et al. (2015) found that the number of roots was closely related to the yield of maize. Studies on root traits are hampered

by their complexity and the inaccessibility of the rhizosphere (Kuijken et al. 2015). However, with the development of root phenotyping platforms and root data analysis tools, our understanding of the root functions is increasing and the prospects for harnessing the potential of root systems to boost and stabilize crop yields are looming (Paez-Garcia et al. 2015). Liu et al. (2018) showed that the representative super hybrid rice cultivars had dense root and higher grain yield. In peanut, root dry weight, root volume, total root absorption area and active root uptake correlated with strong drought resistance (Li et al. 2014).

So far, there has been no specific performance in the sesame root breeding programmes although the root system is crucial for the plant functioning (Dossa et al. 2017). There are no data about the natural variation of sesame root traits and more importantly how the root traits affect the seed yield in sesame is unknown. To fill this gap, we designed this study and examined the morphological and anatomical variability of root traits in 40 sesame genotypes collected from different countries. We compared soil and nutrient solution plant culture systems to recommend an efficient approach to study sesame root traits at the large scale. In addition, we evaluated the contribution of root traits to seed yield in various environments. Also, we performed gene expression profiling in two contrasting sesame genotypes for root traits with the aim to reveal the candidate genes and biological pathways underlying the differential root phenotypes (Fig. 1). The goal of this work is to provide a comprehensive insight into the variability of sesame root systems at the morphological, anatomical and molecular levels for

Fig. 1 Overview of the study



harnessing the potential of root traits in sesame breeding programmes.

Materials and methods

Plant materials

A total of 40 sesame (*Sesamum indicum* L.) genotypes were obtained from the Sesame Germplasm Resource Preserving Center, of the Oil Crops Research Institute-Chinese Academy of Agricultural Sciences (Wuhan, China). The genotypes originated in Asia, Africa and America (Table 1).

Plant growth in soil system

The trial was conducted in the Wuchang experimental station of Oil Crops Research Institute-Chinese Academy of Agricultural Sciences. Seeds were disinfected with absolute ethanol for 5 min and washed with 70% alcohol twice. Sterile seeds were grown in pots (25 cm diameter and 45 cm depth) filled with 6 kg of experimental soil composed of 1.5 kg vermiculite, 2 kg soil, 2 kg sand and 0.5 kg nutritive soil, and were maintained under shelter to avoid rainy days interfering. All plants were grown in a growth chamber under 16 h/8 h (day/night), 28 °C/25 °C (day/night), at a PPFD of 200 μmol m⁻² s⁻¹ and 60% relative humidity and were watered every 2 days to keep good growth conditions.

The experiment was carried out with five replications and two plants per pot, resulting in a total of 400 seedlings. The root of 45-day-old plants was delicately separated from the shoot and thoroughly washed and both samples were put into paper bags for later use.

Plant growth in hydroponic system

Round and uniform sesame seeds were sowed on gauze that was fixed to a plant germination tray (25 × 19 × 7 cm, length × width × height) filled with a quarter-strength modified Hoagland’s solution (Hoagland and Arnon 1950). The modified Hoagland’s solution consisted of: 5 mM Ca(NO₃)₂·4H₂O, 6 mM KNO₃, 2 mM NH₄NO₃, 2 mM MgSO₄·7H₂O, 1 mM KH₂PO₄, 0.5 mM K₂SO₄, 0.16 mM EDTA-Fe, 40 μM H₃BO₃, 9 μM MnCl₂·4H₂O, 0.6 μM ZnSO₄·7H₂O, 0.32 μM CuSO₄·5H₂O and 0.32 μM (NH₄)₆Mo₇O₂₄·4H₂O. The pH value was adjusted to 5.8 with NaOH or HCl using a portable PH meter. Seven days after sowing, uniform seedlings were selected and transplanted into blue plastic basins (34 × 26 × 12 cm, length × width × height) containing 160 seedlings of 40 genotypes (four replicated seedlings for each genotype). The experiment was performed under controlled environmental conditions with the temperature and relative humidity kept at

Table 1 Genotypes of *Sesamum indicum* collected from different countries used for root diversity analysis

No	Genotype name	Sample ID	Original producing area
1	Heizhima	G073	China
2	Baizhima	G098	China
3	Zhima1	G121	China
4	Mazhima	G135	China
5	Zichangma	G142	China
6	Haihuiheizhima	G143	China
7	Bachazhima	G153	China
8	Baizhima	G160	China
9	Heizhima	G164	China
10	Zhima	G221	China
11	Fazhima	G222	China
12	Zhima	G256	China
13	Baizhima	G259	China
14	Batongzhima	G263	China
15	Heizhima	G290	China
16	Dengmingsizhima	G301	China
17	Baizhima	G340	China
18	Baizhima	G347	China
19	Heizhima	G348	China
20	Chama	G349	China
21	Baizhima	G370	China
22	Mazhima	G378	China
23	Zhima	G388	China
24	98n09	G405	China
25	384(7)	G454	Japan
26	Ty-13	G463	India
27	91-2299	G507	Israel
28	912305	G523	Israel
29	Ec-355666	G532	India
30	697	G546	Nepal
31	Zhima1	G557	Vietnam
32	Pi 229668 02	G581	Argentina
33	Baizhima	G608	China
34	Mishuozhima	G614	China
35	L161	G626	Egypt
36	Zhongzhi12	G633	China
37	Yuzhi1hao	G667	China
38	Zhengzhi12hao	G674	China
39	Xiongzhi1hao	G691	China
40	Liaozhi2hao	G698	China

35 °C and 60%, respectively, and under long-day conditions (16 h day/8 h night). A randomized complete block design was used with three replications, resulting in a total of 480 seedlings. After the first week of germination, the seedlings were transferred into a half-strength Hoagland’s nutrient solution for three additional weeks. The nutrient solution

was renewed once a week. One-month-old plants were harvested and the root was delicately separated from the shoot and both samples were put into paper bags for later use.

Root image scanning

At harvest, root samples were scanned using a desktop scanner (EPSON Perfection V800 Photo). Root images were analyzed using an image analysis system (WinRHIZO Pro software). Four root data including the total root length (RL, cm), root surface area (SA, cm²), root volume (RV cm³) and root number (RN) were extracted (see Abbreviations). The taproot length (MRL cm) and diameter per plant (MRD cm) were measured using the vernier caliper (only for soil-grown plants). Shoots and roots were then dried in a drying oven at 80 °C for 72 h and weighed to determine shoot dry weight (SDW g) and root dry weight (RDW g). Root–shoot ratio (RSR) data were then estimated (see Abbreviations).

Field trials and seed yield data recording

The 40 genotypes were planted in three different environments in China, including Wuhan, the Hubei province (30.57 N, 114.30 E), Nanning, the Guangxi province (23.17 N, 107.55 E) and Sanya, the Hainan province (109.187 E, 18.38 N). The field trials were conducted during the year 2017 using a randomized block design with three replications. Each plot had four rows of 2 m long spaced 0.4 m apart. At the four-leaf stage, seedlings were thinned down and eight evenly distributed plants in each row were retained. Five uniform plants for each genotype were randomly selected to collect the seed yield per plant data (g) after maturation.

Root phenotypic data analysis

Descriptive statistics of the experimental data were implemented in the R software (www.r-project.org). Normal distribution of data was determined using the Shapiro–Wilk *W* test, while homogeneity of variances was determined with the Bartlett test. ANOVA results were considered significant at $P < 0.05$ and mean comparisons were done using the Tukey HSD test. Agglomerative hierarchical clustering (AHC) was performed based on the Ward's method using the Euclidean distances. Then, discriminant analysis (DA) was carried out to refine the clustering in order to sort out the different groups of genotypes according to the root trait characteristics. Pearson correlation coefficients of the root traits, the AHC and DA analyses were performed using the software XLSTAT version 2016. To compare the two methods of plant culture systems (Soil vs Hoagland), we employed the MANOVA with the Wilks lambda test.

Root anatomical experiment and data analysis

Roots from plants grown in soil were collected carefully by hand and then washed with deionized water to remove soil particles. Root anatomical characteristics were evaluated in samples. Tissue samples observed under microscopy were collected from 10 mm up of the taproot tip. The root tip was cut into slices, and cross-section was obtained. A few drops of dihydrochloride, DAPI dye solution were added to the prepared glass slides for staining. Pictures were taken using a LEICA microscope. For each root transverse slice, anatomical traits including root diameter, stele diameter, cortical thickness, mean diameter of cortical cells, cortical cell layer number, number of large xylem, number of small xylem were evaluated using the ImageJ software version 1.8.0_172. For each genotype, the average values of these anatomical traits were calculated from three cross sections.

RNAseq experiment and data analysis

Plant materials

Two genotypes including G546 (big root phenotype) and G259 (small root phenotype) were used for RNA-seq study. The whole root samples were harvested from three plants of the same genotype after 1 month growth in Hoagland nutrient solution as described above.

Total RNA isolation and sequencing

Total RNA of the six root samples (50 g with mixed taproot and lateral root) was extracted using an EASYspin Plus kit (Aidlab, Beijing, China). The cDNA libraries generated from RNA samples were pair-end sequenced on an Illumina HiSeq 4000 platform according to the methods described by Dossa et al. (2017).

RNA-seq data analysis

The raw data were first processed with FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) to filter out adapters and low-quality sequences. Then, the clean reads were mapped to the sesame genome v.1.0 (<https://www.ncbi.nlm.nih.gov/genome/?term=sesamum>) (Wang et al. 2014) using HISAT (Kim et al. 2015). The RSEM package (Li and Dewey 2011) was used to calculate gene expression levels for each sample expressed as fragments per kilobase of transcript per million fragments mapped (FPKM). The gene expression levels in G546 were compared with those in G259 to identify the differentially expressed genes (DEG). The DEGs were detected as described by Tarazona et al. (2011) based on the parameters: fold change ≥ 2.00 and Probability ≥ 0.8 with a significant false discovery rate-adjusted *P*

value (FDR) < 0.05 based on the three biological replicates. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for the DEGs were performed using the clusterProfiler version 3.8.

Validation of gene expression using qRT-PCR

The qRT-PCR was performed on RNA extracted from root samples as described by Dossa et al. (2016) using the sesame gene *Actin 7* (*SIN_1006268*) as the internal control. Specific primer pairs of ten selected genes were designed using the Primer Premier 5.0 (Lalitha 2000) (Table S1). Data are presented as relative transcript level based on the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001).

Results

Sesame root traits variation in soil culture system

Forty sesame genotypes were grown in pot containing 6 kg soil for 45 days and nine root traits were assessed (Table 1, see Abbreviations). Table 2 presents the mean value and

Table 2 Variation of root in 40 sesame genotypes grown in soil culture system

Traits	Mean	SD	Range	ANOVA
RDW	0.14	0.06	0.06–0.29	***
SDW	0.77	0.26	0.38–1.43	***
RSR	0.17	0.03	0.12–0.23	***
RL	2050.52	349.14	1205–2625	***
SA	338.66	80.11	194.5–484.7	***
RV	4.58	1.60	2.53–9.22	***
RN	1761.44	298.27	1087–2317	***
MRL	24.04	5.57	13.08–38.54	***
MRD	1.08	0.17	0.70–2.22	***

*** $P < 0.001$

Table 3 Correlation analysis of root traits from soil-grown sesame plants

	RL	SA	RV	RN	RDW	SDW	RSR	RD	MRL
RL	1								
SA	0.91	1							
RV	0.77	0.91	1						
RN	0.85	0.82	0.65	1					
RDW	0.70	0.80	0.79	0.70	1				
SDW	0.60	0.66	0.70	0.57	0.90	1			
RSR	0.34	0.50	0.50	0.30	0.45	0.20	1		
RD	0.30	0.39	0.42	0.43	0.50	0.56	0.08	1	
MRL	0.38	0.44	0.55	0.29	0.51	0.55	0.23	0.32	1

Bold values are Pearson coefficients ≥ 0.8

range of the assayed root traits data. Overall, a large variation for root traits were observed among the sesame germplasm, which is cardinal for harnessing the potential of root traits in sesame improvement programmes. Pearson correlation analysis (R) of the root traits showed that the root surface area (SA) was highly and positively correlated to the total root length (RL), root volume (RV), root number (RN) and root dry weight (RDW), indicating that SA is a good trait that can be used to predict several root components in sesame and could be prioritized for a large-scale root phenotyping. Interestingly, a positive and strong correlation was found between shoot (SDW) and root dry weight (RDW), implying that the sesame plants with extensive aboveground parts tend to develop dense roots (Table 3).

Discriminant analysis of the genotypes based on the root phenotypic data resulted in three different clusters (Fig. 2a). Cluster I contained 22 genotypes and was characterized by the highest mean values for RL, SA, RV, RSR and moderate mean values for RDW, SDW and RN. Cluster II encompassed five genotypes with low mean values for RL, SA, RV, RDW, RSR, RDW, RSR, but moderate mean values for RN. Cluster III contained 13 genotypes and compared with the other clusters, and its genotypes had moderate mean values for RL, SA, RV, RSR and high mean values for RN, SDW and RDW. Cluster I could be designated as big root biomass genotypes, cluster II could be named as small root biomass genotypes and cluster III could be called as moderate root biomass genotypes.

Root traits variation in the Hoagland nutrient solution culture system

Hoagland nutrient solution is a plant culture system widely used to investigate root traits, particularly for a large-scale phenotyping. In the attempt to evaluate the response of sesame root under hydroponic growth condition, the 40 genotypes were grown in 1/4 strength Hoagland nutrient for 1 week and subsequently transferred into 1/2 strength Hoagland nutrient solution for an additional 3 weeks.

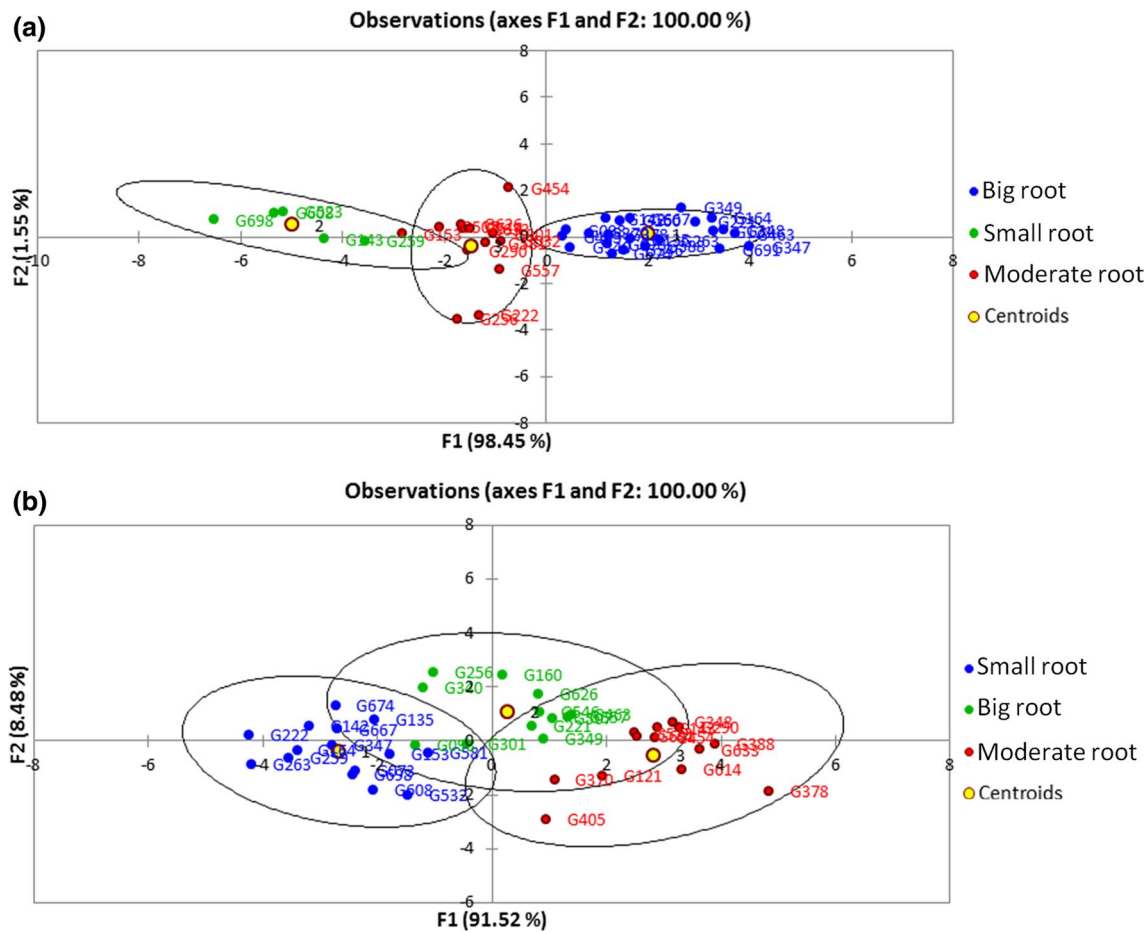


Fig. 2 Discriminant analysis of the studied population. **a** Soil culture system. Blue dots, big root biomass genotypes; green dots, small root biomass genotypes; red dots, moderate root biomass genotypes. **b**

Hoagland solution culture. Green dots, big root biomass genotypes; blue dots, small root biomass genotypes; red dots, moderate root biomass genotypes

Table 4 Variation of root traits in 40 sesame genotypes grown in Hoagland nutrient solution

Traits	Mean	SD	Range	ANOVA
RDW	0.0925	0.0513	0.022–0.257	***
SDW	0.7381	0.1705	0.372–0.994	***
RSR	0.1292	0.0621	0.047–0.285	***
RL	2025.5825	612.8020	1081–3365	***
SA	249.8783	69.8968	136.6–388	***
RV	2.4956	0.6452	1.458–3.922	***
RN	2997.3000	740.7810	1870–4533	***

*** $P < 0.001$

Similar to the soil-grown culture, we observed a high variability for the root traits as summarized in Table 4. Discriminant analysis revealed three groups of genotypes based on their root phenotypic traits (Fig. 2b). Cluster I grouped 15 genotypes characterized by low RDW, SDW, RSR, RL, SA, RV and RN values. Cluster II encompassed 12 genotypes

characterized by high RDW, RSR, RL, SA, RV and RN but moderate SDW values. Finally, cluster III gathered together 13 genotypes which have moderate RDW, SDW, RSR, RL, SA, RV, RN and high SDW. We, therefore, named cluster I as small root biomass genotypes, cluster II as big root biomass genotypes and cluster III as moderate root biomass genotypes.

Comparing the grouping patterns of the genotypes from the two culture systems, we could observe that many genotypes were constitutively classified as big root biomass genotypes (G098, G221, G340, G349, G463, G546) or small root biomass genotypes (G259, G608, G698). This result indicates that highly contrasting genotypes for root traits could be clearly distinguished regardless of the growth medium. Next, the root traits measured from Hoagland solution culture and soil culture systems were individually compared using ANOVA. The results showed that except for SDW and RL, all the remaining traits varied significantly according to the plant culture systems (Table 5). In general, root trait values from the soil culture were significantly higher than

Table 5 Comparison of root trait data from soil and Hoagland-grown plants

Traits	Mean_Soil	Mean_Hoagland	ANOVA	MANOVA
RDW	0.1355	0.0925	***	0.00022***
SDW	0.7746	0.7381	ns	
RSR	0.1681	0.1292	**	
RL	2050.5190	2025.5825	ns	
SA	338.6623	249.8783	***	
RV	4.5832	2.4956	***	
RN	1761.4400	2997.3000	***	
MRL	24.0407	–	–	
MRD	1.0763	–	–	

ns not significant $P > 0.05$

** $P < 0.01$; *** $P < 0.001$

those from the Hoagland solution culture. Only RN showed the opposite trend, suggesting that the Hoagland solution culture tends to increase the overall root number. By integrating all the root traits, the MANOVA test revealed that the two methods of plant culture induce significantly different root phenotypes in sesame.

Anatomical diversity of the sesame root

We selected nine genotypes, including seven with a big root biomass and two with a small root biomass (Fig. 2a) to investigate for the first time the diversity of anatomical features in sesame taproot. The variations of root diameter, stele diameter, cortex cell layer number, number of large xylem, number of small xylem are described in Table 6. Because of the high discrepancy of sample sizes among genotypes with big root biomass and small root biomass, we did not perform comparative statistical tests. Nonetheless, it could be noticed that the big root genotypes had globally higher values for root diameter, stele diameter, cortical thickness, number of large xylem and number of small xylem than the group of small root biomass genotypes. G546 had the thickest root (15.36 mm) followed by G256 (14.48 mm) and G340 (14.30 mm). The ratio of cortical thickness to root diameter of the big root genotypes was slightly smaller than that of the small root genotypes, while the ratio of the stele diameter to the root diameter showed the opposite trend. The reduced ratio of cortical thickness in favor to the stele diameter may be beneficial for nutrient and water transportation in root. The cortical cell layer numbers were similar among genotypes with big root or small root phenotypes, ranging from 5 to 7 layers. Furthermore, the total number of xylem vessels of big root sesame genotypes was larger than that of small roots genotypes. Overall, sesame harbors an important anatomical diversity, which seems to be linked to the global root biomass.

Table 6 Differences in anatomical characters of big root and small root

Genotypes	Root diameter (mm)	Stele diameter (mm)	Cortical thickness (mm)	Mean diameter of cortical cells (mm)	Stele diameter/ root diameter	Cortical thickness/ root diameter	Cortical cell layer number	Number of large xylem	Number of small xylem	Total number of xylem (small + large)
Big root genotypes										
G256	14.48	8.38	3.07	0.44	0.58	0.21	7	12	11	23
G301	11.01	5.54	2.40	0.40	0.50	0.22	6	8	3	11
G340	14.30	8.65	2.51	0.42	0.61	0.18	6	14	4	18
G546	15.36	9.34	2.74	0.39	0.61	0.18	7	17	6	23
G691	13.38	8.09	2.74	0.46	0.61	0.21	6	16	8	24
G221	11.90	6.52	2.80	0.56	0.55	0.24	5	10	1	11
G121	12.10	6.35	2.70	0.39	0.53	0.23	7	9	10	19
Small root genotypes										
G259	10.89	5.31	2.46	0.35	0.49	0.23	7	9	2	11
G674	11.12	5.11	2.71	0.45	0.46	0.24	6	9	7	16

Relationship between root biomass and seed yield in sesame

To determine the contribution of root biomass to seed yield in sesame, the 40 genotypes were planted in three different environments in China, including Wuhan, Nanning, and Sanya. The average seed yield per plant (g) was estimated for each genotype in each environment. We classified the genotypes according to the three clusters obtained from the DA based on the soil culture (Fig. 2a). As shown in Fig. 3, except for Sanya location ($P < 0.3949$), the genotypes with big root biomass had significantly higher seed yield than the genotypes with small root biomass [Nanning ($P < 0.0006$), Wuhan ($P < 0.0283$)], denoting that the root biomass positively contributes to increased seed yield in sesame. It is worth mentioning that all the genotypes did not grow well in Sanya location compared to the other two locations (as

evidenced by the very low seed yield in Sanya) (Fig. 3) and this may explain the lack of significant differences of the seed yield between cultivars with different root types.

Global gene expression profiles in G546 and G259, two contrasting genotypes for root phenotype

Two genotypes including G546 with the biggest root biomass and G259 with the smallest root biomass (Fig. 4), constitutively identified from the soil-grown and Hoagland-grown culture systems, were used for RNA-seq analysis. With three biological replicates, transcriptome sequencing of the six root samples yielded a total of 33.54 Gb clean data with 93.22% of bases scoring Q30 and above (Table S2). Of the total clean reads, 82–93% were unique matches with the *Sesamum indicum* reference genome V1.0 (Wang et al. 2014) (Table S3). A total of 21,801 and 21,679 expressed

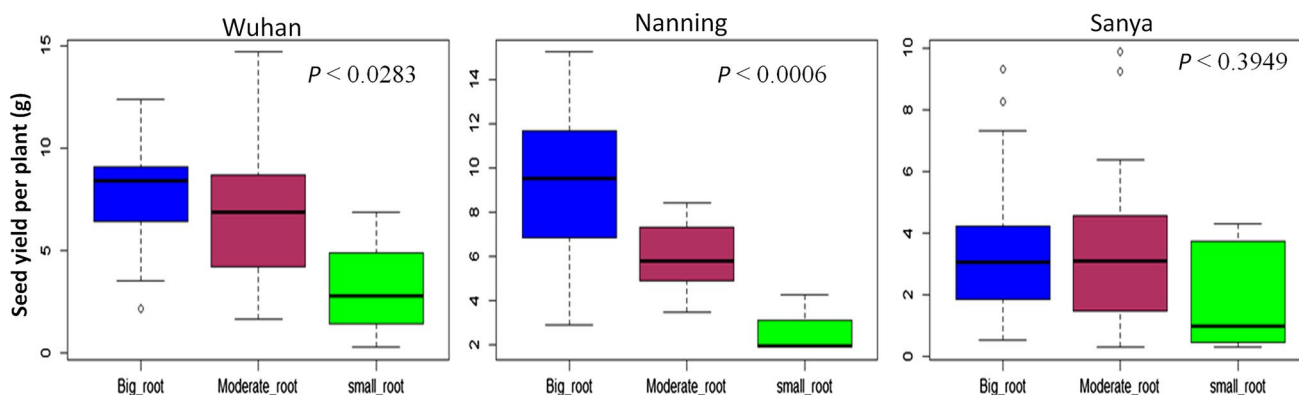


Fig. 3 Seed yield per plant (g) of 40 sesame genotypes classified into three groups according to their root biomass. Data were recorded in three locations in China (Wuhan, Nanning and Sanya) and the P values represent *Tukey HSD* tests from ANOVA

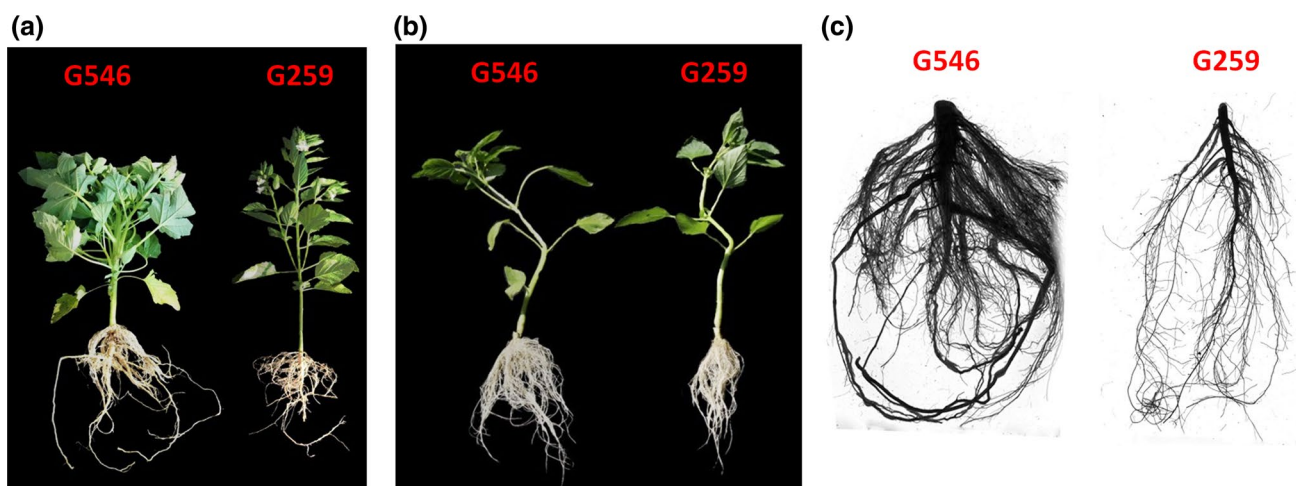


Fig. 4 Two sesame genotypes with contrasting root phenotypes detected after screening. **a** Phenotype of G546 and G259 based on soil culture system after 45 days. **b** Root morphology of G546 and

G259 based on Hoagland solution culture after 1 month. **c** Scanned root of G546 and G259 based on soil culture system after 45 days

genes in G546 and G259 were detected, respectively, resulting in 22,417 unique genes in sesame root. We identified 21,063 genes commonly expressed in both genotypes, while 738 and 616 genes were genotype-specific genes and may participate in the observed root phenotypes (Fig. 5a). Hierarchical clustering of the samples based on the number of fragments per kilobase of exon per million fragments mapped (FPKM) showed that all the biological replicates clustered together, indicating the high reliability of our sequencing data (Fig. 5b). The FPKM values range from 0.00333 to 19900.14667 in G546 and from 0.0033 to 26292.66 in G259. Overall, similar global gene expression levels could be observed in samples from the two genotypes (Fig. 5c).

Differentially expressed genes between G546 and G259

We compared the gene expression profiles in roots of the two genotypes to detect the differentially expressed genes (DEGs), which underlay the differences in root phenotypes. We identified 1831 and 1066 up- and downregulated genes in G259, respectively. These DEGs contributed principally to the GO terms related to sequence-specific DNA-binding transcription factor activity, heme binding, protein kinase activity and iron binding (Fig. 6a), highlighting the key roles of transcription factors in the root phenotype in sesame. We further investigated the possible physiological and biochemical pathways involved in sesame root phenotypes through KEGG database. The DEGs between the two genotypes are enriched in several pathways, predominantly

phenylpropanoid biosynthesis, starch and sucrose metabolism, stilbenoid, diarylheptanoid and gingerol biosynthesis, and flavonoid biosynthesis (Fig. 6b), indicating that these pathways are crucial for the root growth and development in sesame. The top up- and downregulated genes include several unannotated genes but also TFs and some structural genes, which could be functionally characterized using systems biology approaches (Fig. 7a). To validate our RNA-seq data, we selected five upregulated genes and five downregulated genes (Table S1) and performed qRT-PCR using the cDNAs from root of the two genotypes (G546 and G259). The qRT-PCR results were strongly correlated with the RNA-seq data ($r^2=0.8$, Fig. S1). This result confirms well the high reliability of the RNA-seq data obtained in the present study.

Enriched transcription factor families in the DEGs between G546 and G259

Transcription factors (TF) coordinate the expression levels of target genes to maintain a particular phenotype (Feng et al. 2018). To identify the main regulators of gene expression between the two genotypes, we investigated the enriched TF families among the DEGs. Prediction of the total unigenes encoding TF in sesame resulted in 58 TF families with a total of 2326 TFs. Among the upregulated genes in G259, 196 are TFs belonging to 35 families, predominantly ERF, WRKY, MYB and NAC, implying that these genes positively participate in the small root biomass phenotype (Fig. 7b). Likewise, 54 downregulated genes in

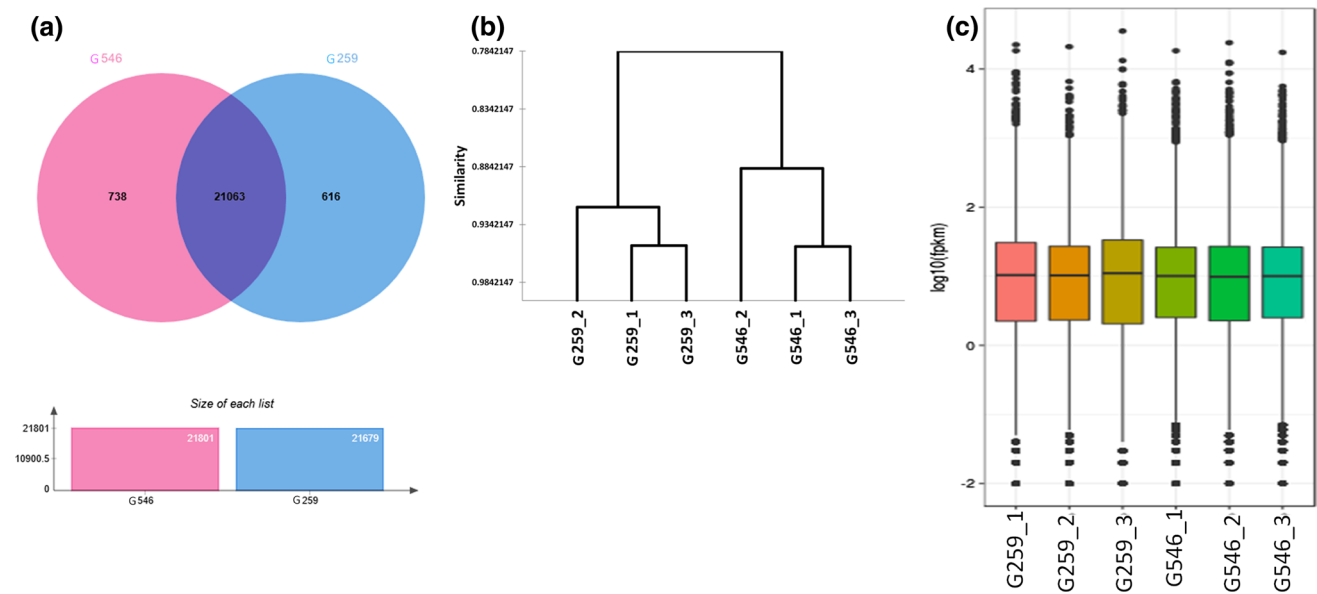


Fig. 5 Transcriptome sequencing in two sesame genotypes with contrasting root phenotypes. **a** Shared and specific expressed genes between G546 and G259. **b** Tree showing that the three replicates

were very close and clearly separated the two genotypes. **c** The log₁₀ FPKM values range in G546 and G259

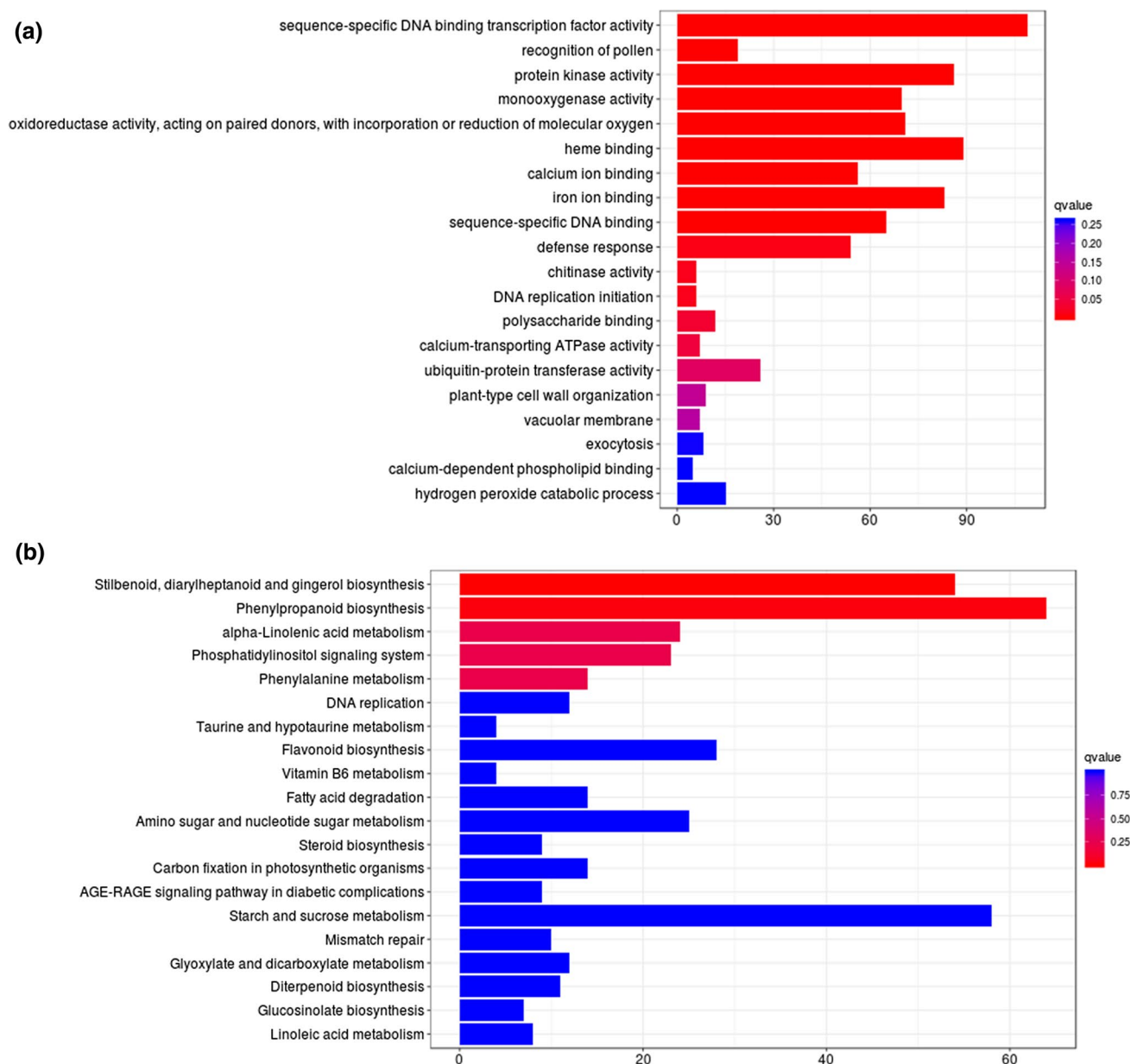


Fig. 6 Functional classification of the assembled unigenes. **a** Functional classification based on GO enrichment. **b** Pathway assignment based on the KEGG enrichment

G259 are TFs belonging to 21 families with bHLH, HD-ZIP, GRAS and LBD as the most enriched ones (Fig. 7c). These TFs may play key roles in the rapid root growth and high root biomass in sesame.

Discussion

The root system plays an important role in the growth and development of plants, including holding plants to the soil, absorbing water and nutrients for plant growth and acting

as storage organs (Paez-Garcia et al. 2015). Illuminating the variability of sesame root system at the morphological, anatomical and molecular levels traits could provide desirable root characteristics and candidate genes for breeding programmes. Root traits have been extensively studied and harnessed to improved yield and resistance to biotic and abiotic stress in soybean (Fried et al. 2018), rice (Xu et al. 2018a, b), oilseed rape (Wang et al. 2017), wheat (Wasson et al. 2014), maize (Ju et al. 2018), etc. However, there has been no specific performance in the root breeding programmes in sesame. In this study, the selected 40 sesame genotypes

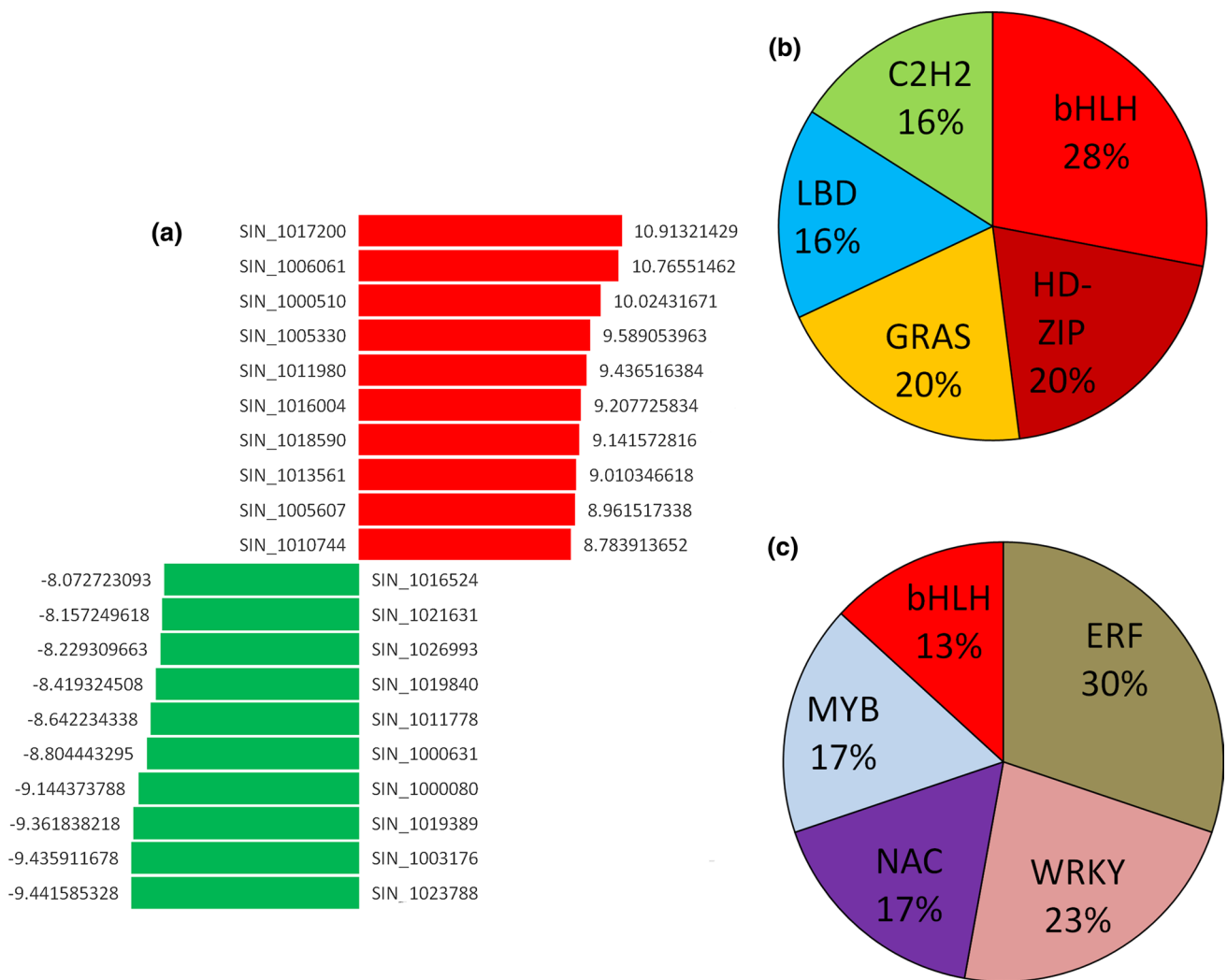


Fig. 7 Differentially expressed genes between G546 and G259 **a** Top 20 up- and downregulated genes between G546 and G259. Values represent the fold changes in gene expression. **b** Major transcription

factor families upregulated in G259. **c** Major transcription factor families downregulated in G259

are from various countries over the world (Table 1), so they have a good representativeness and genetic diversity, which can reflect the different root types of sesame. Through the statistical analysis of the assayed root traits in the 40 sesame genotypes, we found that sesame holds a large diversity (Table 2). This is very interesting because this natural variation will allow the detection of SNPs/candidate genes/QTLs, which can be directly employed in molecular breeding programmes (Wei et al. 2015; Dossa et al. 2019). Since the observed root variability in this study was mainly based on plants grown in controlled conditions, in future study, it is important to confirm these results in field non-controlled conditions.

The root research in crops has been mainly hampered by the difficulty to access the rhizosphere (Kuijken et al. 2015). Much time is required to wash plant roots from soil and

separate the living plant roots from organic debris. In particular, for a large-scale plant root phenotyping, it is almost impossible to investigate accurately thousands of soil-grown plants. Therefore, various platforms and growth mediums have been proposed to study the root system, each with its advantages and drawbacks (Atkinson, et al. 2019). The effect of different culture conditions on the root systems of sesame was compared using two culture systems: Hoagland nutrient solution and soil culture (Fig. 1). MANOVA revealed that the two systems of plant culture induce significantly different root phenotypes in sesame, the root traits from the soil culture being significantly higher than those from the Hoagland solution culture except for the root number (Table 5). Our results are consistent with the study of You and Zhao (2016) who showed that hydroponic roots formed a growth characteristic that adapt to the water environment with smaller

root diameter and lower root surface area. Similarly, Liu et al. (2017) reported in maize seedlings that the hydroponics system is characterized by a higher crown root number compared to the vermiculite culture system. In fact, the root structure changed significantly under hydroponic conditions in our experiment, with the formation of a large number of adventitious roots, leading to a higher number of roots in hydroponics culture than in soil culture. Young roots from hydroponics cultured sesame plants have no friction of soil particles and are not constrained by the soil resistance, so the epidermis gets thinner and their SA, RDW and RV are smaller than plants from the soil culture (Table 5). Importantly, the duration of the plant growth in soil was 45 days while in the Hoagland solution, it was 30 days and this may have also contributed to the higher values of root parameters in soil condition. Nonetheless, based on the clustering of the genotypes in the two methods (Fig. 2a, b), we found that similar genotypes were commonly clustered either in the small-root or in the big-root group, indicating that high-contrasting genotypes for root traits could be clearly distinguished regardless of the method used. In this regard, we can recommend the Hoagland solution culture for a large-scale root phenotyping in sesame.

Anatomical variation was associated with differences in functional efficiency and stress response of several crops, including maize (Zhu et al. 2010), Erianthus (Shiotsu et al. 2015) and rice (Singh et al. 2013). Kondo et al. (2000) reported that root diameter was highly correlated with stele diameter, which perfectly matched our results. Shiotsu et al. (2015) showed that compared with other cereal crops, Erianthus root were larger, correlating with a stronger water absorption and transportation. Because the genotypes with big root phenotypes had higher values for root and stele diameter and vessel number in the present study (Table 6), it is tempting to speculate that the sesame root biomass is tightly associated with the anatomical features. Selection and breeding for better root phenotypes based on the root anatomical features can improve acquisition of soil resources and crop production in marginal environments. Hence, the observed anatomical diversity may be essential in sesame root breeding programmes.

Mounting evidences demonstrate the positive contribution of root traits to crop yield (Ali et al. 2016; Sandhu et al. 2017; Xie et al. 2017; Beyer et al. 2019). Accordingly, in this study, we showed that sesame genotypes with a big root phenotype had significantly higher seed yield than those with a small root phenotype (Fig. 3). This extremely exciting result represents a solid proof that increasing sesame root biomass through breeding techniques will positively contribute to the seed yield improvement. Future investigations are needed to uncover the specific root traits to prioritize in sesame root breeding programmes. For example, in the present study, we found that the root surface area (SA) was highly and

positively correlated with many other root traits (Table 3) and could, therefore, be the target in sesame root breeding programmes. However, how the big root biomass aids sesame plants to improve the seed yield is still elusive. We observed a positive and strong correlation between shoot and root dry weights traits (Table 3), suggesting that dense and big root biomass may help the plant to acquire more nutrient and water from the soil, which will ultimately promote the aboveground biomass formation. Since the shoot dry weight (SDW) is an easier trait to record compared to SA, SDW can be used as a proxy to detect sesame genotypes with dense root phenotype.

Transcriptome sequencing technology has been successfully applied to understand the molecular mechanisms underlying important agronomic traits in plants (Chen et al. 2018; Wan et al. 2018; Zeng et al. 2018; Arro et al. 2019; Wang et al. 2019; Xiao et al. 2019). In the present study, RNA-seq technology was employed to analyze the transcriptome of the genotypes G546 (big root phenotype) and G259 (small root phenotype). In organisms, different gene products coordinate with each other to perform biological functions, and annotation analysis of differentially expressed genes helps to interpret gene function (Cheng et al. 2018; Wu et al. 2018). The KEGG enrichment analysis of the differentially expressed genes between G546 and G259 highlighted important pathways involved in the differential root phenotypes, including phenylpropanoid biosynthesis, starch and sucrose metabolism and stilbenoid, diarylheptanoid and gingerol biosynthesis (Fig. 6b), which were reported to contribute to root growth and development in plants (Li and Zhang 2003; Sun et al. 2015; Jiang et al. 2017). Jiang et al. (2017) indicated that gingerols and diarylheptanoids were predominantly synthesized in fibrous roots of ginger and then transported to rhizomes, where they accumulate. Li and Zhang (2003) showed that development of sweet potato storage root coincided with starch accumulation. Also, most of the transcripts encoding sucrose synthase in *Rehmannia glutinosa* were upregulated in the developing tuberous roots (Sun et al. 2015). The GO functional classification of the differentially expressed genes showed that transcription factors (TF) are active in sesame root development (Fig. 6a). It is well known that TFs play an important role in the regulation of plant growth, evolution, and stress resistance (Sun et al. 2009; Gujjar et al. 2014; Feng et al. 2018). ERF and WRKY TFs were highly active in the small root genotype, while the bHLH and HD-ZIP were upregulated in the big root phenotype (Fig. 7b, c), denoting that some TF families participate in the growth and root biomass accumulation while some play the opposite role. The top five genes in this study that are upregulated in G546 with transcription factor activity include *SIN_1000080* (*bHLH*), *SIN_1008247* (*B3*), *SIN_1010728* (*ERF*), *SIN_1001533* (*HSF*), *SIN_1022669* (*B3*), revealing that the mentioned genes may positively

regulate the growth, development, and metabolism of sesame roots. The role of these key genes in root growth and development and the association with the seed yield performance in sesame remain to be further studied.

Author contribution statement XZ and KD conceived, designed and supervised the experiment; RZ, MAM, DL, LQ, AL, JW, YG, MW, LS, JY, ZW, XZ, KD performed the experiments. KD and RS conducted data analyses; KD and RS wrote the manuscript; All authors read and approved the final version of the manuscript.

Acknowledgements This work was supported by the China Agriculture Research System (CARS-14), the Agricultural Science and Technology Innovation Project of Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2016-OCRI), the Central Public-interest Scientific Institution Basal Research Fund (1610172018007) and the Peanut and Sesame Industry Technology System of Jiangxi, China (JXARS-18).

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw RNA-seq data are freely available at NCBI Sequence Read Archive: www.ncbi.nlm.nih.gov/bioproject/PRJNA552167.

Compliance with ethical standards

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

References

- Ali ML, Luetchens J, Singh A et al (2016) Greenhouse screening of maize genotypes for deep root mass and related root traits and their association with grain yield under water-deficit conditions in the field. *Euphytica* 207(1):79–94
- Anilakumar K, Pal A, Khanum F et al (2010) Nutritional, medicinal and industrial uses of sesame (*Sesamum indicum* L.) seeds—an overview. *Agric Consp Sci* 75(4):677–685
- Arro J, Yang Y, Song GQ et al (2019) RNA-Seq reveals new DELLA targets and regulation in transgenic GA-insensitive grapevines. *BMC Plant Biol* 19(1):80
- Atkinson JA, Pound MP, Bennett MJ, Wells DM (2019) Uncovering the hidden half of plants using new advances in root phenotyping. *Curr Opin Biotechnol* 55:1–8
- Beyer S, Daba S, Tyagi P et al (2019) Loci and candidate genes controlling root traits in wheat seedlings—a wheat root GWAS. *Funct Integr Genom* 19(1):91–107
- Chen L, Bian J, Shi S et al (2018) Genetic analysis for the grain number heterosis of a super-hybrid rice *WFYT025* combination using RNA-Seq. *Rice* 11(1):37–50
- Cheng FC, Jinn T-R, Hou RCW et al (2006) Neuroprotective effects of sesamin and sesamol in gerbil brain in cerebral Ischemia. *Int J Biomed Sci* 2(3):284–288
- Cheng Q, Bai S, Ge G et al (2018) Study on differentially expressed genes related to defoliation traits in two alfalfa varieties based on RNA-Seq. *BMC Genom* 19(1):807–815
- Dossa K, Wei X, Li D et al (2016) Insight into the AP2/ERF transcription factor superfamily in sesame (*Sesamum indicum*) and expression profiling of the DREB subfamily under drought stress. *BMC Plant Biol* 16:171
- Dossa K, Li D, Wang L et al (2017) Dynamic transcriptome landscape of sesame (*Sesamum indicum* L.) under progressive drought and after rewatering. *Genom Data* 11:122–124
- Dossa K, Li D, Yu J et al (2019) The genetic basis of drought tolerance in the high oil crop *Sesamum indicum*. *Plant Biotechnol J*. <https://doi.org/10.1111/pbi.13100>
- Feng ZJ, Xu SC, Liu N et al (2018) Soybean TCP transcription factors: evolution, classification, protein interaction and stress and hormone responsiveness. *Plant Physiol Biochem* 127:129–142
- Fried HG, Narayanan S, Fallen B et al (2018) Characterization of a soybean (*Glycine max* L. Merr.) germplasm collection for root traits. *PLoS One* 13(7):e0200463
- Gebregergis Z, Assefa D, Fitwy I (2018) Sesame sowing date and insecticide application frequency to control sesame webworm *Antigastra catalaunalis* (Duponchel) in Humera, Northern Ethiopia. *Agric Food Secur* 7:39
- Gharby S, Harhar H, Bouzoubaa Z et al (2017) Chemical characterization and oxidative stability of seeds and oil of sesame grown in Morocco. *J Saudi Soc Agric Sci* 33(D5):256–289
- Gujjar RS, Akhtar M, Singh M (2014) Transcription factors in abiotic stress tolerance. *Ind J Plant Physiol* 19(4):306–316
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. *Calif Agric Exp Stn Circ* 347(5406):357–359
- Hsu DZ, Su SB, Chien SP et al (2005) Effect of sesame oil on oxidative stress associated renal injury in endotoxemic rats: involvement of nitric oxide and proinflammatory cytokines. *Shock* 24(3):276–280
- Jiang Y, Liao Q, Zou Y et al (2017) Transcriptome analysis reveals the genetic basis underlying the biosynthesis of volatile oil, gingerols, and diarylheptanoids in ginger (*Zingiber officinale* Rosc.). *Bot Stud* 58:41
- Ju C, Zhang W, Liu Y et al (2018) Genetic analysis of seedling root traits reveals the association of root trait with other agronomic traits in maize. *BMC Plant Biol* 18:171–186
- Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* 12(4):357–360
- Kondo M, Aguilar A, Abe J et al (2000) Anatomy of nodal roots in tropical upland and lowland rice varieties. *Plant Prod Sci* 3(4):437–445
- Kuijken RCP, Eeuwijk FAV, Marcelis LFM et al (2015) Root phenotyping: from component trait in the lab to breeding. *J Exp Bot* 66(18):389–401
- Lalitha S (2000) Primer premier 5. *Biotechnol Softw Internet Rep* 1:270–272
- Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform* 12:323–339
- Li XQ, Zhang D (2003) Gene expression activity and pathway selection for sucrose metabolism in developing storage root of sweet potato. *Plant Cell Physiol* 44(6):630–636
- Li D, Wang L, Zhang Y et al (2012) Biological characteristics of Fusarium Wilt pathogens in main sesame production areas of China. *Chin Agric Sci Bull* 28(03):245–252 (in Chinese with English abstract)
- Li GG, Wan YS, Liu FZ et al (2014) Root morphology and physiological characteristics of peanut varieties with different drought resistance. *Acta Agron Sin* 40(3):531–541 (in Chinese with English abstract)
- Liu Z, Gao K, Shan S et al (2017) Comparative analysis of root traits and the associated QTLs for maize seedlings grown in paper roll, hydroponics and vermiculite culture system. *Front Plant Sci* 8:436–449
- Liu K, He A, Ye C et al (2018) Root morphological traits and spatial distribution under different nitrogen treatments and their relationship with grain yield in super hybrid rice. *Sci Rep* 8(1):1–9

- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* 25:402–408
- Nakano D, Kurumazuka D, Nagai Y et al (2010) Dietary sesamin suppresses aortic NADPH oxidase in DOCA salt hypertensive rats. *Clin Exp Pharmacol Physiol* 35(3):324–326
- Paez-Garcia A, Motes CM, Scheible W-R, Chen RJ, Blancaflor EB, Monteros MJ (2015) Root traits and phenotyping strategies for plant improvement. *Plants* 4(2):334–355
- Sandhu N, Subedi SR, Yadav RB et al (2017) Root traits enhancing rice grain yield under alternate wetting and drying condition. *Front Plant Sci* 8:1879. <https://doi.org/10.3389/fpls.2017.01879>
- Sarkar PK, Khatun A, Singha A (2016) Effect of duration of water-logging on crop stand and yield of sesame. *Int J Innov Appl Stud* 14(1):1–6
- Shiotsu F, Abe J, Doi T et al (2015) Root morphology and anatomy of field-grown *Erianthus arundinaceus*. *Am J Plant Sci* 6(1):103–112
- Singh A, Shamim M, Singh KN (2013) Genotypic variation in root anatomy, starch accumulation, and protein induction in upland rice (*Oryza sativa*) varieties under water stress. *Agric Res* 2(1):24–30
- Sun LF, Xing SC, Zhang J et al (2009) Function of the transcription factors in plant domestication and stress resistance. *Genom Appl Biol* 28(03):569–577
- Sun P, Xiao X, Duan L et al (2015) Dynamic transcriptional profiling provides insights into tuberous root development in *Rehmannia glutinosa*. *Front Plant Sci* 6:396–410
- Svacina P, Streda T, Chloupek O (2014) Uncommon selection by root system size increases barley yield. *Agron Sustain Dev* 34(2):545–551
- Tarazona S, Garcia-Alcalde F, Dopazo J et al (2011) Differential expression in RNA-seq: a matter of depth. *Genome Res* 21(12):2213–2223
- Wan L, Li B, Lei Y et al (2018) Transcriptomic profiling reveals pigment regulation during peanut testa development. *Plant Physiol Biochem* 125:116–125
- Wang L, Yu S, Tong C et al (2014) Genome sequencing of the high oil crop sesame provides insight into oil biosynthesis. *Genome Biol* 15(2):R39
- Wang ZG, Ma B-L, Gao J, Sun J (2015) Effects of different management systems on root distribution of maize. *Can J Plant Sci* 95:21–28
- Wang X, Chen Y, Thomas CL et al (2017) Genetic variants associated with the root system architecture of oilseed rape (*Brassica napus* L.) under contrasting phosphate supply. *DNA Res* 24(4):407–417
- Wang M, Qu H, Zhang H et al (2019) Hormone and RNA-seq analyses reveal the mechanisms underlying differences in seed vigour at different maize ear positions. *Plant Mol Biol* 99(4–5):461–476
- Wasson AP, Rebetzke GJ, Kirkegaard JA et al (2014) Soil coring at multiple field environments can directly quantify variation in deep root traits to select wheat genotypes for breeding. *J Exp Bot* 65(21):6231–6249
- Wei X, Liu K, Zhang Y et al (2015) Genetic discovery for oil production and quality in sesame. *Nat Commun* 33:8609
- Witcombe JR, Hollington PA, Howarth CJ et al (2008) Breeding for abiotic stresses for sustainable agriculture. *Philos Trans R Soc Lond B Biol Sci* 363(1492):703–716
- Wu H, Shi N, An X et al (2018) Candidate genes for yellow leaf color in common wheat (*Triticum aestivum* L.) and major related metabolic pathways according to transcriptome profiling. *Int J Mol Sci* 19(6):1594
- Xiao Y, Du Z, Qi X et al (2019) RNA-sequencing analysis reveals transcriptional changes in the roots of low-cadmium-accumulating winter wheat under cadmium stress. *Acta Physiol Plant* 41:13
- Xie Q, Fernando KMC, Mayes S et al (2017) Identifying seedling root architectural traits associated with yield and yield components in wheat. *Ann Bot* 119(7):1115–1129
- Xu G, Lu D, Wang H et al (2018a) Morphological and physiological traits of rice roots and their relationships to yield and nitrogen utilization as influenced by irrigation regime and nitrogen rate. *Agric Water Manag* 203:385–394
- Xu T, Yang R, Hua X et al (2018b) Improvement of the yield and flavour quality of sesame oil from aqueous extraction process by moisture conditioning before roasting. *Int J Food Sci Technol* 54(2):471–479
- Yang M, Huang FH (2009) Current situation, existing problems, development trend and countermeasures of sesame industry in China. *China Oils Fats* 34(1):7–12 (in Chinese with English abstract)
- You F-L, Zhao S-N (2016) Comparison of the root structures of *Sansevieria trifasciata* in Hydroponic culture and soil culture. *J Anhui Agric Sci* 44(16):18–19
- Zeng H, Zhang X, Zhang X et al (2018) Early transcriptomic response to phosphate deprivation in soybean leaves as revealed by RNA-sequencing. *Int J Mol Sci* 19(7):21–45
- Zhu J, Brown KM, Lynch JP (2010) Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). *Plant Cell Environ* 33(5):740–749

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.