



Article

Characterizing Root Morphological Traits in 65 Genotypes of Foxtail Millet (*Setaria italica* L.) from Four Different Ecological Regions in China

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Abstract: As an indispensable grain crop, foxtail millet (*Setaria italica* L.) is becoming a functional food in China because of its abundant nutrients. However, low rainfall and uneven precipitation limit its growth and production, especially in northwest China. Understanding the root phenotypic characteristics of foxtail millet is critical for utilizing its root characteristics to breed new millet varieties with improved resource-use efficiency and better adaptation to harsh environments. The present study characterized the variability of the root morphological traits at the seedling stage of 65 millet genotypes selected from four ecological regions across seven provinces in China. The plants were assessed after 21 days of growth in the germination pouches, and 48 root and shoot traits were measured. The results showed a large variation among the genotypes in the growth and functional traits. Among the measured traits, root dry mass, maximal root length, root surface area, and root tissue density contributed the most to the principal components. Root surface area ratio and root volume ratio were significantly correlated with root length ratio, respectively, while root length density and root weight density were negatively correlated with root diameter ($p < 0.01$). Shoot dry mass, root number, root length, root dry mass, and total dry mass were closely correlated with each other. Root length and root surface area in each 5-cm sections decreased from the top to the bottom along the root system. Among the four ecological regions, genotypes from the early maturing area of northwest China had the greatest root length, shoot dry mass, root dry mass, and root density, while those from the early maturing area of northwest China generally had the largest root system, with about 2.2 times more root length and dry weight than those from the early maturing area of northeast China (the shortest root system). The genotypic variability in root traits and correlations among shoot and root traits form a basis for breeding new millet cultivars.

Keywords: millet; root characteristics; phenotype variation; ecological regions; breeding



Citation: Yang, X.; Tian, Q.; Yan, J.; Chen, Y. Characterizing Root Morphological Traits in 65 Genotypes of Foxtail Millet (*Setaria italica* L.) from Four Different Ecological Regions in China. *Agronomy* **2022**, *12*, 1472. <https://doi.org/10.3390/agronomy12061472>

Received: 13 April 2022

Accepted: 15 June 2022

Published: 18 June 2022

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1. Introduction

In the current climate disaster scenarios, seasonal droughts induced by uneven rainfall and rapidly increasing population have highlighted food insecurity problems [1–5]. To cope with the increased population and to alleviate hunger, the global grain yield production must be increased by 70% [6,7]; thus, the increasing demand for food conflicts with the shortage of food [8]. In the face of such a great contradiction between supply and demand, many crops with strong resistance to environmental stress, including foxtail millet, are increasingly being planted and replanted [9]. Foxtail millet (*Setaria italica* L.), which has evolved from *Setaria viridis* L., is widely cultivated in the world, with a long history of planting [10], especially in northwest China [11,12]. Its grains are rich in a variety of trace elements and proteins [13], and it is consumed by more than half of the world's population, especially women and children [14].

The growth of plants is strongly associated with the morphological and physiological characteristics of their roots [15–17]. Environmental stresses can cause big changes in the development and form of plant roots, which are the interaction point between plants and soil [18], and they significantly affect many important agronomic traits, including drought tolerance [19]. According to previous reports, at the early growth stage of corn, the grain yield was found to be closely related to the root traits [20]. The root system is the main organ used for obtaining underground resources and plays a key role in water and nutrient absorption [6,8,21–25]. The root is usually the most sensitive organ for sensing and responding to miscellaneous soil stresses, such as soil drought, salinity, waterlogging, and nutritional deficiency [15,26–28]. Under abiotic stress, crop yield can be affected by the root phenotype through changes in the metabolic efficiency of soil exploration and the redirection of roots to the soil area with the highest amount of limited resources [29–31]. In each crop species, investigating the association of different types of roots with the environment is the first stage of research; this includes studies on the adaptation of crop species with deep roots to drought-prone environments [32]. Adapting to the intrinsic heterogeneity of soil resources, plants can modulate their root phenotype at different comprehensive levels [20], change the relative input of aerial and root biomass at the individual level, and adjust their root structure or make changes at the organ level [21,33]. The root system promotes aerial growth, and the root tip is the most active part of the root system, which is essential for plant yield [34]. Root morphology covers basal characteristics, such as root length, root length density, diameter, surface area, and volume, all of which affect the spatial structure of the root system [35,36]. Root length, surface area, and volume affect the spatial distribution of the roots, while the root diameter is related to intense soil penetration and drought tolerance. The relationship between the size of the aboveground plant parts and the size of the roots (S/R ratio) is critical to the plant water balance. A lower S/R is more beneficial to plants in a water-deficient environment and is an important indicator for evaluating plant drought tolerance [37]. Some researchers have found a correlation between the phenotypic traits and drought resistance [38]. Root system architecture (RSA) is a relatively complex quantitative trait which differs in different environments, and it is difficult to determine the phenotype of underground roots [39,40]. Thus, relatively few studies on millet root traits have been carried out to date. Applying the genetic information of root traits and root structure to the practice of millet breeding is expected to improve its resource efficiency and stress resistance [27].

In millet, a good performance has been shown to be associated with drought resistance, but its yield remains lower than that of other cereals [36]. Research on millet root-structure characteristics would not only improve our knowledge of its agronomic traits, but also enable plant breeders to enhance its tolerance to various stress conditions [35]. The knowledge on root phenotypic diversity and the interrelationships among root traits provides guidance for follow-up research and will make better sense of these traits in order to formulate breeding strategies [41]. It is extremely important to update local millet varieties with improved adaptation to environmental stress and climate changes. However, millet root morphological traits in different ecological regions have not previously been studied. Some early studies proved the practicability of semi-hydroponic phenotypic systems under a wide variety of environments, including the field [42], and significant correlations in root traits were discovered between wheat seedlings grown on moist germination paper and field-planted wheat seedlings [43]. The present study examined the variability of root morphological traits among 65 genotypes of foxtail millet from four ecological regions of China, cultivated in seed germination pouches. The objectives of this study were to (1) assess the variations in root system architecture among a set of millet germplasm and (2) to evaluate root trait correlations among the different ecological regions. The results will provide references for millet breeders.

2. Materials and Methods

2.1. Plant Materials

Sixty-five millet genotypes were selected from seven provinces (see Figure 1 for the distribution and the average rainfall in 2020) that belong to four millet planting ecological areas in China (Table S1). In the early maturing area of northeast China, the climate is the coldest, with long hours of sunshine during the growing season and low evaporation in the spring. Moreover, the area of the early maturing northwest is characterized by low rainfall with frequent drought seasons, high evaporation in spring, a high amount of wind and sand, and low soil fertility. In the middle and late maturing areas of northwest China, millet is not sensitive to temperature because these areas are characterized by a mild climate, drought, and a long sunshine duration. The summer maturing region is characterized by low latitude, high temperature, and rainy weather, as well as a small temperature difference between day and night and few sunshine hours.

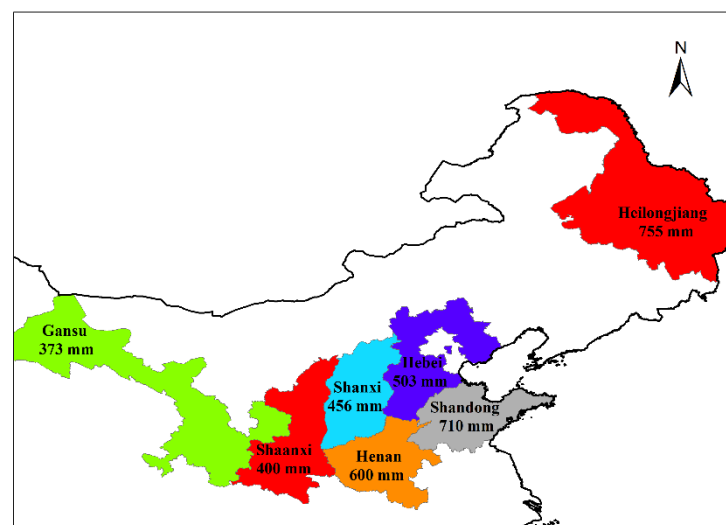


Figure 1. Distribution map of average rainfall of 65 genotypes from 7 provinces in China (2020).

2.2. Plant Culture

An incubator cultivation experiment was implemented from December 2020 to May 2021. Millet seeds were sterilized with 3% NaClO for 20 min and germinated on moist germination paper and kept at 16 °C for 10 h in the dark, after which they were placed at 24 °C, with 30,000 Lx, and an environmental air humidity of 65% for 14 h. The seeds were then cultivated in seed germination pouches (160 × 125 mm²) with 40 mL of distilled water. Four evenly located positions were selected at the groove of the germination paper, and two seeds were planted at each position. The port was sealed with a paper clip to reduce water loss.

Each genotype was cultivated in one pouch, and there were four replications of the pouches per genotype. Ten genotypes were placed on each shelf within an incubator. On the seventh day of the growing season, two leaves were visible; the paper clips were then removed, and four seedlings were established. The rest of the distilled water was poured out, and 40 mL of 25% Hoagland's nutrient solution was added; the position of the pouches was randomly changed daily. After a week, a fresh nutrient solution replacement was made, and the seedlings were harvested on day 21 for the assessments.

2.3. Root Trait Measurements and Calculations

Leaf number, root number, root width, maximal root depth, and maximal root length were measured manually prior to the harvest. The root width, maximal root depth, and maximal root length of each plant were measured using a ruler, and the root angle was measured with a protractor.

Root width is the maximal gap between the primary and the lateral roots, and maximal root depth is the maximal vertical length from the root top to the root tip. Maximal root length is the longest root of a single plant. After manual measurements, the roots were separated from the shoots, and the roots were divided into 5 cm sections from the base for scanning. There were 3 sections in each plant. Subsamples of every 5 cm section in each pouch were placed in distilled water in a plastic tray (22 cm × 31 cm × 2 cm), and they were scanned on a grey scale at 150 dpi using a desktop scanner (Epson Perfection V800, Long Beach, CA, USA). The images were analyzed using the WinRHIZO software (Regent Inc., Quebec, QC, Canada) to determine the length, diameter, surface area, and volume of the roots. The shoot samples were deactivated in a baking oven for 20 min at 105 °C, after which all the root and shoot samples were dried at 80 °C for 48 h, and the dry weights of the roots and shoots were determined.

The first 5 cm layer of roots and the remaining part of the roots of each root system were defined as the “top-root layer” and the “sub-root layer”, correspondingly. The functional traits were calculated by the following formulae:

$$\text{Root to shoot mass ratio (RSM)} = \text{root dry mass} / \text{shoot dry mass}$$

$$\text{Specific root length (SRL)} = \text{root length} / \text{root dry mass (cm mg}^{-1}\text{)}$$

$$\text{Root growth rate (RGR)} = \text{deepest root length per plant} / 21 \text{ days of growth (cm d}^{-1}\text{)}$$

$$\text{Root length density (RLD)} = \text{root length} / \text{root volume (cm cm}^{-3}\text{)}$$

$$\text{Root weight density (RWD)} = \text{root dry mass} / \text{root volume (mg cm}^{-3}\text{)}$$

$$\text{Root length ratio (RLR)} = \text{top-root length (0-5 cm)} / \text{sub-root length (>5 cm)}$$

$$\text{Root area ratio (RAR)} = \text{top-root area (0-5 cm)} / \text{sub-root area (>5 cm)}$$

$$\text{Root volume ratio (RVR)} = \text{top-root volume (0-5 cm)} / \text{sub-root volume (>5 cm)}$$

Detailed descriptions of the 48 traits, including 17 global traits (three shoot traits and 14 root traits) and 31 local traits, are shown in Table 1.

Table 1. Description of global traits and local traits in 65 millet genotypes.

Traits	Abbreviation	Description	Unit
<i>Global traits</i>			
Root width	RW	Maximum distribution of roots in the horizontal direction	Cm
Maximal root depth	MRD	Maximum depth of the root system in the vertical direction	Cm
Maximal root length	MRL	Maximum length of the seminal or primary root	Cm
Root number	RN	Seminal and primary root number	Number
Root dry mass	RDM	Total root dry mass	Mg
Root length	RL	Total root length	Cm
Root diameter	RD	Average root diameter	Mm
Root area	RA	Total root surface area	mm ²
Root volume	RV	Total root volume	cm ³
Root length density	RLD	Average root length density	cm cm ⁻³
Root weight density	RWD	Average root weight density	mg cm ⁻³
Specific root length	SRL	Total root length per unit root dry mass	cm mg ⁻¹
Root to shoot dry mass ratio	RSM	Total root dry mass divided by the shoot dry mass	
Root growth rate	RGR	Average daily root growth (based on the longest seminal or primary root growth at 15 days after seed sowing)	cm d ⁻¹
Root length ratio	RLR	Root length in Section 1 (top-root layer) over sub-root layer	
Root surface area ratio	RSAR	Root surface area in Section 1 (top-root layer) over sub-root layer	
Root volume ratio	RVR	Root volume in Section 1 (top-root layer) over sub-root layer	
Leaf number	LN	Total leaf number of each plant	
Shoot dry mass	SDM	Dry weight of the above-ground part	Mg
Total dry mass	TDM	Total dry mass (sum of root and shoot dry mass)	Mg

Table 1. Cont.

Traits	Abbreviation	Description	Unit
<i>Local traits</i>			
Root dry mass s1	RDM_s1	Total root dry mass in Section 1	Mg
Root dry mass s2	RDM_s2	Total root dry mass in Section 2	Mg
Root dry mass s3	RDM_s3	Total root dry mass in Section 3	Mg
Root dry mass in sub-root layer	RDM_sub	Combined root dry mass in sub-root layer (s2 and s3)	Mg
Root length s1	RL_s1	Total root length in Section 1	Cm
Root length s2	RL_s2	Total root length in Section 2	Cm
Root length s3	RL_s3	Total root length in Section 3	Cm
Root length in sub-root layer	RL_sub	Combined root length in sub-root layer (s2 and s3)	Cm
Root diameter s1	RD_s1	Average root diameter in Section 1	Mm
Root diameter s2	RD_s2	Average root diameter in Section 2	Mm
Root diameter s3	RD_s3	Average root diameter in Section 3	Mm
Root diameter in sub-root layer	RD_sub	Average root diameter in sub-root layer (s2 and s3)	Mm
Root area s1	RA_s1	Total root surface area in Section 1	mm ²
Root area s2	RA_s2	Total root surface area in Section 2	mm ²
Root area s3	RA_s3	Total root surface area in Section 3	mm ²
Root area in sub-root layer	RA_sub	Combined root surface area in sub-root layer (s2 and s3)	mm ²
Root volume s1	RV_s1	Total root volume in Section 1	cm ³
Root volume s2	RV_s2	Total root volume in Section 2	cm ³
Root volume s3	RV_s3	Total root volume in Section 3	cm ³
Root volume in sub-root layer	RV_sub	Combined root volume in sub-root layer (s2 and s3)	cm ³
Root length density s1	RLD_s1	Average root length density in Section 1	cm cm ⁻³
Root length density s2	RLD_s2	Average root length density in Section 2	cm cm ⁻³
Root length density s3	RLD_s3	Average root length density in Section 3	cm cm ⁻³
Root length density in sub-root layer	RLD_sub	Combined root length density in sub-root layer (s2 and s3)	cm cm ⁻³
Root weight density s1	RWD_s1	Average root t weight density in Section 1	mg cm ⁻³
Root weight density s2	RWD_s2	Average root t weight density in Section 2	mg cm ⁻³
Root weight density s3	RWD_s3	Average root t weight density in Section 3	mg cm ⁻³
Root weight density in sub-root layer	RWD_sub	Combined root weight density in sub-root layer (s2 and s3)	mg cm ⁻³

Note: Root Section 1(S1), Section 2(S2) and Section 3(S3) indicate root distribution at 0–5 cm, 6–10 cm and > 10 cm, respectively.

2.4. Statistical Analysis

The data processing of the 48 traits (Table 1) was performed using Excel 2019. A distribution map with average rainfall in 2020 (Figure 1) was performed in ArcGIS (version 10.8, Environmental Systems Research Institute, Inc, Redlands, CA, USA). A descriptive statistics analysis was performed for minimum, maximum, medium, mean, and standard deviation; one-way analysis of variance (ANOVA, $p < 0.05$) was carried out with the genotypes as the variables, and the Pearson correlations of 18 traits with $CV \geq 0.2$ ($p < 0.05$) were performed using SPSS Statistics (version 23, IBM, New York, NY, USA). The data of four replications of each genotype were calculated as means and then classified by province and ecological region, respectively. The mean values of root length in three layers and the root dry mass of each province were selected and ordered with the total root length in ascending order; the mean data of each ecological region were processed by the same method. The data of the two groups were used to draw double Y-axis column plots. The root lengths of the different layers and the biomass dry mass were plotted as stacked histograms, and the significant differences were noted. The selected 18 root traits of the genotypes were plotted as box charts (grouped by layer, ecological region, and province). The boxes were based on the median values of the defined traits by the first and third quartiles, and the individual data points that fell outside the whiskers (1.5 times the interquartile range from the median) were considered as outliers. A General Linear Model multivariate analysis was performed for the genotype and ecological region when $p < 0.01$. The 18 selected traits were used for principal component analysis (PCA) for both the global and the local traits to identify the determinants of the root morphological variability across the genotypes. The PCA comprehensive values were ordered in an ascending order with the ecological regions and drawn into a column plot. Cluster centers of the six identified groups were generated by a dendrogram of the agglomerative hierarchical analysis. All the

figures were plotted using Origin Pro 2021b graphing packages (Origin Lab, Northampton, MA, USA).

3. Results

3.1. Variation of Root Phenotypic Characteristics at Seedling Stage

3.1.1. Global Traits

Global traits are important indicators for measuring the changes of root phenotypic characteristics among the 65 genotypes. Most of the traits were significantly different among cultivars ($p < 0.05$, $CV \geq 0.2$; Table 2), such as root diameter (RD, $CV = 0.21$), maximal root length (MRL, $CV = 0.22$), root number (RN, $CV = 0.23$), and leaf number (LN, $CV = 0.24$).

Table 2. Minimum, maximum, mean, medium, standard deviation (SD); coefficient of variations (CV); and p -value of global and local traits in 65 millet genotypes.

Traits	Minimum	Maximum	Median	Mean	SD	CV	p -Value
<i>Global traits</i>							
RW	1.5	14.5	8.50	7.36	3.65	0.50	0.000
MRD	0.6	11.1	3.15	4.70	3.07	0.65	0.000
MRL	4.0	13.4	9.83	9.61	2.16	0.22	0.000
RN	5.1	16.4	11.8	11.43	2.60	0.23	0.000
RDM	0.10	32.0	7.85	9.83	6.01	0.61	0.000
RL	69.4	779	325	338.08	137.80	0.41	0.000
RD	0.25	0.66	0.42	0.42	0.09	0.21	0.000
RA	12.6	84.75	42.63	43.91	17.69	0.40	0.000
RV	0.10	1.12	0.44	0.48	0.23	0.48	0.000
RLD	383	1469	712	7706	265.67	0.34	0.000
RWD	0.7	53.9	19.0	22.56	12.38	0.55	0.068
SRL	4.00	78.3	38.8	38.73	14.05	0.36	0.000
RSM	0.03	2.61	0.53	0.59	0.39	0.66	0.000
RGR	0.04	0.74	0.21	0.31	0.20	0.65	0.000
LN	3.00	19.00	11.3	11.14	2.69	0.24	0.000
SDM	3.50	69.1	16.0	18.49	10.68	0.58	0.000
TDM	3.50	80.9	25.6	28.32	14.13	0.50	0.000
RLR	0.31	1.95	0.66	0.80	0.37	0.46	0.001
RSAR	0.33	1.85	0.59	0.76	0.35	0.46	0.050
RVR	0.34	1.72	0.55	0.72	0.34	0.47	0.001
<i>Local traits</i>							
RDM_s1	0.001	16.6	5.45	6.00	2.62	0.44	0.000
RDM_s2	0.00	23.8	2.33	3.14	3.70	1.18	0.196
RDM_s3	0.00	7.33	0.30	0.72	1.17	1.63	0.000
RDM_sub	0.0	24.4	2.48	3.86	4.31	1.12	0.037
RL_s1	69	202	135	135	30.9	0.23	0.000
RL_s2	0.00	235	114.7	118	43.0	0.36	0.000
RL_s3	0.00	449	95.7	85.7	85	0.99	0.006
RL_sub	0.00	660	197	203	116	0.57	0.000
RD_s1	0.30	0.58	0.41	0.41	0.06	0.15	0.000
RD_s2	0.29	0.58	0.42	0.42	0.07	0.17	0.000
RD_s3	0.27	0.73	0.41	0.44	0.11	0.25	0.000
RD_sub	0.28	0.63	0.43	0.43	0.08	0.19	0.000
RA_s1	8.35	26.92	17.2	17.2	4.82	0.28	0.000
RA_s2	0.00	26.94	15.5	15.4	6.14	0.40	0.000
RA_s3	0.00	42.5	12.7	11.3	9.85	0.87	0.000
RA_sub	0.00	63.56	26.04	26.7	14.1	0.53	0.000
RV_s1	0.06	0.34	0.18	0.19	0.07	0.37	0.000
RV_s2	0.00	0.36	0.17	0.17	0.08	0.47	0.000
RV_s3	0.00	0.41	0.13	0.13	0.12	0.92	0.000
RV_sub	0.00	0.77	0.28	0.30	0.18	0.60	0.000
RLD_s1	383	1503	823	885	288	0.33	0.000

Table 2. Cont.

Traits	Minimum	Maximum	Median	Mean	SD	CV	p-Value
RLD_s2	0.00	1602	752	853	357	0.42	0.000
RLD_s3	0.00	1746	480	579	519	0.90	0.004
RLD_sub	325	1635	691	761	299	0.39	0.000
RWD_s1	0.007	79.56	30.63	36.3	18.0	0.50	0.000
RWD_s2	2.42	97.1	15.1	18.7	14.6	0.78	0.000
RWD_s3	0.25	27.6	4.82	6.53	6.72	1.03	0.095
RWD_sub	2.42	51.9	10.8	13.29	9.46	0.71	0.001

After 21 days of planting, the total root length (TRL) of 8 genotypes was less than 200 cm; that of 40 genotypes was between 200 and 400 cm; that of 15 genotypes was between 400 and 600 cm; and that of 2 genotypes was greater than 600 cm (Figure 2). Maximal root depth (MRD) was 11.13 cm (18#); root growth rate (RGR) ranged from 0.04 cm per day (33#) to 0.74 cm per day (18#); and root width (RW) ranged from 1.5 cm to 14.5 cm (mean value 7.36 cm). Root length (RL) ranged from 69.4 cm to 779 cm pouch⁻¹ (mean value 338 cm), and total root dry mass (RDM) ranged from 0.1 mg to 32.03 mg (mean value 9.83 mg). YuGu 11 (44#), from the summer maturing region, had the longest RL (778.97 cm), whereas LongGu 26 (#38), from the early maturing area of northeast China, had the shortest RL (69.37 cm). The RDM of Nong 2019 (11#), from the mid-late maturing region in northwest China, was the highest (32.03 mg pouch⁻¹) and that of LongGu 39 (33#), from northeast China, was the lowest (0.1 mg pouch⁻¹). At the same depth among the root layers, the roots varied greatly between genotypes and ecological regions. Among the seven provinces, the genotypes in Heilongjiang and Hebei had the shorter RLs. Gansu and Henan had longer RLs, while Shaanxi and Shanxi had the higher RDM (Figure 2a).

The root system was large in the genotypes from the early maturing area of northwest China (mean value 447 cm pouch⁻¹) and small in the genotypes from the early maturing area of northeast China (mean value 204 cm pouch⁻¹) (Figure 3), the largest one being 2.2 times greater than the smaller. The TDM in the maturing area of northwest China was the highest (mean value 38.5 mg pouch⁻¹) and that in the early maturing area of northeast China was the lowest (mean value 13.7 mg pouch⁻¹). The root length density (RLD) ranged from 383 cm cm⁻³ to 1469 cm cm⁻³ (mean value 770.6 cm cm⁻³). The root length ratio (RLR) ranged from 0.31 to 1.95, showing that there were different root distributions and morphological patterns among the 65 genotypes. The root diameter (RD) ranged from 0.25 mm to 0.66 mm (mean value 0.42 mm). The specific root length (SRL) ranged from 4.0 cm mg⁻¹ (38#, LongGu 26) to 78.3 cm mg⁻¹ (33#, LongGu 39), with a mean value of 38.7 cm mg⁻¹, and the genotypes from the early maturing area of the northwest China genotype had low a SRL (Figure S1).

3.1.2. Local Traits

Some local root traits had a CV value higher than 0.2, and the local traits of the genotypes in Section 1 had lower CV values than those in the other sections (Table 2). The results showed that the RL, RDM, RV, RLD, surface area (RA), and weight density (RWD) of the genotypes in Sections 2 and 3 were significantly different from those in the other sections, indicating that the difference in the root traits of the different genotypes is closely related to the depth of the root. Affected by the traits in Section 1, the root length ratio (RLR), root surface area ratio (RSAR), and root volume ratio (RVR) were also significantly different in all the local traits.

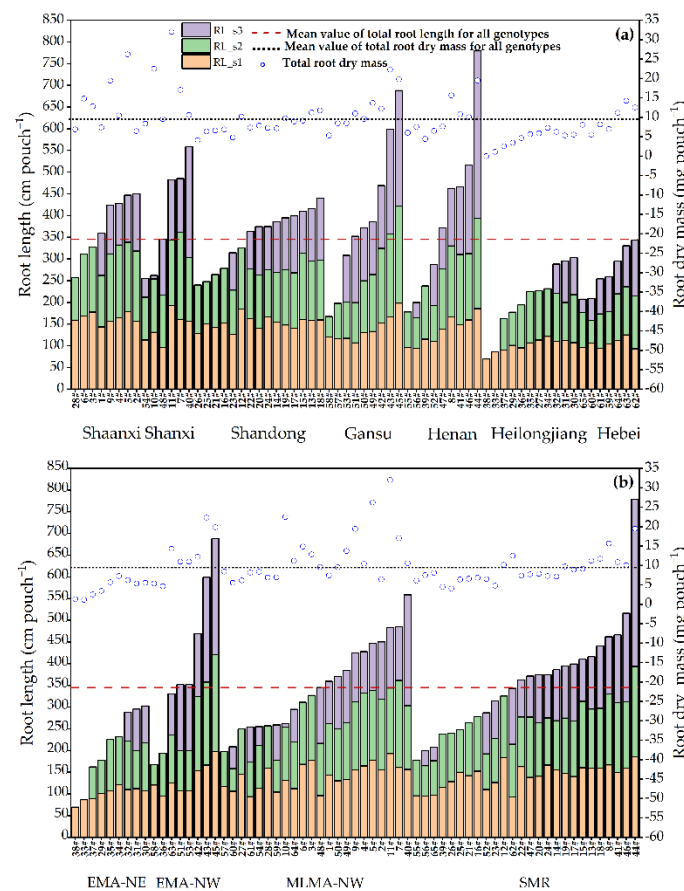


Figure 2. Variation of 65 millet genotypes shown by cumulative histograms: (a) root length and root dry mass ordered by seven provinces and (b) root length and root dry mass ordered by four ecological regions. Data were plotted from the lowest to the highest total root length (TRL) value; root lengths for the three sections were plotted in three different colors; RL_S1: root length in Section 1 (0–5 cm, orange bars); RL_S2: root length in Section 2 (5–10 cm, green bars); RL_S3: root length in Section 3 (>10 cm, purple bars). The abbreviations of four ecological regions: early maturing area of northeast China (EMA-NE), early maturing area of northwest China (EMA-NW), mid-late maturing region in northwest China (MLMA-NW), summer maturing region (SMR).

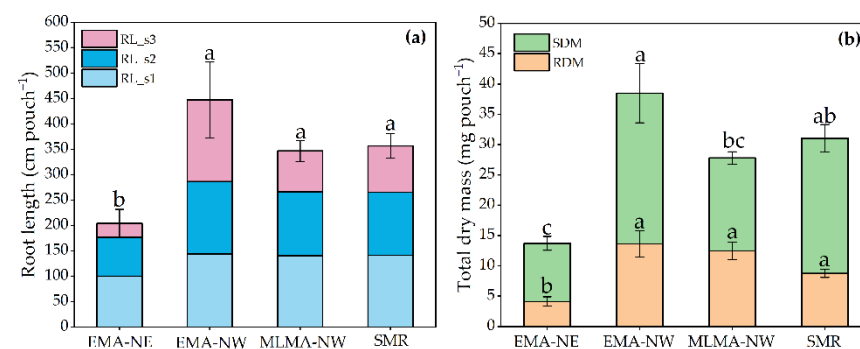


Figure 3. Variation of root length and total dry mass in 4 ecological regions: (a) root length in three layers and (b) shoot dry mass and root dry mass. Root data are the means for each ecological region. Root lengths in Section 1 (RL_S1, 0–5 cm), Section 2 (RL_S2, 5–10 cm), Section 3 (RL_S3, >10 cm) $\pm$ SE of total root length of all sections are presented. Ecological regions are ordered by the geographic location from north to south. Data are in mean $\pm$ SE. Bars of total root length or total root mass followed by different letters are significantly different, respectively ($p < 0.05$).

3.2. General Variation and Correlation among Traits

Forty-three traits were significantly different among the 65 genotypes ($p < 0.01$), and 45 traits had CV values higher than 0.2. Forty-four traits had greater variation ($p < 0.05$, $CV \geq 0.2$) in the global traits of root morphology (Table 2). RL, RA and RV decreased, while RD increased from top to bottom in the three layers (Figure 4). The RLs in the s1 (0–5 cm), s2 (5–10 cm), and s3 layer (>10 cm) were 1345, 118, and 86 cm, respectively (Table 2). The differences in RDM, shoot dry mass (SDM), SRL, and RL were significant among the four ecological regions (Figures S1 and S2), as also shown in the seven provinces (Figure S3).

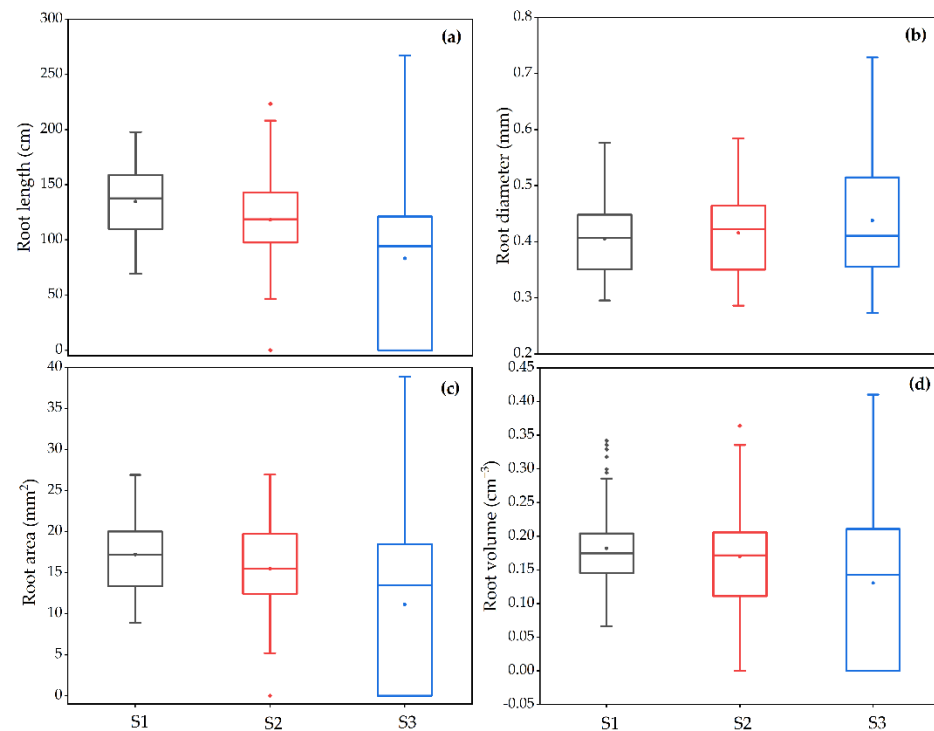


Figure 4. Variations of root phenotypic in the three root sections: (a) root length, (b) root diameter, (c) root area, and (d) root volume. Boxplots are limited to the first and third quartiles, and the middle line is the median. Four root traits were plotted. Three root sections: s1, 0–5 cm, colored grey; s2, 5–10 cm, colored red; s3 > 10 cm, colored blue.

Out of the 48 investigated traits, 18 traits with higher coefficients of variation ($CV \geq 0.2$) were selected to perform the Pearson correlation analysis (Table S2). Sixteen traits were correlated with each other ($p < 0.01$). RL was strongly correlated with SDM ($R^2 = 0.25$, $p < 0.01$, Figure 5a), RDM ($R^2 = 0.45$, $p < 0.01$, Figure 5b), MRL ($R^2 = 0.46$, $p < 0.01$, Figure 5c), and root number (RN) ($R^2 = 0.5$, $p < 0.01$, Figure 5d). The RL in each layer also had significant correlations with SDM, RDM, MRD, and RN ($p < 0.01$, Figure 5). The root length ratio (RLR) was positively correlated with the root surface area ratio (RSAR) ($R^2 = 0.96$, $p < 0.01$, Figure 6a) and the root volume ratio (RVR) ($R^2 = 0.81$, $p < 0.01$, Figure 6b). RD was negatively correlated with RLD ($R^2 = 0.86$, $p < 0.01$, Figure 6c) and RWD ($R^2 = 0.54$, $p < 0.01$, Figure 6d). RDM was positively correlated with RSR ($R^2 = 0.46$, $p < 0.01$, Figure S4a) and TDM ($R^2 = 0.47$, $p < 0.01$, Figure S4b).

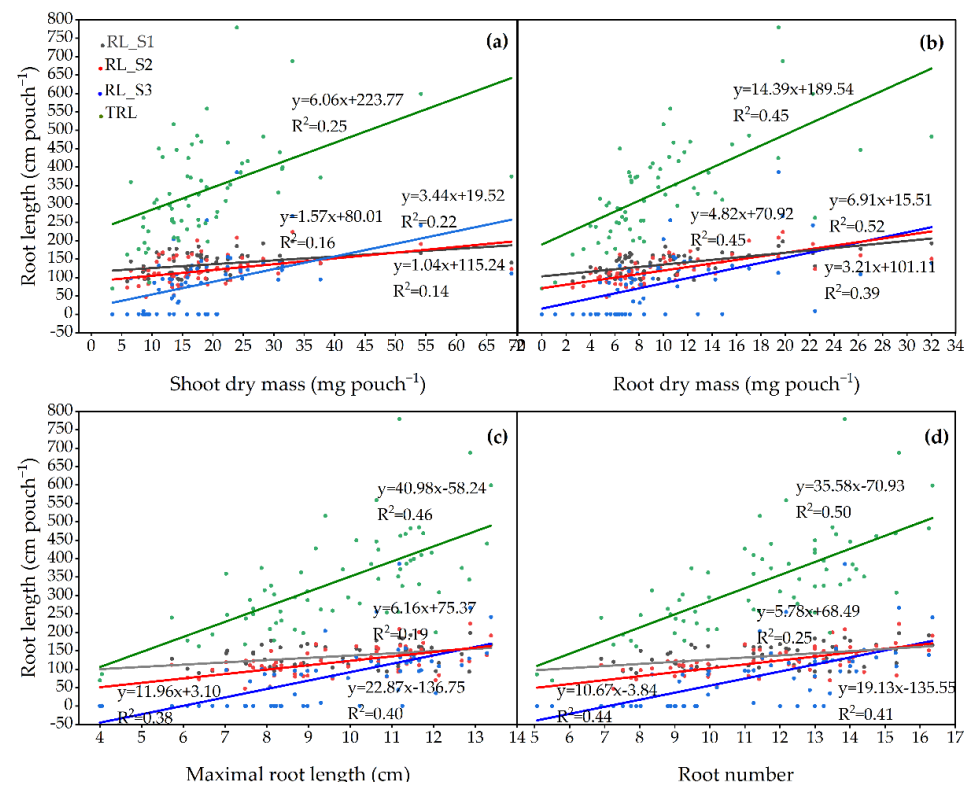


Figure 5. Linear fitting of root phenotypic characteristics: (a) root length vs. shoot dry mass; (b) root length vs. root dry mass; (c) root length vs. maximal root length; (d) root length vs. root number. Total root length (TRL) and root length in three sections (RL_S1, RL_S2, RL_S3) were used in the analysis.

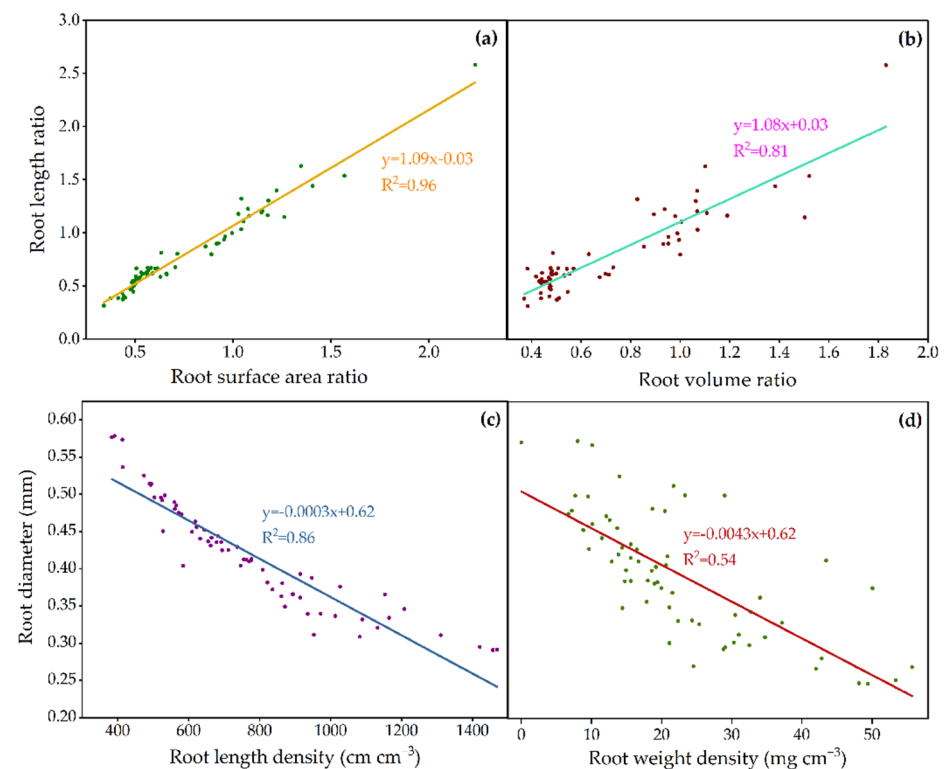


Figure 6. Linear fitting of root phenotypic characteristics: (a) root length ratio vs. root surface area ratio; (b) root length ratio vs. root volume ratio; (c) root diameter vs. root length density; (d) root diameter vs. root weight density.

3.3. Principal Component Analysis of Selected Traits

A principal component analysis (PCA) of the 18 selected traits ($p < 0.05$, $CV \geq 0.2$) was performed (Figure 7a), and it revealed that four principal components have eigenvalues greater than 1, which captured 86.6% of the variability (Table 3). Among the global traits, SDM, MRL, RN, RDM, and TDM were strongly associated with the ecological regions; meanwhile, RLR, RSAR, and RVR were strongly affected by the ecological regions (Figure 7a). Four traits, namely MRL, TRDM, RA, and root tissue density (RTD), made the greatest contribution to the PCA. In PC1 and PC2 of the PCA, the marginal cultivars had larger deviations from the mean values; they possibly had special traits and will be used for further research (Figure 7b). The PCA of seven local traits on the top layer accounted for 59.7% for PC, and the sub-layer accounted for 50.5% for PC (Figure 8). The composite values of the PCA were arranged from largest to smallest by ecological region (Figure S5); the varieties with higher composite values in each ecological region will be selected for future drought-stress studies.

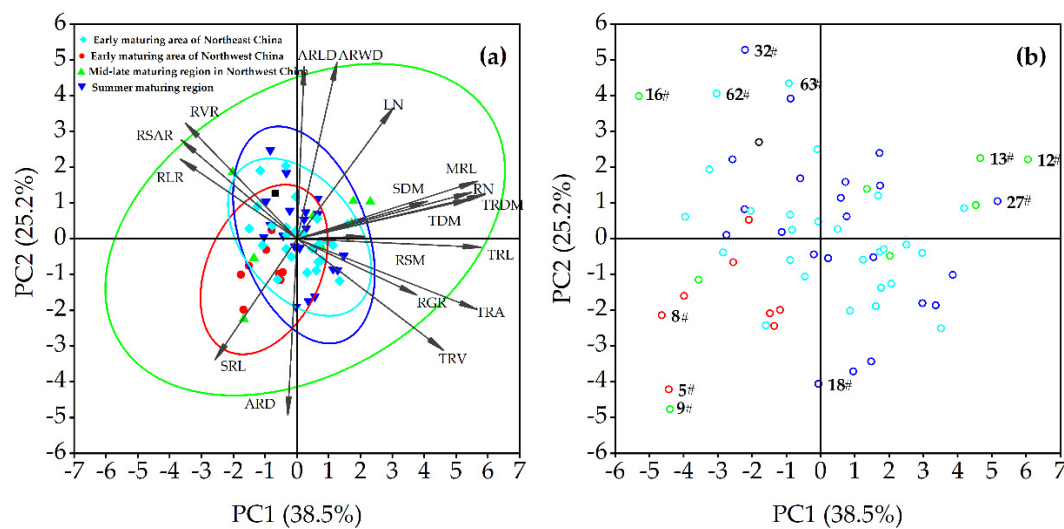


Figure 7. Principal component analysis of root and shoot phenotypes in four ecological regions: (a) distribution of 18 selected traits ($CVs \geq 0.2$) in principal component 1 and principal component 2 and (b) the distribution of 65 genotypes in principal component 1 and principal component 2. Different ecological regions were colored with different colors, respectively.

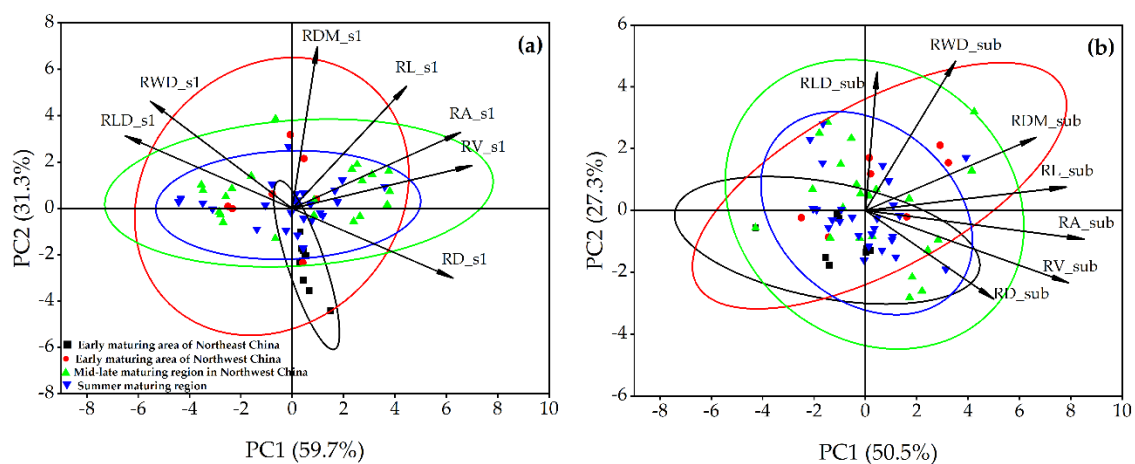


Figure 8. Principal component analysis of root phenotypes in the top layer (0–5 cm section) and the sub-layer (below 5 cm section): (a) seven root traits in the top layer and (b) seven root traits in the sub-layer, with genotypes presented by ecological regions.

Table 3. Principal component analysis (PCA) of 18 selected traits in 65 millet genotypes ($p < 0.05$, $CV \geq 0.2$).

Traits	Abbreviation	PC1	PC2	PC3	PC4
Leaf number	LN	0.31	0.71	−0.22	−0.05
Maximal root length (cm)	MRL	0.81	0.36	−0.07	0.18
Root number	RN	0.86	0.29	0.09	−0.06
Root dry mass (mg)	RDM	0.75	0.24	0.53	−0.07
Shoot dry mass (mg)	SDM	0.57	0.29	−0.30	0.66
Root length (cm)	RL	0.86	0.02	−0.13	−0.09
Root area (mm ²)	RA	0.87	−0.35	0.05	−0.03
Root volume (cm ³)	RV	0.73	−0.60	0.20	−0.01
Root diameter (mm)	RD	0.13	−0.91	0.25	0.13
Root length density (cm cm ^{−3})	RLD	−0.12	0.89	−0.22	−0.17
Root weight density (mg cm ^{−3})	RWD	0.01	0.88	0.38	−0.15
Specific root length	SRL	−0.22	−0.55	−0.71	−0.01
Root to shoot dry mass ratio	RSM	0.26	−0.08	0.82	−0.41
Root growth rate (cm d ^{−1})	RGR	0.55	−0.34	0.43	0.38
Total dry mass (mg)	TDM	0.77	0.33	−0.01	0.48
Root length ratio	RLR	−0.78	0.03	0.39	0.37
Root surface area ratio	RSAR	−0.80	0.14	0.39	0.35
Root volume ratio	RVR	−0.79	0.25	0.36	0.30
Variation proportion					
Eigenvalue		6.92	4.54	2.61	1.51
Variability (%)		38.46	25.20	14.50	8.40
Cumulative (%)		38.46	63.66	78.16	86.56

Note: Four principal components with eigenvalues >1 were extracted and considered significant.

3.4. Genotypic Population Identification Based on the Selected Traits

Sixty-five millet genotypes with 18 selected traits were separated into three main branches at a rescaled distance of 80, using the Euclidean mean distance method as the mileage pacer trigger (Figure S6). They were further separated into six groups when a rescaled distance of 60 was used. Group 1 included three groups with almost half of the genotypes (32 genotypes, 49.2%); group 2 included three groups of 17 genotypes (26.2%); group 3 included only one group with two cultivars (10# and 11#); group 4 included two groups with nine genotypes; and group 5 included two groups of four cultivars and one (20#) genotype, followed by group 6. The most striking observation to emerge from the data comparison was that the genotypes from the same province or ecological region were not put together in a group.

4. Discussion

Recently, the interest in root structure affecting crop yields to improve food security has been increasing [30,32,44,45]. In many ecosystems, air dehydration precedes soil dehydration [46]. Usually, the shoots of the plant are first exposed to reduce the water potential, after which the environment around the root system becomes increasingly arid [47,48]. In wheat, root biomass has been shown to decrease at high soil temperatures [49], and a deeper and more effective root system has been shown to be essential for absorbing more water from the soil and for significantly increasing wheat yield under drought stress [39,50,51]. Root growth is dynamic and spatially and temporally heterogeneous [52]; therefore, the changes in root structure depend on the environment [42]. The germination and the seedling growth of plants are necessary stages for the growth of plant populations [53,54]. During seedling development, plant functional traits can rapidly respond to altered environments; for example, the depth of the plant roots affects the ability of the ecosystem to recover from drought stress [55]. Deep roots link the deep soil and groundwater to the atmosphere, further affecting the hydrological cycle and climate [40]. Water and nutrient acquisition are closely related to these measured root traits [56] of MRD [57], RL, RDM, and

RLD in the three layers [26,44,58]. Therefore, it is very important to study the root system to improve the stress resistance and yield of plants [59].

In the present study, the traits of millet from the four ecological regions showed different performances. Millet cultivars from the early maturing area of northwest China had the greatest RL, RDM, SDM, and TDM (Figure 2). RL is an indicator of a plant's yield potential [60]. Root size, length, and architecture could improve water uptake, which were associated with increased drought tolerance in crop plants [61,62]. The RL and RDM were about two-fold higher than those in the cultivars from the early maturing area of northeast China. A moderate water deficit can promote the increase in root and shoot dry mass [63]; meanwhile, a plant will pass on the characteristics of a larger root system to the next generation and obtain higher yields [37,64]. The correlation coefficient between the RN and RL of the same root type, as well as among the RL, RV, and RA in hydroponics [65], was high, which is in line with those of previous studies [66]. A strong positive correlation was observed between RLR and the root ratio (RSR and RVR, Figure 6a,b), which illustrated that the root in the top and sub-layers had similar trends of RL, RA, and RV (Figure 4a,c,d). The high correlations between RDM, RSM, and RL indicated that root changes affect the ratio of aboveground and underground biomass, which is an important manifestation of a plant's response to environmental stress [67].

The root system architecture (RSA) is systematically shaped by the diversity of characteristics of a species and the evolutionary processes [65]. The longer RL and the greater RA indicate that the ability of a root to obtain nitrogen from the soil profile increased [68]. This study came to the same conclusion: millet cultivars from the early maturing area of northwest China had the largest RL among all the regions (Figure 3), which was significantly positively correlated with RA ($R = 0.901$, $p < 0.01$, Table S2). SRL reflects the absorption capacity of roots per unit of biomass input and is also a measure of root thickness, and thus, it is considered to be one of the most important traits affecting the exploitation and uptake of water and nutrients, taking into account the economic cost of root establishment [69–71]. In a field experiment, it was found that genotypes with lower SRL, from a 0–60 cm depth, produced higher yields, suggesting that thicker roots in the topsoil represent a favorable feature for obtaining deep water in soil [72,73]. Millet cultivars from the early maturing area of northwest China had low SRL (Figure S1c), which could be associated with high yield. Future studies will validate these genotypes in the field conditions.

Root diameter is a powerful predictor of penetration strength in hard soils, and greater RD may result in greater hydraulic conductivity [74]. A comparison of the findings with those of other studies confirms that RD was negatively correlated with root density (RLD and RWD, Figure 6c,d). The cultivars from different ecological regions showed some variation, reflecting the potential for their ecological and climatic linkage and the need for further research. Mild water stress can expand the root system and improve RWD and RLD. The development of longer roots and higher dry weights in the upper soil saves irrigation water [63]. Moreover, water deficiency leads to the redistribution of carbon during root growth, as well as a decrease in RLD in the topsoil layer, but an increase in RL. Thus, RLD is the key factor for assessing the amount of nutrients and water absorbed by the root system per unit volume and is an important symbol of the drought-tolerant varieties [75]. Drought treatments can increase the density of roots in deep soil; however, the pearl millet with deep roots cannot grow well in a moist environment [37,76].

Because the growth of a field plant is irreversible, the root morphology and topological structure encode traits derived from “morphological memory” and provide clues to unlock the secrets of the plant–environment interaction [30,77,78]. The exploration of 65 millet genotypes at the seedling stage did not allow us to classify them by root diameter due to the smaller differences in this factor. However, there are still some limitations in transforming the phenotype data obtained from hydroponic systems at the early stage into field conditions in the breeding programs. Modern root imaging technology and root modeling simulations could provide efficient tools to assist in root phenotyping studies in different environments.

5. Conclusions

The root system morphological traits of foxtail millet at the seedling stage from different ecological regions differed significantly. Millet cultivars from the early maturing area of northwest China had the largest root systems (root length, shoot dry mass, root dry mass, and root density), while those from the early maturing area of northeast China had the smallest root systems. The outcomes of this study will facilitate our follow-up studies on the response of millet to drought and other abiotic stresses for improved grain yield under field conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12061472/s1>, Figure S1: Variations of root and shoot phenotypes among the 4 ecological regions, Figure S2: Variations of root phenotypic among the 4 ecological regions, Figure S3: Variations of root and shoot phenotypes traits in the 7 provinces, Figure S4: Linear fitting of root phenotypes, Figure S5: Comprehensive values of 18 selected traits, Figure S6: Dendrogram of agglomerative hierarchical clustering (AHC) of 18 selected root traits, Table S1: Cultivar, origin, seed provider, ecological region of 65 millet genotypes, Table S2: Pearson's correlation matrix for 18 selected traits of 65 millet genotypes.

Author Contributions: Conceptualization, J.Y. and Y.C.; methodology, X.Y., J.Y. and Y.C.; software, X.Y.; formal analysis, Q.T.; data curation, X.Y. and Q.T.; writing—original draft preparation, X.Y. and Q.T.; writing—review and editing, Y.C. and J.Y.; supervision, J.Y.; funding acquisition, J.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, 31960223; the Key Research and Development Program of Shaanxi Province, 2021NY-101; and the Science and Technology Program of the Yulin Science and Technology Bureau, CXY-2020-100.

Data Availability Statement: Not applicable.

Acknowledgments: We gratefully acknowledge Ling Qin of Shandong Academy of Agricultural Sciences; Zhijiang Li of Heilongjiang Academy of Agricultural Sciences; Miao Jing of Yulin Academy of Agricultural Sciences, who provided seed materials for this study. We also acknowledge Rui Liu, Mengjia Zhang, Zexu Zhou, and Yu Li for their assistance with sample measurement.

Conflicts of Interest: The authors declare no conflict of interest.

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