

## Article

# Comprehensive Evaluation of Root Morphological Indices of 204 *Gossypium hirsutum* Germplasm in Seedling Stage

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## Abstract

The root phenotype of cotton seedlings serves as a criterion for selecting high-vigour varieties, yet systematic evaluations of Upland Cotton germplasm resources remain limited. We measured nine root- and shoot-related traits in 204 *Gossypium hirsutum* accessions from diverse ecological regions. Correlation analysis revealed that root surface area, volume, and fresh weight were closely interrelated indicators. Principal component analysis extracted two biologically meaningful factors—‘absorptive capacity’ and ‘morphological strategy’—with a cumulative contribution of 55.72%. Cluster analysis classified the accessions into three functional groups: high-yield potential ( $n = 15$ ), balanced adaptation ( $n = 135$ ), and fine-root dominance ( $n = 54$ ). These clusters corresponded with ecological origins and breeding periods, reflecting local adaptation. We propose root surface area, volume, and fresh weight as cost-effective screening indicators and identified 15 elite accessions as potential parents for root-centred breeding. These findings are particularly relevant for the arid production environments of Northwestern China.

**Keywords:** cluster analysis; *Gossypium hirsutum*; morphological indicators; principal component analysis; seedling root system

## 1. Introduction

In order to cultivate cotton varieties with a high yield and stress tolerance, it is necessary to deeply understand the characteristics of root variation. However, it is surprising that few studies have systematically described the root morphological characteristics of different germplasm resources in the early development stage. This knowledge gap is particularly prominent in Xinjiang—in 2025, Xinjiang’s cotton production accounted for 92.2% of the country’s total (5.686 million tons, 2.4619 million hectares of planting area; National Bureau of statistics, 2025). The root system affects the efficiency of water and nutrient absorption, as well as the accumulation of above-ground biomass, boll development and fibre quality [1]. Varieties with more developed root systems have stronger drought and salinity tolerance [2], and the distribution of photosynthetic products to the reproductive organs is relatively favourable. Therefore, research on the morphological diversity of roots and their genetic basis should be carried out to develop suitable varieties for the arid production environment in Xinjiang.



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Root Structure Analysis (RSA) generally refers to all kinds of morphological indices of the primary root, such as the length and number of lateral roots, surface area and volume [3,4], branch structure, and the number of root tips; physiological parameters generally include root activity, absorption area, secretion components, hormone synthesis and translocation (e.g., cytokinin and abscisic acid), and nutrient absorption kinetics. Together, the above-mentioned structural and functional characteristics determine the plants' ability to obtain soil resources and face environmental stresses for above-ground growth; thus, they have been the subjects of research in root biology and crop ecology.

Sun Si Min and others [5] used 220 natural populations and 325 introduced populations, combined with the effect of interspecific heterosis, to demonstrate that the introduced populations had significant advantages in root traits and provided high-quality parent resources for genetic improvement. Li Tingting [6] showed that the cotton taproot system consists of a main taproot, side roots and fine roots; it has an inverted cone shape, the mature root can reach a depth of about 2 metres, and the lateral roots are about 1.2 metres long horizontally. Due to the problems associated with the large-scale collection of phenotypic data for cotton roots, genetic analysis is still relatively limited [7]. Previous research has mainly studied the shape and physiology of roots [8]; Zhang Zhongbo et al. [9] investigated the different growth dynamics of cotton roots in various ecological environments and cultivation systems, and Fry et al. [10] studied the structure of cotton roots under drought stress, finding that the root length density increased, the average root diameter decreased, and the ratio of root surface area to root volume rose under drought stress; also, by altering root topology (e.g., extending the depth of branches) and enhancing the proportion of deep soil moisture utilisation, the drought tolerance of the plant was increased. Maurel et al. [11] carried out related studies. A root hydraulic model was constructed to study the impact of structural regulation on plant growth; Zhang et al. [12] have built a virtual cotton root system using the GREENLAB functional structure model and realised visual simulations of root spatial structure; through correlation analysis, other studies have determined that root length, root surface area and root volume can be employed as potential screening indicators for cotton heat resistance [13–16].

A total of 204 natural populations of upland cotton at home and abroad were taken as the subjects for measuring and an all-encompassing assessment of root correlation at the seedling stage in this study. This provides a theoretical basis and high-quality resources for the genetic improvement of strong-root cotton to increase cotton yield and enhance water- and fertiliser-use efficiency. Characterisation of Genetic Variation in Seedling Root Morphological Indices of 204 Upland Cotton Germplasm, Identification of Core Root-Related Indices Associated with Biomass Accumulation, Analysis of Adaptability Differences of Root Structure in Different Ecological Cotton Areas, Elucidation of the Internal Correlation Mechanism between Root Morphology and Ecological Adaptability, Statistical Classification of Root Types, and Selection of Parents with Good Root Traits for Genetic Improvement.

## 2. Materials and Methods

### 2.1. Test Materials

A total of 204 *Gossypium hirsutum* varieties from different sources were tested. Among them, 95 were from the Northwest Inland Cotton Region of China, 54 from the Yellow River Basin cotton region, 25 from the Yangtze River Basin cotton region, 11 from the northern ultra-early cotton region, 6 from the former Soviet Union, 2 from Africa, 10 from the United States, and 1 from Central Asia.

All cotton varieties used in this study were provided by the Cotton Research Institute, Xinjiang Academy of Agricultural Sciences, Key Laboratory of Cotton Genetic Improvement and Smart Production of Xinjiang. These varieties cover four breeding periods from

1950 to 2022: 1950–1969, 1970–1989, 1990–2009, and 2010–2022. For each variety, detailed background information—including pedigree, release year, breeding region, parental combinations, and primary breeding objectives—was obtained from the research group’s variety registration and breeding records. All root-related phenotypic data presented in this study (e.g., root surface area, volume, fresh weight, dry weight, and other morphological traits) were collected through our own experiments following the protocols described below. By combining the historical background data with our experimental measurements, we analysed the temporal trends in root system characteristics across different breeding eras.

## 2.2. Experimental Design

Select 60 plump seeds from each material sample, surface sterilise, then perform germination using the three-layer filter paper method (3 replicates): roll the filter paper into a cylindrical shape and place it vertically in a culture box, with the upper part wrapped in a sterile plastic bag for moisture retention. Cultivate at 28 °C and 80% humidity in a dark incubator for 7 days. Subsequently, transfer the seeds to a hydroponic system using 1/2 Hoagland nutrient solution, replacing the nutrient solution every 3 days. We nurtured the seeds under a temperature of 28 °C, 85% humidity, and a 16 h light period followed by an 8 h dark cycle. Each variety was represented by three biological replicates, with 10 individual plants per replicate. Light was provided by full-spectrum LED lamps at a photosynthetic photon flux density of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

## 2.3. Investigated Traits and Methods

Samples were collected at the three-leaf stage, and the fresh weight of roots was determined after cleaning. Specifically, roots were rinsed three times with deionised water, and any visible debris was manually removed with forceps during spreading. The root system was scanned using a root scanner (EPSON Perfection V800, Epson (China) Co., Ltd., Beijing, China) (3 replicates per material). The cleaned root system was placed in an organic glass box lined with thin water and carefully spread with tweezers to avoid overlapping or crossing. For two-dimensional array preparation, the primary root was aligned along the tray, lateral roots were spread to both sides in a herringbone pattern, and adjacent roots were kept at least three diameters apart to avoid overlap. Photographs were taken and saved, followed by quantitative analysis using the WinRhizo (Wseen (China) Co., Ltd., Hangzhou, China) root system analysis software.

The specific traits investigated are as follows:

**Root Surface Area (RSA):** The root system was laid flat on the scanner tray (avoiding crossing or overlapping) and scanned in grayscale mode with a resolution of  $\geq 600$  dpi. The software automatically calculated the total root surface area ( $\text{mm}^2$ ).

**Root Volume (RV):** Measured synchronously with root surface area, it is calculated by the software as the total volume ( $\text{mm}^3$ ) based on root diameter distribution and length.

**Root Average Diameter (RAD):** The software automatically measures the diameter of each root segment and calculates the weighted average (mm).

**Taproot Length (TRL):** The software automatically calculates the length (in cm) by marking the main root path on the scanned image.

**Root Fresh Weight (RFW):** After washing the root system, the sample surface was dried with absorbent paper (to remove all residual moisture) and it was weighed using a 0.1 g analytical balance (g).

**Root Dry Weight (RDW):** After measuring the fresh weight of the root system, it was placed in a 105 °C oven for 30 min to inactivate enzymes, then dried at 80 °C until a constant weight was achieved. The weight was recorded using a 0.1 g analytical balance (g).

Stem Length (SL): The linear distance measured with a ruler from the cotyledon node to the stem apex growth point (cm).

Stem and Leaf Fresh Weight (SLFW): The stem and leaves were separated, the surface moisture was absorbed with absorbent paper, and the weight was recorded using a 0.1 g analytical balance (g).

Stem and Leaf Dry Weight (SLDW): After determining the fresh weight of stems and leaves, they were heated in a 105 °C oven for 30 min to inactivate enzymes, then dried at 80 °C until a constant weight was achieved. The weight was recorded using a 0.1 g analytical balance (g).

#### 2.4. Data Processing

Data were organised using Excel 2019, with correlation analysis, principal component analysis, and cluster analysis performed using SPSS 26.0 software.

### 3. Results

#### 3.1. Correlation Among Traits During the Seedling Stage of *Gossypium Hirsutum*

The correlation analysis in Figure 1 demonstrates a highly significant positive correlation between root surface area, volume, and stem and leaf fresh weight, indicating that the root system serves as the foundation for above-ground growth. The more ideal the root development, the higher the biomass of the stem and leaves. There is a strong positive correlation among root surface area, root volume and root fresh weight; these three can be taken as the main indicators for assessing root growth, and the correlations with root dry weight, stem and leaf dry weight, and most other indicators are weak and not significant. Dry weight reflects the amount of organic matter, and other indicators show the current growth status and thus have different physiological meanings. The results showed that the indicators of plant root growth (surface area, volume, and fresh weight) were highly synergistic with those of stem and leaf biomass (fresh weight); root fresh weight and stem and leaf fresh weight were the key indices reflecting the overall growth of the plant, and root dry weight showed strong independence and may need to be combined with other physiological indicators (such as lignin content) for separate analysis. No substantial negative correlations were observed among the measured growth indicators, suggesting that plant development is promoted through synergistic interactions among these traits, rather than through trade-offs.

#### 3.2. Principal Component Analysis

The KMO value was 0.705 ( $>0.7$ ), and the results of the Bartlett sphericity test were significant ( $p < 0.001$ ); therefore, root surface area, root volume, root diameter, root length and root fresh weight were suitable for principal component analysis. Principal component analysis (Table 1) found three principal components that together accounted for 68.674% of the total variance in the original nine indicators, thus covering most of the information. The first principal component (PC1) has the largest proportion of variance (39.665%) and serves as a primary explanation factor; it is positively correlated with root surface area, root volume, root fresh weight and stem and leaf fresh weight, indicating the biomass accumulation capacity of the root system, and is thus named the “comprehensive biomass factor”. The contribution rate of the second principal component (PC2) was 16.053%, and it was positively correlated with the dry weight of stems and leaves but negatively correlated with the average root diameter; therefore, it reflected the features of root morphological differentiation and dry matter distribution and was designated as the “morphological differentiation factor”. The contribution rate of the third principal component (PC3) is 12.956%, and it is positively correlated with root length (0.592) and stem length (0.552) (absolute

load value >0.5), so it has vertical growth potential and is referred to as a “vertical growth factor”. Root surface area, root volume and root fresh weight all showed the highest load value (>0.85) in PC1, and correlation analysis indicated a significant positive correlation ( $p < 0.01$ ); thus, they were selected as the core indices for seedling root identification.



**Figure 1.** Phenotypic correlation analysis of 204 *Gossypium hirsutum* varieties. RSA: root surface area; RV: root volume; RAD: root average diameter; TRL: taproot length; RFW: root fresh weight; RDW: root dry weight; SL: stem length; SLFW: stem and leaf fresh weight; SLDW: stem and leaf dry weight. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**Table 1.** Principal component analysis results of *G. hirsutum* seedling stage traits.

| Traits                           | Principal Component 1 | Second Principal Component | Third Principal Component |
|----------------------------------|-----------------------|----------------------------|---------------------------|
| RSA                              | 0.863                 | −0.059                     | −0.378                    |
| RV                               | 0.861                 | −0.211                     | −0.325                    |
| ARD                              | 0.397                 | −0.613                     | 0.210                     |
| RL                               | 0.517                 | −0.269                     | 0.592                     |
| RFW                              | 0.876                 | 0.157                      | −0.102                    |
| RDW                              | −0.017                | 0.350                      | −0.434                    |
| SL                               | 0.458                 | 0.475                      | 0.552                     |
| SLFW                             | 0.818                 | 0.233                      | 0.040                     |
| SLDW                             | 0.103                 | 0.722                      | 0.130                     |
| Eigenvalue                       | 3.570                 | 1.445                      | 1.166                     |
| Contribution rate (%)            | 39.665                | 16.053                     | 12.956                    |
| Cumulative contribution rate (%) | 39.665                | 55.718                     | 68.674                    |
| Weight (%)                       | 57.758                | 23.376                     | 18.866                    |

RSA: root surface area (mm<sup>2</sup>); RV: root volume (mm<sup>3</sup>); RAD: root average diameter (mm); TRL: taproot length (cm); RFW: root fresh weight (g); RDW: root dry weight (g); SL: stem length (cm); SLFW: stem and leaf fresh weight (g); SLDW: stem and leaf dry weight (g). PC1, PC2, and PC3 represent the first, second, and third principal components, respectively. Contribution rate (%) indicates the proportion of total variance explained by each principal component. Cumulative contribution rate (%) is the sum of variances explained by the selected principal components. Weight (%) denotes the relative weight of each principal component in the comprehensive evaluation, calculated as the ratio of its contribution rate to the cumulative contribution rate.

### 3.3. Cluster Analysis

Based on nine morphological indices of root and stem (root surface area, root volume, average root diameter, root length, root fresh weight, root dry weight, stem length, stem and leaf fresh weight, stem and leaf dry weight), 204 *Gossypium hirsutum* varieties were clustered and divided into three groups with significant characteristics (Table 2).

**Table 2.** Clustering analysis of seedling stage indices of 204 *Gossypium hirsutum* germplasm.

|                        | Group 1 (n = 15) | Cluster Category<br>Group 2 (n = 135) | Group 3 (n = 54) | F       | p         |
|------------------------|------------------|---------------------------------------|------------------|---------|-----------|
| RSA (cm <sup>2</sup> ) | 294.044 ± 68.275 | 61.201 ± 15.493                       | 110.965 ± 24.364 | 603.004 | 0.000 *** |
| RV (cm <sup>3</sup> )  | 11.52 ± 5.333    | 1.699 ± 0.613                         | 2.978 ± 1.12     | 251.315 | 0.000 *** |
| ARD (mm)               | 0.634 ± 0.123    | 0.524 ± 0.133                         | 0.489 ± 0.137    | 8.303   | 0.000 *** |
| RL (cm)                | 29.64 ± 2.904    | 25.143 ± 4.923                        | 24.184 ± 4.239   | 9.972   | 0.000 *** |
| RFW (g)                | 4.683 ± 1.131    | 1.931 ± 0.71                          | 2.649 ± 1.177    | 74.538  | 0.000 *** |
| RDW (g)                | 0.066 ± 0.021    | 0.068 ± 0.023                         | 0.075 ± 0.044    | 1.072   | 0.344     |
| SL (cm)                | 31.56 ± 4.656    | 26.927 ± 6.231                        | 27.42 ± 4.846    | 5.162   | 0.007 *** |
| SLFW (g)               | 14.427 ± 4.607   | 6.023 ± 2.975                         | 7.602 ± 2.869    | 57.766  | 0.000 *** |
| SLDW (g)               | 0.405 ± 0.111    | 0.424 ± 0.145                         | 0.539 ± 0.135    | 15.406  | 0.000***  |

Note: \*\*\* represent significance levels of 1%.

Group 1 (High-yield Potential Type, 15 samples): Root system indicators (root surface area, root volume, fresh weight) and stem/leaf fresh weight were all significantly higher than in other groups ( $p \leq 0.01$ ). The roots had a small average diameter and were relatively long. ‘Xinlu Zao 78’ and ‘Zhongmiansuo 63’ are typical materials with strong biomass accumulation capabilities. It is expected that these traits can increase the area for water and nutrient absorption by roots, thereby enhancing the efficiency of utilisation in high-fertility cotton-growing areas.

Cluster 2 (Equilibrium-adapted Type, 135 samples): All indicators were at moderate levels, there was good synergy between root length and average root diameter, and a significant positive correlation existed between stem length and fresh root weight ( $r = 0.354^{**}$ ). This cluster accounted for the highest proportion of materials (66.2%) and included ‘Lumianyan 28’ and ‘Jimian 169’, which have broad ecological adaptability and are suitable for extensive cultivation in a conventional water and fertiliser environment.

Group 3 (Fine-root Dominant Type, 54 samples): The average root diameter was significantly smaller than in the other groups ( $p \leq 0.05$ ), but it had a high root length density and a larger root surface area/volume ratio. Representative materials are ‘Yumian 11’ and ‘Chuancotton 234’. A large number of fine roots help to take up nitrogen from the soil efficiently and thus have an advantage in low-nitrogen soils.

The clustering results are in agreement with the “root system size” and “morphological differentiation” factors determined by principal component analysis, and thus the core indicator screening is considered reasonable. Differences in the ecological adaptability of the various taxons provide concrete materials for regional cotton breeding.

### 3.4. Differences in Root System Characteristics Across Ecological Cotton Regions

Through phenotypic analysis and multivariate statistics of nine root and shoot traits at the seedling stage of 204 *Gossypium hirsutum* germplasm accessions, we revealed the genetic variation characteristics of cotton root traits. Correlation analysis showed that root surface area, root volume, and root fresh weight were highly significantly positively correlated with each other ( $p < 0.01$ ) and also with shoot and leaf fresh weight, indicating that these indicators effectively reflect the overall biomass accumulation capacity of the plant. Principal component analysis extracted two core factors—“root system size” and

“morphological differentiation”—with a cumulative contribution rate of 55.72%. Among these, root surface area, root volume, and root fresh weight had loadings >0.86 on the first principal component, confirming them as key indicators for evaluating seedling root system size. Cluster analysis classified the 204 accessions into three types: high-yield potential (15 accessions), balanced adaptation (135 accessions), and fine-root dominance (54 accessions), corresponding to high biomass accumulation, drought-resistant efficiency, and nitrogen absorption characteristics, respectively. Differences in root traits among the three types were highly significant, while root dry weight showed no significant difference among types, further confirming that root dry weight is more influenced by post-transplant environmental conditions and dry matter translocation, with relatively lower genetic stability. These genetic analyses provide a phenotypic basis for future QTL mapping and gene discovery related to root architecture.

### 3.4.1. Differences in Root Morphological Characteristics Across Ecological Cotton Regions

In general, the root system indices in the various ecological cotton areas differed significantly from one another. The Northwest Inland Cotton Region consistently had the most developed root system, and among them, it had the largest root surface area, root volume and fresh root weight. The Yangtze River Basin had the least extensive root system and exhibited lower values for most of the parameters. The Yellow River Basin and the foreign cotton areas showed intermediate or different characteristics, and the Northern Early Maturity Cotton Region was marked by a long root. The reasons for the above differences are shown in Table 3.

**Table 3.** Comparison of root system indices in *Gossypium hirsutum* seedlings across different ecological cotton regions.

| Cotton-Growing Area                             | n  | Root Surface Area (cm <sup>2</sup> ) | Root Volume (cm <sup>3</sup> ) | Average Root Diameter (mm) | Root Length (cm) | Fresh Root Weight (g) |
|-------------------------------------------------|----|--------------------------------------|--------------------------------|----------------------------|------------------|-----------------------|
| Northern Early Maturity Cotton Region           | 11 | 72.872                               | 1.797                          | 0.467                      | 26.696           | 2.462                 |
| Cotton-growing areas in the Yellow River Basin  | 54 | 75.315                               | 1.987                          | 0.500                      | 24.732           | 2.123                 |
| Northwest Inland Cotton Region                  | 96 | 123.936                              | 4.135                          | 0.544                      | 25.699           | 2.699                 |
| Cotton-growing areas in the Yangtze River Basin | 25 | 67.607                               | 1.543                          | 0.455                      | 23.984           | 2.005                 |
| Foreign cotton regions                          | 18 | 75.203                               | 2.328                          | 0.604                      | 25.288           | 2.094                 |

Note: The first column lists the names of different ecological cotton regions from which *Gossypium hirsutum* seedlings were collected. These regions represent major cotton production areas used for comparative analysis of root system indices.

#### Regional Analysis in Detail:

1. Northwest Inland Cotton Region: It has the most developed root system of all cotton-growing areas, with a root surface area of 123.936 cm<sup>2</sup>, a root volume of 4.135 cm<sup>3</sup> and a root fresh weight of 2.699 g; all of these are higher than those in other areas for growing cotton in China. This area is a hot, sunny place with little rain. A big root system can draw water and nutrients from a wide area to reduce the damage of drought and live in an arid environment.
2. Cotton-growing Areas in the Yellow River Basin: The root surface area (75.315 cm<sup>2</sup>) and root volume (1.987 cm<sup>3</sup>) are the second highest among all regions, second only to those in the Northwest Inland Cotton Area, and have a mean root diameter of 0.500 mm. The root system is relatively round, so it is suitable for the soil conditions of a semi-humid area and can meet the requirements of nutrient absorption and plant support.

3. Foreign Cotton Regions: The average root diameter was the largest (0.604 mm), and the root system was more robust; however, root volume and fresh weight were not significantly higher, suggesting fewer root branches or a slower growth rate.
4. Northern Early Maturity Cotton Area: It has a relatively long root length (26.696 cm), is capable of penetrating deep into the soil by forming a longitudinal root system and can access nutrients from deep soil layers relatively early in a cold, shallow-soil environment. Given that it is an “early-maturing” type, its root system likely has a relatively short growth cycle and needs to establish a high-efficiency absorption system quickly to meet the early developmental demands.
5. Cotton-growing Areas in the Yangtze River Basin: The root system is the most fragile, with a low root surface area (67.607 cm<sup>2</sup>), an average root diameter of 0.455 mm, and a root volume of 1.543 cm<sup>3</sup>. In a humid climate, fine roots can quickly absorb water from the upper part of the soil to avoid hypoxia caused by waterlogging and are thus suitable for high-humidity environments.

#### 3.4.2. Correlation Between Root System Characteristics and Environment

Overall, the root system characteristics of *Gossypium hirsutum* seedlings varied distinctly across different ecological cotton regions, reflecting adaptive strategies to local climatic and soil conditions. In the Northwest Inland Cotton Region, the root system tends to be “broad and deep”, enhancing water absorption capacity in arid environments by increasing surface area and volume. In the Yellow River Basin, the root system develops in a balanced way, suitable for a semi-humid climate. In the Yangtze River Basin, the root system is slender and shallow, and it is adapted to moist environments with sufficient shallow water, which helps avoid waterlogging and hypoxia. The Northern Early Maturity Cotton Region prioritises vertical growth, rapidly acquiring deep nutrients by extending root length to adapt to a short-season climate. In foreign cotton regions, the coarse-root characteristics might be related to specific cultivation needs, but the comprehensive growth indices are not significantly better than those in the Northwest Inland Cotton Region of China.

Detailed analysis by region:

1. In the Northwest Inland Cotton Region, the root system tends to be “broad and deep”, which enhances water absorption capacity in arid environments by increasing surface area and volume.
2. The root system of cotton in the Yellow River Basin developed in a balanced way, which was suitable for a semi-humid climate.
3. Cotton-growing areas in the Yangtze River Basin: The root system is slender and shallow, adapted to moist environments with sufficient shallow water, and avoids waterlogging and hypoxia.
4. Northern Early Maturity Cotton Region: Root system prioritises vertical growth and rapidly acquires deep nutrients by extending root length to adapt to a short-season climate.
5. Foreign cotton regions: The coarse-root characteristics might be related to specific cultivation needs, but the comprehensive growth indices were not significantly better than those in the Northwest Inland Cotton Region of China.

#### 3.5. Evolution of Root System Characteristics in Different Varieties Across Generations

In general, the development of the root system of *Gossypium hirsutum* in China from the 1950s to now has gradually changed due to changes in the cultivation area, breeding goals and technological progress. From 1950 to 1969, the root system was relatively small and had a weak absorption capacity; therefore, most of the cotton cultivation was

concentrated in the Yellow River Basin and the Northern Early Maturity Cotton Region. Varieties introduced from the former Soviet Union showed slightly larger root system indicators than those at home. From 1970 to 1989, the scale of the root system increased evenly in both diameter and length. Cultivation areas expanded to the Yangtze River Basin and the Northwest Inland, and breeding objectives shifted to high yields, increasing root-expansion capacity for support of above-ground growth. From 1990 to 2009, Xinjiang cotton varieties in the Northwest Inland Cotton Region showed excellent root system performance and had robust, deep-rooted systems with explosive biomass growth and coordinated development of roots and shoots. Given the scarcity of water in Xinjiang, many varieties have been selected for their deep-rooted systems and high absorption efficiency in water-saving agriculture for high-yield production. From 2010 to 2022, Xinjiang cotton varieties have shown excellent root system performance and formed an extended network of functional specialisation. Molecular breeding technology was used to modify the root structure for high yields and low resource consumption under climate change. See Table 4.

**Table 4.** Comparison of root system indices in seedling stage of different *Gossypium hirsutum* varieties across different years.

| Year Period | n   | Root Surface Area (cm <sup>2</sup> ) | Root Volume (cm <sup>3</sup> ) | Average Root Diameter (mm) | Root Length (cm) | Fresh Root Weight (g) |
|-------------|-----|--------------------------------------|--------------------------------|----------------------------|------------------|-----------------------|
| 1950–1969   | 23  | 88.3                                 | 2.1                            | 0.44                       | 24.3             | 2.6                   |
| 1970–1989   | 10  | 92.5                                 | 2.4                            | 0.48                       | 23.8             | 2.3                   |
| 1990–2009   | 50  | 132.8                                | 4.3                            | 0.55                       | 24.7             | 3.1                   |
| 2010–2022   | 121 | 163.6                                | 7.2                            | 0.56                       | 27.2             | 3                     |

Detailed analysis by period:

1. 1950–1969: The root system was relatively small and had poor absorption abilities. The two main areas of cotton production were the Yellow River Basin (Shaanxi and Shanxi) and the Northern Early Maturity Cotton Area in Liaoning Province. Varieties introduced from the former Soviet Union (e.g., KK-1543, C-4744) made up a relatively large proportion and had slightly larger root system indicators than domestic varieties. At that time, breeding efforts focused on early maturity and adaptability; root growth was still relatively low due to technological limitations at the time. The introduction of Soviet varieties provided good genetic resources for the following improvements.
2. 1970–1989: The root system increased in scale and developed evenly in both diameter and length. Cotton production areas have been expanded to include the Yangtze River Basin (Hubei, Jiangsu) and the Northwest Inland (Xinjiang); although the Yellow River Basin is still relatively large, variety differentiation is gradually increasing. Breeding goals changed to high yields, and root expansion capacity was increased to support above-ground growth. Varieties in the Yangtze River Basin have relatively fine root systems (adapted to a humid environment), and those in the Yellow River Basin are more balanced in terms of absorption and stress resistance.
3. 1990–2009: Xinjiang cotton varieties in the Northwest Inland Cotton Region (e.g., Xinlu Zao series) showed excellent root system characteristics, having strong and deep roots that could expand biomass rapidly, and the roots and shoots developed together. During this time, due to the dry climate of Xinjiang, varieties have evolved to have “deep-rooted systems and high absorption efficiency” for water-saving irrigation and high-yield cultivation. Transgenic technology and hybrid breeding have been employed to increase the tolerance to root stress in salt-alkali.
4. 2010–2022: Xinjiang cotton varieties achieved a high-performance root system and developed extensive root networks with functional specialisation. Pest-resistant vari-

eties increased absorption efficiency by enlarging the root surface area, and drought-tolerant types (e.g., Xinlu Zhong series) expanded the length of roots for deep-root penetration. Varieties in the Yellow River Basin have optimised root system indices for water and nutrient use, reduced redundant growth, etc. Molecular breeding technology was employed to improve the root-structure system for higher-yield, resource-use-efficient crops. Given climate change, one of the breeding goals now is to strengthen root resistance to extreme weather.

#### 4. Discussion

Plants have developed various types of root systems in their lives to meet different living needs and obtain various resources. The arrangement and form of these areas affect the efficiency of root contact with soil resources for whole-plant growth [17]. As an important link for underground resources and above-ground production, the root system actively absorbs and metabolically transports nutrients (N, P, K) and water for cross-organ allocation [18], and at the same time, it also participates in long-distance regulation of above-ground growth and development by synthesising signalling substances such as cytokinins and abscisic acid [19]. Increases in biomass and changes in the turnover of soil organic carbon pools have also been observed [20]. Thus, by exploring the genetic differences and ecological adaptation mechanisms of root architecture, it may be possible to improve crop root traits specifically for enhanced tolerance to abiotic stress and better resource utilisation efficiency [21].

In particular, the 15 selected strong-root accessions (e.g., ‘Xinlu Zao 78’, ‘Zhongmian-suo 63’) can serve as elite parents for root genetic improvement. Notably, the genetic grouping based on phenotypic clustering in this study also showed a strong association with the distribution of ecological cotton regions: for instance, accessions from the Northwest Inland Cotton Region mainly fell into the high-yield potential or balanced adaptation types, and those from the Yangtze River Basin were predominantly classified as fine-root dominance type, indicating genetic differences in root architecture among different ecotypes.

The comparison of root system indices of *Gossypium hirsutum* seedlings across different ecological cotton regions showed that the Northwest Inland Cotton Region had the most developed root system, and its root surface area, root volume and root fresh weight were all significantly higher than those in other regions. This area is relatively dry and sunny; therefore, it has an extensive root system that can absorb more water and is thus adapted to a relatively arid environment. The Yellow River Basin had medium-sized roots and, among those in the Northwest Inland, had the second largest root surface area and root volume, as well as a relatively narrow average root diameter; thus, it used a balanced strategy of absorption efficiency and mechanical support under semi-humid conditions. The Yangtze River Basin had the most dispersed root system. In a humid climate, a slender and shallow root system can quickly absorb water from the upper soil layer, avoid waterlogging and hypoxia, and is thus adapted to a high-moisture-content soil. The Northern Early Maturity Cotton Region had the longest roots, and thus it could access deeper soil layers for low-temperature adaptation and a thin topsoil, and it had a short growing season; at the same time, it achieved early maturity and an efficient nutrient supply. Foreign cotton areas had a larger mean root diameter but no significant advantage in root volume or fresh weight; therefore, they may have been bred more for mechanical support or storage purposes, and their overall absorption efficiency did not exceed that of varieties from China’s Northwest Inland Cotton Region.

From the perspective of temporal evolution (1950–2022), the root system scale of Chinese *Gossypium hirsutum* varieties has shown a progressive increase. During 1950–1969, varieties were mainly concentrated in the Yellow River Basin and the Northern Early

Maturity Cotton Region, with relatively low root indices; the introduced Soviet varieties were slightly better than the domestic ones. During 1970–1989, cultivation expanded to the Yangtze River Basin and the Northwest Inland, breeding objectives shifted toward high yields, and root expansion capacity improved. During 1990–2009, varieties from the Northwest Inland Cotton Region (e.g., ‘Xinlu Zao’ series) showed explosive root growth, forming a “robust and deep-rooted” architecture with coordinated root–shoot development, adapting to water-saving irrigation and high-yield demands in Xinjiang. During 2010–2022, Xinjiang varieties achieved record-breaking root performance with significantly increased root length, forming extensive root networks with functional specialisation. Molecular breeding techniques enabled targeted improvement of the root structure. Under climate change, enhancing root resistance to extreme weather has become a key breeding objective. Varieties from the Yellow River Basin have stabilised their root indices and now focus more on efficient water and nutrient use and reducing redundant growth. Although foreign varieties had coarse roots, their comprehensive growth indices did not surpass those of Chinese Northwest Inland varieties, indicating that Chinese-bred varieties have developed advantages in root resource acquisition efficiency.

Compared with previous studies, Sun Simin et al. [5] revealed the significant superiority of introduced populations in root traits through an analysis of 220 natural populations and 325 introduced populations but did not perform cluster analysis based on ecological adaptation. On the other hand, the present study classified the 204 accessions into three groups using cluster analysis, clarified the ecological adaptation features of these groups, and extended the association mechanism between root architecture and ecological cotton distribution. Liu Tingting et al. [6] indicated that although high-throughput acquisition of cotton root phenotypes has been achieved, genetic analysis has been lacking. WinRhizo root analysis software was employed in this study to accurately measure nine indices, and principal component analysis was used to identify the key indices for phenotypic data support of root genetic analysis. Fry et al. [10] have also found that root-architecture plasticity in drought-tolerant plants is required. At present, studies in the Northwest Inland Cotton Area have revealed that cotton varieties have adapted to an arid environment by expanding the root surface area and volume; thus, the findings of Fry et al. have been verified, and root architecture is known to regulate abiotic stress. Many factors influence the structure of cotton roots, such as features of the roots themselves (number, length, diameter and space distribution of roots at different orders), geographical environment, soil conditions and cultivation practices. Notably, the residual mulch film has been found to change the distribution of soil moisture and thus affect root system architecture in cotton fields [22]. At present, most research on cotton root systems has focused on how fertilisers affect them, what different irrigation methods do to the development of roots, and how planting arrangements influence root shape and yield. For example, research has investigated the regulation effect of mild drought stress on cotton morphology and yield during the flowering and boll stage [23], as well as the response of different cotton lines to irrigated and rainfed conditions [24]. In addition, the impact of water-deficiency stress under potassium-fertiliser addition on seed-cotton yield has also been studied [25]. Research on architectural typing is lacking, a specific typing system has not been established, and further studies are needed to optimise the root research method, clarify the signal-pathway regulatory mechanism, and apply molecular breeding. Due to the complex structure of the root system and its adaptive response mechanism under drought stress, research in this area is still developing and integrating results from multiple experimental paths [26]. However, it should be noted that most existing studies, including the present work, have not fully reconciled the differences between controlled environments and actual field conditions. Seedling root architecture was studied in a hydroponic system in this

paper, and field conditions were not fully replicated. In the future, field phenomics and molecular biology can be integrated to further explore the genetic regulatory networks of root architecture, and field screening validation should be added to the breeding pipeline to improve reliability.

## 5. Conclusions

Analysis of the root morphology in 204 *Gossypium hirsutum* germplasm seedlings shows that different varieties have different root systems, and these systems are related to the ecological distribution of cotton. Core indices of seedling root identification are root surface area, root volume and fresh weight. According to the above parameters, 15 high-root-material-line parents were selected as the best parents for root genetic improvement in cotton. Based on the above results, this paper has provided a theoretical foundation for root genetic improvement in *Gossypium hirsutum*, identified essential traits for stress-tolerant and high-yield root breeding, and offered references for eco-adaptive breeding and regional cultivation strategies. Hydroponic experiments have some limitations; therefore, future research will need to integrate field phenomics and molecular biology to explore the genetic regulatory networks of root architecture in more depth.

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