Postprint: Phenotypic Trait Diversity of Rhodomyrtus tomentosa from Different Provenances

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Abstract

To analyze the phenotypic trait diversity of Rhodomyrtus tomentosa and its inter-provenance relationships, this study examined 20 different provenances of Rhodomyrtus tomentosa under common garden cultivation conditions. Phenotypic traits of vegetative and floral organs were observed, and variance analysis, variation analysis, Shannon-Wiener diversity index analysis, and cluster analysis were employed to investigate the phenotypic trait diversity among different provenances. The results showed that: (1) Phenotypic traits of Rhodomyrtus tomentosa from different provenances exhibited significant differences ($P<0.05$), with the mean Shannon-Wiener diversity index exceeding 1.35, indicating rich phenotypic trait diversity. (2) The mean coefficient of variation for phenotypic traits within provenances ranged from 10.81% to 63.75%, while among provenances it ranged from 13.08% to 74.04%. Variation among provenances (23.33%) was higher than variation within provenances (19.79%), and variation in vegetative organs (29.52%) was higher than that in floral organs (14.06%). (3) Some traits showed extremely significant or significant correlations; plant height exhibited an extremely significant negative correlation with branch number and significant positive correlations with leaf length, leaf width, and leaf area. (4) At a Euclidean distance of 10, the 20 provenances could be divided into three groups: A, B, and C. Group A contained 8 provenances characterized by tall plants, few branches, large leaves, and large flowers. Group B contained 11 provenances characterized by medium plant height, relatively large leaves, and medium-sized flowers. Group C contained only 1 provenance characterized by short plants, many branches, small leaves, and small flowers. This study provides a theoretical basis and material resources for the breeding of new Rhodomyrtus tomentosa varieties and research on target traits.

Full Text

Preamble

Study on Diversity of Phenotypic Traits of *Rhodomyrtus tomentosa* from Different Provenances

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Abstract: To elucidate the phenotypic trait diversity and inter-provenance relationships of *Rhodomyrtus tomentosa*, we investigated 20 provenances cultivated under homogeneous garden conditions. Vegetative and floral organ phenotypic traits were measured and analyzed using variance analysis, variation analysis, Shannon-Wiener diversity index analysis, and cluster analysis. The results revealed: (1) Significant differences in phenotypic traits among provenances ($P < 0.05$), with mean Shannon-Wiener diversity indices exceeding 1.35, indicating rich phenotypic diversity. (2) The mean coefficient of variation for phenotypic traits within provenances ranged from 13.08% to 74.04%, with inter-provenance variation (23.33%) exceeding intra-provenance variation (19.79%). Vegetative organ variation (29.52%) was higher than floral organ variation (14.06%). (3) Several traits showed highly significant or significant correlations; plant height exhibited extremely significant negative correlations with branch number and significant positive correlations with leaf length, leaf width, and leaf area. (4) At Euclidean distance 10, the 20 provenances clustered into three groups: A, B, and C. Group A comprised 8 provenances characterized by tall plants, few branches, large leaves, and large flowers. Group B contained 11 provenances with medium plant height, relatively large leaves, and medium-sized flowers. Group C included only 1 provenance, showing dwarf plants, numerous branches, small leaves, and small flowers. This study provides a theoretical basis and germplasm materials for new variety breeding and target trait research in *R. tomentosa.*

Keywords: *Rhodomyrtus tomentosa*, phenotypic trait, diversity, coefficient of variation, cluster analysis

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Introduction

Rhodomyrtus tomentosa (Myrtaceae) is the only shrub species of the genus *Rhodomyrtus* naturally distributed in China. With abundant wild resources and strong ecological adaptability, it is widely distributed across tropical and subtropical regions, including the Indochinese Peninsula, Philippines, India, Sri Lanka, Malaysia, Japan, and Indonesia, and is considered an ethnic plant in Thailand (Geetha et al., 2010). In China, *R. tomentosa* occurs in Taiwan, Guangdong, Guangxi, Hainan, Yunnan, Jiangxi, Hunan, Fujian, and Guizhou (Si et al., 2012; Liu et al., 2013). The plant serves as a traditional folk medicine in southern China, with its fruits, leaves, and roots all having medicinal value (Dai et al., 2014). The fruit is sweet and sour, rich in vitamins, and represents a valuable wild fruit resource (Wei et al., 2005). Additionally, *R. tomentosa* is a rare color-changing ornamental species in China, featuring long flowering periods, dense blossoms, evergreen foliage, and compact tree form, making it an integrated ornamental plant for flowers, leaves, and fruits (Chen, 2008) and an important native landscaping material. Currently, *R. tomentosa* resources remain underutilized and predominantly wild. While domestic scholars have investigated its biological characteristics, medicinal value, and nutritional properties, breeding improvement research remains limited.

Phenotypic trait observation represents the most intuitive method for studying plant genetic diversity (Yang et al., 2019), directly reflecting genetic structure and resource richness. Previous genetic diversity studies on *R. tomentosa* have primarily employed molecular markers such as SRAP (Ferriol et al., 2003), ISSR (Qiu et al., 2021), and SNP (Hu, 2020), with relatively few investigations on phenotypic trait diversity. Although *R. tomentosa* possesses numerous phenotypic traits, its vegetative and floral organ morphologies are easily observable, show clear differences, and yield stable results, providing an authentic representation of comprehensive species characteristics. Observing these traits constitutes a fundamental method for germplasm resource surveys and the initial pathway for superior variety selection. Therefore, this study examined 20 *R. tomentosa* provenances, measuring vegetative organs (plant height, branch number, leaf length, leaf width, leaf area) and floral organs (pedicel length, corolla diameter, filament length). Through multiple comparisons, variation analysis, Shannon-Wiener diversity indices, correlation analysis, and cluster analysis, we aimed to: (1) characterize phenotypic diversity patterns in *R. tomentosa* germplasm resources, and (2) elucidate genetic variation 规律 among tested provenances, thereby providing theoretical support for germplasm collection, conservation, utilization, and new variety breeding.

1. Materials and Methods

1.1 Materials

Based on the distribution of *R. tomentosa* in China and field surveys, we selected 20 representative provenances from Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hunan, and Jiangxi provinces (Table 1). Mature fruits were collected and processed to obtain seeds. After seed propagation, seedlings were transplanted to the experimental planting base at Huizhou University (114°42 E, 23°05 N) for homogeneous garden cultivation. The experimental site features abundant rainfall, ample sunshine, and a mild climate, with annual precipitation of approximately 2,200 mm and mean annual temperature of 22 °C. A randomized block design was employed, with 5 plants per provenance in each plot at 0.5 m × 0.5 m spacing. Each provenance was planted in 3 plots as 3 replicates. Standard water-fertilizer management and pest control were applied throughout the growth period.

1.2 Methods

From March to June 2021, phenotypic traits of all test plants were measured. Plant height (distance from ground to highest point, excluding inflorescences) was measured with a ruler (precision 0.1 cm), and branch number (total branches at 0–10 cm from stem base) was counted. Thirty intact, healthy mature leaves were randomly selected to measure leaf length (distance from leaf base to tip) and leaf width (width at middle of leaf). During flowering, 15 flowers per provenance were sampled to measure pedicel length (short stalk connecting flower and stem), corolla diameter (longer and shorter diameters), and filament length (stamen length). Leaf shape index (leaf length/leaf width) and leaf area (leaf length × leaf width × 2/3) were calculated from these measurements (Tian et al., 2021).

1.3 Data Processing and Analysis

Data were organized using Excel 2010, with variation analysis and Shannon-Wiener index calculations performed for different provenances. SPSS 23.0 software was used for one-way ANOVA, multiple comparisons (Tukey HSD), correlation analysis, and cluster analysis of phenotypic traits.

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2. Results

2.1 Comparison of Phenotypic Traits Among Different Provenances

Phenotypic trait measurements for different provenances are presented in Table 2 . The 20 provenances showed significant differences in plant height, branch number, leaf length, leaf width, leaf area, and leaf shape index ($P < 0.05$), with overall means of 45.04 cm, 4.39, 6.69 cm, 3.17 cm, 14.49 cm^2, and 2.14, respectively. Provenances with greater plant height included P1, P8, P10, P12, P15, P16, and P17 (all >50 cm). Provenances P13 and P6 had more branches (>6), while P1, P8, P11, and P15 had fewer branches (mean = 2). Provenances with longer leaves included P7, P8, P10, P12, P15, P17, and P18 (all >7.0 cm). Provenances with leaf width >3.4 cm included P1, P8, P11, P12, P15, P16, P17, and P18, with P15 showing the smallest leaf width. Provenances with larger leaf area included P1, P7, P8, P11, P12, P17, and P18, while P13 had the smallest leaf area. Overall, provenances P8, P12, and P15 exhibited tall plants with large leaves and few branches, whereas P13 showed dwarf plants with small leaves but numerous branches.

Floral organ traits also differed significantly among most provenances ($P < 0.05$). Provenance P14 had the longest pedicel and largest corolla diameter, significantly different from most other provenances ($P < 0.05$). The shortest pedicel occurred in P6, and the smallest corolla diameter in P13. Provenance P3 had the longest filaments (1.16 cm), while P16 had the shortest.

2.2 Variation Analysis of Phenotypic Traits

Variation coefficients for phenotypic traits are shown in Table 3 . Both vegetative and floral organ traits varied within and among provenances. Branch number showed the highest variation coefficient (45.79%-99.76%, mean = 63.75%), with P18 showing maximum variation and P20 minimum. Leaf shape index showed the lowest variation (7.58%-19.92%, mean = 10.81%), with P15 showing maximum variation and P16 minimum. Among floral organs, pedicel length showed the highest variation (7.44%-28.57%, mean = 13.55%), with P6 maximum and P15 minimum. The smallest floral variation occurred in longer corolla diameter (5.23%-21.93%, mean = 11.39%), with P5 maximum and P14 minimum. Overall, mean variation coefficient for vegetative organs (29.52%) exceeded that for floral organs (14.06%), and inter-provenance variation (23.33%) exceeded intra-provenance variation (19.79%).

2.3 Diversity Analysis of Phenotypic Traits

Shannon-Wiener diversity indices for phenotypic traits are presented in Table 4 . All measured traits showed rich diversity, with substantial variation among traits. Leaf area and leaf shape index showed the highest diversity indices (means = 3.45 and 3.32, respectively), while filament length, pedicel length, and branch number showed lower indices (means = 1.35, 1.69, and 1.79, respectively). Other traits ranged from 2.04 to 2.91.

2.4 Correlation Analysis of Phenotypic Traits

Correlation coefficients among phenotypic traits are shown in Table 5 . Several traits showed highly significant ($P < 0.01$) or significant ($P < 0.05$) positive or negative correlations, indicating interdependence among traits. Plant height showed extremely significant negative correlation with branch number ($P < 0.01$)

and significant positive correlations with leaf length, leaf width, and leaf area. Branch number showed significant negative correlations with leaf length, leaf width, and leaf area. Corolla diameter showed significant positive correlations with pedicel length and filament length. However, vegetative organ traits showed no significant correlations with floral organ traits.

2.5 Cluster Analysis of Phenotypic Traits

Based on vegetative and floral organ observations, Euclidean distance cluster analysis grouped the 20 provenances into three categories (A, B, and C) at distance 10 (Figure 1 [Figure 1: see original paper]). Group A included 8 provenances: P8 (Guangdong Dapu), P12 (Guangxi Guiping), P11 (Guangxi Youjiang), P15 (Guangxi Luchuan), P1 (Fujian Liancheng), P17 (Hainan Chengmai), P18 (Hainan Lingao), and P16 (Guizhou Libo). These provenances exceeded groups B and C in mean plant height, leaf length, leaf width, leaf area, pedicel length, and corolla diameter. Group B comprised 11 provenances: P9 (Guangdong Nanxiong), P7 (Guangdong Kaiping), P10 (Guangdong Leizhou), P5 (Guangdong Boluo), P6 (Guangdong Huiyang), P4 (Guangdong Lianping), P3 (Guangdong Chaoan), P19 (Hunan Rucheng), P2 (Fujian Xianyou), P20 (Jiangxi Nankang), and P14 (Guangxi Xiangzhou), characterized by medium plant height, numerous branches, and relatively large leaves and flowers. Group C contained only P13 (Guangxi Lingui), which showed lower values than groups A and B for all traits except branch number.

At Euclidean distance 7.5, groups A and B further subdivided: A into A1 and A2, and B into B1 and B2. Subgroup A1 included P8, P12, P15, P1, P17, and P18, showing tall plants with few branches and large leaves and flowers. Subgroup A2 included P11 and P16, showing relatively tall plants with few branches, wide leaves, but small flowers and short filaments. Subgroup B1 included P9, P7, P10, and P19, characterized by long leaves and large leaf area. Subgroup B2 included P2, P5, P6, P4, P3, P20, and P14, showing dwarf plants with numerous branches and small flowers.

Discussion

Phenotypic traits represent the comprehensive external characteristics of plants, and phenotypic variation constitutes the most direct manifestation of genetic variation, reflecting species genetic diversity and variation patterns (Zhang, 2008). This study demonstrates substantial phenotypic variation and rich diversity among *R. tomentosa* provenances. All 10 measured traits showed mean variation coefficients exceeding 10%, ranging from 10.81% to 63.75%. Research indicates that variation coefficients above 10% represent considerable sample differences, with larger coefficients indicating more pronounced phenotypic differences and greater genetic variation potential (Li et al., 2019). Thus, the tested provenances exhibit rich phenotypic morphology and high genetic diversity.

In addition to genetic factors, phenotypic traits are influenced by environmental conditions. Different adaptive capacities of traits to environment can generate multiple variation types within a single provenance (He et al., 2021). Zeng et al. (2008) found that shoot length-to-width ratio showed the greatest variation among all traits in natural populations of *Amygdalus ledebouriana*, likely due to high environmental sensitivity. Our study similarly found that, except for plant height, vegetative organ traits (branch number, leaf length, leaf width, leaf area) showed higher variation than floral organ traits, indicating vegetative organs are more environmentally sensitive and prone to variation, consistent with He et al. (2021). Furthermore, inter-provenance variation coefficients exceeded intra-provenance coefficients, suggesting that phenotypic variation in *R. tomentosa* occurs primarily among rather than within provenances.

Shannon-Wiener diversity index analysis revealed mean diversity indices above 1.35 for all traits, with leaf area showing the highest index (3.45). Variation coefficient and diversity index are both important biodiversity indicators, but their patterns are inconsistent. For example, branch number showed the highest variation coefficient (74.04%) but a relatively low diversity index (1.79), while leaf shape index showed low variation (13.46%) but high diversity (3.32). This inconsistency reveals that these metrics reflect different aspects: variation coefficient indicates data dispersion and correlates positively with trait variation range, while Shannon-Wiener index reflects germplasm diversity, with higher values indicating richer phenotypic diversity, and the two are not correlated (Li et al., 2016). Similar patterns were reported in studies of mungbean agronomic traits (Qiao et al., 2015).

During adaptation and evolution, different morphological traits often interact to achieve environmental fitness, with changes in one trait directly or indirectly affecting others (Yang and Yang, 2011). Our study found highly significant or significant correlations among some traits, but low correlations between vegetative and floral organ traits, possibly because vegetative organs are more environmentally labile while floral organs are more stable.

Cluster analysis at Euclidean distance 10 divided the 20 provenances into three groups. Group A, characterized by tall plants, few branches, large leaf area, long pedicels, and large flowers, represents excellent germplasm for breeding ornamental single-plant varieties. Group B, with medium plant height, numerous branches, and relatively large leaves and flowers, is suitable for hedge-type breeding. Group C, featuring dwarf plants with numerous branches and small leaves and flowers, is appropriate for groundcover breeding. The clustering did not strictly follow geographic distribution, consistent with Qiu et al. (2021), possibly because *R. tomentosa* adapts to local environmental changes, preserving adaptive genetic variations through its breeding system (Si et al., 2012; Gao et al., 2017). Additionally, seed dispersal by birds may facilitate cross-regional germplasm distribution (Wei et al., 2004). These clustering results enable regional classification of wild provenances, grouping similar phenotypes to provide theoretical guidance for targeted trait collection and differential conservation.

Rich germplasm resources provide a solid genetic foundation for plant breeding. This study demonstrates rich phenotypic diversity among *R. tomentosa* provenances, with significant variation in both vegetative and floral organs, though vegetative organ variation exceeds floral organ variation, and inter-provenance variation exceeds intra-provenance variation. Different provenances exhibit distinct characteristics in plant height, branching, leaf, and flower traits, enabling directional selection based on landscape use and ornamental objectives. Targeted breeding can optimize resource combinations to develop superior varieties with different utilization values.

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