# Postprint: A Method for Screening High-Quality Ramie Germplasm Resources Based on UAV Remote Sensing Phenotyping

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## Abstract

Ramie is one of the important fiber crops. Due to factors such as the shortage of land resources and the promotion and application of superior varieties, genetic variation and genetic diversity in ramie have decreased, leading to an increasingly urgent demand for the investigation and conservation of ramie germplasm resources diversity. UAV remote sensing-based crop phenotyping methods can frequently, rapidly, non-destructively, and accurately monitor the growth characteristics of different genotypes, enabling crop germplasm resources investigation and screening of specific superior varieties. To achieve efficient comprehensive evaluation of ramie germplasm resources phenotypes and assist in screening superior ramie varieties, this study proposes a method for monitoring and screening ramie germplasm resources phenotypes based on UAV remote sensing imagery. First, based on UAV remote sensing imagery, Pix4dmapper software was used to generate a Digital Surface Model (DSM) and orthophoto of the experimental area. Then, key phenotypic parameters of ramie germplasm resources (plant height, plant count, Leaf Area Index, leaf chlorophyll content, water content) were estimated. Plant height was extracted from the DSM using the "differential method", plant count was extracted from the orthophoto using an object detection algorithm, and machine learning methods were used to estimate ramie Leaf Area Index (LAI), leaf chlorophyll content (SPAD value), and water content. Finally, based on the extracted remote sensing phenotypic parameters, genetic diversity analysis of ramie germplasm resources was conducted using variability analysis and Principal Component Analysis. The results showed that: (1) The estimation of ramie phenotypes based on UAV remote sensing achieved good performance, with a fitting accuracy of 0.93 and RMSE of 5.65 cm for plant height; the fitting metrics for SPAD value, water content, and LAI reached 0.66, 0.79, and 0.74 respectively, with RMSE values of

2.03, 2.21, and 0.63; (2) Significant differences existed in remote sensing phenotypes of ramie germplasm resources, with coefficients of variation for estimated LAI, plant height, and plant count reaching 20.82%, 24.61%, and 35.48% respectively; (3) Principal Component Analysis clustered ramie germplasm resources phenotypes into Factor 1 (plant height, LAI) and Factor 2 (LAI, SPAD value), where Factor 1 can be used for evaluating structural characteristics of ramie germplasm resources, and Factor 2 can serve as a screening indicator for high photosynthetic efficiency ramie resources. This study will provide a reference for crop germplasm resources phenotypic monitoring and breeding-related analysis.

## Full Text

## High-Quality Ramie Germplasm Resource Screening Method Based on UAV Remote Sensing Phenotype Monitoring

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### Abstract

Ramie is an important fiber crop. Due to the shortage of land resources and the promotion of excellent varieties, the genetic variation and diversity of ramie have decreased, increasing the need for investigation and protection of ramie germplasm resources diversity. The crop phenotype measurement method based on UAV remote sensing can conduct frequent, rapid, non-destructive and accurate monitoring of growth characteristics of different genotypes, enabling crop germplasm resources investigation and screening of specific high-quality varieties. To achieve efficient comprehensive evaluation of ramie germplasm phenotype and assist in screening dominant ramie varieties, this study proposes a method for monitoring and screening ramie germplasm phenotype based on UAV remote sensing images. First, based on UAV remote sensing images, Pix4dmapper software was used to generate the digital surface model (DSM) and orthophoto of the test area. Then, key phenotypic parameters (plant height, plant number, leaf area index, leaf chlorophyll content, water content) of ramie germplasm resources were estimated. Plant height was extracted from DSM using the "subtraction method," plant number was extracted from orthophoto images using target detection algorithms, and machine learning methods were used to estimate leaf area index (LAI), leaf chlorophyll content (SPAD value), and water content. Finally, based on the extracted remote sensing phenotypic parameters, genetic diversity analysis of ramie germplasm resources was conducted using variability analysis and principal component analysis. The results showed that: (1) The ramie phenotype estimation based on UAV remote sensing was effective, with the fitting accuracy of plant height 0.93, and the root mean square error (RMSE) 5.65 cm. The fitting indexes of SPAD value, water content and LAI were 0.66, 0.79 and 0.74, respectively, and RMSE were 2.03, 2.21 and 0.63, respectively; (2) The remote sensing phenotypes of ramie germplasm were significantly different, as the coefficients of variation of LAI, plant height and plant number reached 20.82%, 24.61% and 35.48%, respectively; (3) Principal component analysis was used to cluster the remote sensing phenotypes into factor 1 (plant height and LAI) and factor 2 (LAI and SPAD value). Factor 1 can be used to evaluate the structural characteristics of ramie germplasm resources, and factor 2 can be used as the screening index of high-light efficiency ramie resources. This study could provide references for crop germplasm phenotypic monitoring and breeding correlation analysis.

**Keywords:** ramie; diversity of germplasm resources; phenotype; UAV remote sensing; digital surface model; machine learning

## 1 Introduction

Ramie belongs to the Urticaceae family and Boehmeria genus. As a traditional natural textile raw material, it has many advantages such as antibacterial, breathable, and cool properties [1]. In recent years, ramie' s unique cultural, medicinal, and ecological values have been gradually explored, and it has been widely applied in medical research, feed production, soil remediation, and other aspects. China is a country with abundant ramie variety variation types and wild species of Boehmeria genus. However, with the shortage of land resources and the promotion of excellent varieties, this valuable crop resource has experienced serious variety simplification, threatening ramie germplasm resources and increasing the need for diversity investigation and protection.

Crop germplasm resources contain rich genetic diversity. Under the interaction of long-term environmental adaptation and artificial selection, they have formed phenotypic trait diversity, such as agronomic traits, yield traits, resistance traits, and quality traits, which constitute the material basis for breeding new crop varieties [2]. Facing thousands of germplasm resources, phenotypic data measurement is a huge and cumbersome task. Traditional manual measurement methods are not only time-consuming and labor-intensive, but also significantly affected by environmental and observer subjective factors, making it difficult to achieve multi-location and multi-temporal germplasm resources phenotypic measurement. High-throughput phenotyping technology provides a new approach for crop phenotypic trait evaluation [3]. For example, Marefatzadeh et al. [4] used semi-automatic indoor high-throughput technology to analyze 589 tomato germplasm resources from different regions, demonstrating the application potential of high-throughput phenotyping technology in genetic diversity research of crop germplasm resources.

With the maturity of indoor high-throughput phenotyping technology, its application has gradually shifted from controlled environments to complex field environments. More and more researchers have begun to focus on the application of high-throughput phenotyping technology in field crop growth monitoring. Field phenotyping technology, relying on the development of UAV remote sensing, spectral technology, and artificial intelligence, has made breakthroughs in crop plant height measurement [5], variety identification [6], nutrient diagnosis [7], yield estimation [8], and pest monitoring [9]. For example, Jiang et al. [10] used vegetation indices obtained from UAV multispectral remote sensing and random forest regression to estimate quinoa leaf area index (LAI) and chlorophyll content, with estimation accuracies of 0.977-0.980 and 0.983-0.986, respectively. Weiss and Baret [11] used UAV remote sensing to obtain grapevine canopy images and extracted grapevine height data using 3D reconstruction technology, achieving 88% accuracy. Ganeva et al. [12] accurately extracted winter durum wheat LAI, fraction of absorbed photosynthetically active radiation, vegetation coverage fraction, leaf chlorophyll content (SPAD value), canopy chlorophyll content, tillering, and yield based on UAV remote sensing images, and analyzed the genetic diversity, proximity, and similarity of the studied genotypes.

Tanger et al. [13] studied the potential of high-throughput phenotyping technology for identifying genetic variation of important traits in rice and found that the quantitative trait loci (QTL) results obtained from high-throughput phenotyping were consistent with those from manual observation.

Currently, research on high-throughput monitoring of crop germplasm resources in the field based on UAV remote sensing and its application in assisted breeding is still in the preliminary exploration stage. Previous studies are often limited by crop varieties or focus on monitoring single phenotypic information, lacking comprehensive consideration of key crop phenotypes. Therefore, this study takes more types of ramie germplasm resources as objects, explores the feasibility of UAV remote sensing technology in ramie germplasm resources diversity investigation and evaluation according to commonly used phenotypic evaluation indicators in ramie germplasm resources assessment, and explores the feasibility of using crop phenotypic analysis results obtained by remote sensing technology to screen high-quality varieties and assist crop breeding. The main work includes using a UAV remote sensing system to obtain field ramie canopy images, extracting main phenotypes of ramie germplasm resources (plant height, plant number, LAI, SPAD value, water content) using image processing and machine learning technologies, and finally conducting genetic diversity and difference studies on ramie germplasm resources based on remote sensing phenotypes.

## 2 Materials and Methods

### 2.1 Study Area

The study area is located at the Yunyuan planting base of Hunan Agricultural University in Furong District, Changsha City, Hunan Province (28°11’ 02” N, 113°04’ 10” E). The area contains 154 ramie varieties [Figure 1: see original paper], which were seeded and transplanted in December 2017 and first harvested in June 2018. The area consists of 154 plots, each with an area of 3.6 m$^2$. Each plot contains 8 ramie plants (2 rows × 4 plants), with 0.6 m spacing between genotypes and 0.5 m drainage ditch width. The experimental area has uniform soil composition and fertile soil, with convenient irrigation and drainage, and consistent water and fertilizer management. To facilitate later image reconstruction and improve the accuracy of latitude, longitude, and elevation position calibration, 6 ground control points were arranged in the experimental field.

### 2.2 Data Collection

Data collection was conducted 6 times during the ramie seedling stage (March 15, March 23), row closure stage (March 29, April 7), and vigorous growth stage (April 12, April 20) in 2022, including ground measurement data and UAV image data. To ensure data timeliness, ground data measurement and UAV aerial photography were synchronized on the same day.

**2.2.1 Ground Data Collection** Ground data including plant height, SPAD value, LAI, water content, and plant number were collected. Plant height was measured using a ruler from the bottom to the top of the canopy. SPAD value was measured using a SPAD-502 chlorophyll meter produced by KONICA MINOLTA, Japan. LAI was measured using an LAI-2200 canopy analyzer. Leaf water content was the difference between fresh leaf weight and dry leaf weight. All data were obtained by randomly selecting 10 ramie plants from each plot and calculating the average. On April 20, 2022, the number of ramie plants in each plot was obtained through manual visual counting.

**2.2.2 UAV Data Collection** Ramie canopy RGB images were obtained using DJI Inspire 2 equipped with a Zenmuse X5s high-definition digital camera, and ramie canopy multispectral images were obtained using DJI Phantom 4. To ensure stable and sufficient solar radiation, flight operations were conducted between 12:00-14:00 local time. DJI-GS Pro was used to automatically generate flight routes in the designated area, with a forward overlap rate of 80%, side overlap rate of 70%, flight altitude of 20 m, gimbal pitch angle of 90°, and camera exposure mode set to automatic.

### 2.3 Remote Sensing Image Processing

**2.3.1 Remote Sensing Image Stitching** Pix4dmapper software was used to generate orthophoto images and digital surface model (DSM) images of the study area [Figure 2: see original paper]. During the stitching process, to obtain accurate geographic references, the three-dimensional spatial position information of imported ground control points was used for georeferencing.

**2.3.2 Remote Sensing Feature Extraction** Remote sensing features extracted from UAV images included texture features, spectral features, and HDSM (elevation data). The gray-level co-occurrence matrix was selected as texture features. Vegetation indices were used as spectral features, which are formed by linear or nonlinear combination of reflectance from different bands [14,15]. HDSM was extracted by differential operation of DSM obtained at different stages. Specific calculation formulas are shown in Table 1 .

Rectangular Areas of Interest (AOI) were drawn using the raster tool in ArcGIS 10.2 software along the plot boundaries. When drawing, 10% of the plot edge was reserved to eliminate edge effect interference.

### 2.4 Ramie Phenotypic Parameter Monitoring

**2.4.1 Ramie Plant Number Monitoring** In previous studies, using ramie germplasm resources canopy images obtained in 2019 and 2020, a ramie plant counting model was constructed using the Fully Convolutional One-Stage Object Detection (FCOS) algorithm. The FCOS model used ResNet101 as the backbone, and Feature Pyramid Networks (FPN) were used to extract ramie plant features from images, with training epochs of 20. Figure 3 [Figure 3: see original paper] shows the ramie plant detection results, with blue boxes marking actual plant samples and red boxes marking identified samples.

**2.4.2 Ramie Physiological Parameter Monitoring** Samples collected at different stages were mixed and divided into training and validation sets at a 7:3 ratio. Previous studies have shown that crop physiological and biochemical indices respond differently to remote sensing features. For example, crop LAI has been proven to be linearly correlated with Relative Vigor Index (RVI) and Transform Soil Adjusted Vegetation Index (TSAVI) [16], and more suitable for quadratic equation fitting with Green Vegetation Index (GVI) and Perpendicular Vegetation Index (PVI). Water content has extremely significant correlation with vegetation indices such as ExG and ExR [17]. In this study, field-measured phenotypic data and remote sensing features were used as input parameters, sensitive features were screened using recursive feature elimination method, and four widely used machine learning methods were employed: Linear Regression (LR), Random Forest Regression (RF), Support Vector Machines (SVM), and Partial Least Squares Regression Analysis (PLSR) to construct ramie physiological parameter estimation models. Coefficient of Determination ($R^2$) and Root Mean Square Error (RMSE) were used as model evaluation metrics. Higher $R^2$ and lower RMSE indicate higher model fitting accuracy.

### 2.5 Ramie Germplasm Resources Genetic Diversity Analysis

Traditional ramie germplasm resources evaluation methods have defects such as being time-consuming, labor-intensive, and destructive. Therefore, this study used phenotypic data obtained by remote sensing monitoring to analyze phenotypic genetic diversity and genetic variation of ramie germplasm resources, aiming to achieve efficient evaluation and screening of crop germplasm resources. Coefficient of variation and diversity index [18] provide information support for genetic diversity. Higher coefficient of variation and higher diversity index indicate more significant genetic diversity. Principal component analysis can not only provide correlation information among phenotypic traits but also provide genotype positioning according to individual trait values of main components. All analyses were implemented in SPSS software. The calculation formulas for coefficient of variation and diversity index are as follows:

$$CV = \frac{Sd}{Mean} \times 100\%$$
$$H' = -\sum (P_i \times \ln P_i)$$

Where CV is coefficient of variation; H' is diversity index; P__i is the percentage of individuals in the ith level of a phenotypic trait relative to the total number of individuals; Sd is standard deviation; Mean is average value.

## 3 Results

### 3.1 Statistical Description of Ramie Germplasm Resources Phenotypic Traits

Based on ground-measured data obtained at different growth stages, statistical description of ramie germplasm resources phenotypic traits was conducted, with results shown in Table 2 . From seedling stage to vigorous growth stage, SPAD values and water content of ramie germplasm resources showed an overall decreasing trend, which is consistent with ramie growth patterns. Leaf water content is an important indicator reflecting the balance between water supply and transpiration. From seedling stage to vigorous growth stage, temperature gradually increased, and under drought stress, ramie leaf wilting increased and relative water content decreased. With crop growth, changes in leaf number and area caused LAI to be in continuous dynamic change. In this study, from seedling stage to vigorous growth stage, ground-measured LAI gradually increased. This is because as the stem grows, the total number of leaves gradually increases, leaf area gradually expands, shading between ramie rows intensifies, and thus LAI continues to increase. Ramie plant height generally showed a trend of gradually increasing with later growth stages.

In terms of phenotypic differences existing in ramie germplasm resources at different stages, SPAD values of ramie leaves were generally in the range of 24.70-46.51 during the first three growth stages. SPAD values at the seedling stage showed obvious variety differences, with variation range of 25.50-46.51 and standard deviation of 4.02, indicating large variation in light efficiency utilization among ramie germplasm resources at the seedling stage. The seedling stage may be a critical period affecting the final accumulation of organic matter in ramie. With ramie growth, differences in SPAD values among varieties decreased, with standard deviations of 3.39 at row closure stage and 3.36 at vigorous growth stage. Water content of ramie germplasm resources ranged from 60.81% to 95.84% during the first three stages. Ranking by standard deviation of water content at different stages showed vigorous growth stage > row closure stage > seedling stage. Water content variation range at vigorous growth stage was 60.81%-85.06%, at row closure stage was 71.31%-93.49%, and at seedling stage was 79.69%-95.84%. The high water content differences shown by ramie germplasm resources at different growth stages are helpful for screening drought-resistant varieties under drought conditions. LAI of ramie germplasm resources ranged from 0.91 to 7.64 during the first three stages, with little variation amplitude among stages. With later growth stages, differences in plant height among ramie germplasm resources gradually increased, with standard deviation changing from 10.28 at seedling stage to 22.42 at vigorous growth stage.

### 3.2 Monitoring Results

**3.2.1 Ramie Germplasm Resources Plant Height Monitoring** Using measured ramie plant height as the dependent variable and HDSM as the independent variable, linear regression, exponential regression, and polynomial regression were used for fitting, with results shown in Table 3 . Plant height monitoring accuracy $R^2$ was 0.70-0.86, with RMSE controlled within 4.59-6.86 cm. The fitting effects of the three regression models differed, with overall accuracy showing polynomial regression > linear regression > exponential regression. Using polynomial regression model to fit plant height data of ramie germplasm resources throughout the whole growth stage, the extraction accuracy of ramie plant height based on the whole growth stage was very high, with $R^2$ of 0.93 and RMSE of 5.65 cm between measured plant height and HDSM, indicating the feasibility of using UAV remote sensing to monitor ramie plant height.

Data from each growth stage were randomly divided into training and validation sets at a 7:3 ratio, and training set data were used to construct plant height correction models for different growth stages. The seedling stage, row closure stage, and vigorous growth stage plant height correction models are formulas (20)-(22), respectively. Table 4 shows the correction effect of ramie plant height correction models. As can be seen from Table 4, the accuracy of the validation set was significantly improved after correction, with high fitting degree between corrected HDSM and measured plant height.

**3.2.2 Ramie Germplasm Resources Physiological Parameter Monitoring** The estimation results of four machine learning algorithms (LR, RF, SVM, PLSR) for ramie physiological parameters are shown in Table 5 . LR performed best in estimating SPAD value and water content of ramie, with $R^2$ of 0.66 and RMSE of 2.03 for SPAD value, and $R^2$ of 0.79 and RMSE of 2.21 for water content. Compared with the estimation accuracy of the training set ($R^2$ = 0.78, RMSE = 2.29), the fitting metrics of the test set were improved, indicating strong generalization ability and stability of the model. SVM performed best in estimating ramie LAI, with $R^2$ of 0.74 and RMSE of 0.60 for the training set, and $R^2$ of 0.74 and RMSE of 0.63 for the test set. The similar accuracy between training and test sets indicates that the model is reliable and stable. Therefore, the ramie physiological parameters extracted based on remote sensing images have high accuracy, and subsequent studies will analyze ramie germplasm resources based on the best model estimations.

### 3.3 Remote Sensing Phenotypic Differences of Ramie Germplasm Resources

Variability analysis was conducted on remote sensing phenotypes of ramie germplasm resources [Figure 4: see original paper]. The coefficient of variation range of LAI of ramie germplasm resources at different stages was 18.94%-23.42%, with an average coefficient of variation of 20.83%. The coefficient of variation range of HDSM was 20.06%-27.27%, with an average coefficient of variation of 24.61%. The coefficient of variation of plant number was 35.48%. These results indicate that ramie germplasm resources have large differences in LAI, plant height, and plant number extracted by remote sensing. In actual production, plant height and effective plant number are the main factors constituting ramie yield [19], and LAI can reflect ramie population canopy structure and photosynthesis capacity, which is also closely related to final yield. Therefore, investigating differences in plant height, plant number, and LAI of ramie resources will be helpful for ramie yield evaluation and high-yield variety breeding.

The coefficient of variation range of water content was 2.3%-4.59%, and the differences among different germplasm resources gradually increased with later growth stages, reaching a maximum value of 4.95% at vigorous growth stage (April 20). This is because temperature rises and precipitation decreases during this period, soil water content decreases, and compared with early ramie growth, this period can be regarded as drought stress. The increased variation range of water content among different ramie germplasm resources indicates differences in variety drought tolerance.

### 3.4 High-Quality Ramie Germplasm Resources Phenotypic Screening

Principal component analysis was conducted on remote sensing phenotypes of ramie germplasm resources [Figure 5: see original paper]. The first two principal components explained more than 43.39% of the total variation of all germplasm resource traits. In the first principal component, plant height and LAI had high loadings, both being important indicators reflecting ramie structural characteristics. Therefore, principal component 1 (factor 1) can be used for structural characteristic evaluation of ramie germplasm resources. LAI can reflect ramie light interception ability, and chlorophyll content is also closely related to plant photosynthesis. Therefore, principal component 2 (factor 2) can be used as a screening index for high light efficiency ramie resources.

Based on the analysis of ramie germplasm resources diversity, principal component analysis was further used to extract comprehensive indicators for screening specific ramie varieties. According to comprehensive traits, supplementary information about genotype differences can be obtained. The distribution of ramie germplasm resources in the factor 1-factor 2 coordinate system is shown in Figure 6 [Figure 6: see original paper]. Based on the quadrant where germplasm resources are located, the strongest influence corresponding to specific traits can be determined. The closer to the coordinate origin, the more balanced the germplasm resources are in the first and second traits, while germplasm resources at the edges are affected by specific traits. For example, variety 5-5 and variety 1-9 have similar traits on factor 1, but 5-5 has lower factor 2 characteristic values while 1-9 has higher factor 2 characteristic values, indicating that 1-9 has higher light efficiency utilization than 5-5. The results show that by locating the distribution of different ramie varieties in the factor 1-factor 2 coordinate system, the corresponding structural-related traits and light efficiency utilization-related traits can be determined, providing references for ramie germplasm resources screening.

## 4 Discussion

Crop germplasm resources phenotypic diversity is the driving force for variety breeding. This study proposes a rapid and non-destructive method for evaluating ramie germplasm resources phenotypic diversity by combining UAV remote sensing technology and machine learning technology. The main conclusions are as follows:

(1) Ramie germplasm resources phenotypic estimation based on UAV remote sensing is feasible. The fitting effect between field-measured phenotypic data and remote sensing phenotypes is good, with ramie plant height estimation accuracy of 0.93 and RMSE of 5.65 cm; SPAD value $R^2$ of 0.66 and RMSE of 2.09; water content $R^2$ of 0.79 and RMSE of 2.21%; and LAI $R^2$ of 0.74 and RMSE of 0.63. This indicates that low-altitude high-resolution image information obtained by UAV remote sensing can not only provide real-time and high-frequency data support for analyzing crop growth status, but also serve as a supplement to existing ground, aerial, and satellite remote sensing to build a more comprehensive and three-dimensional agricultural remote sensing monitoring system [20].

(2) LR, SVM, RF, and PLSR were used to construct ramie physiological parameter estimation models based on UAV remote sensing. The estimation effects of the four machine learning algorithms were comparable. LR performed best in estimating SPAD value and water content of ramie, while SVM performed best in estimating LAI.

(3) The extracted remote sensing phenotypes have the potential to reflect genotype differences in ramie germplasm resources, with coefficients of variation of LAI, plant height, and plant number reaching 20.83%, 24.61%, and 35.48%, respectively. This demonstrates the feasibility of using UAV remote sensing technology for crop germplasm resources phenotypic diversity investigation.

(4) Principal component analysis of remote sensing phenotypes showed that factor 1 can be used to evaluate structural characteristics of ramie germplasm resources, and factor 2 can be used as a screening index for high light efficiency ramie resources. The principal component analysis results create possibilities for large-scale efficient identification, genotype analysis, and breeding utilization of ramie germplasm resources.

Using UAV remote sensing technology to rapidly, non-destructively, and efficiently extract large-scale field phenotypic data is of great significance for crop variety screening and accelerating crop breeding processes. However, how to mine remote sensing data and extract reliable and meaningful biological knowledge remains the focus of future research. In future studies, the latest artificial intelligence methods such as machine learning and deep learning should be introduced to improve the estimation accuracy of remote sensing phenotypes according to actual breeding targets of crops under specific environments. In addition, crop variety breeding highly depends on genotype and environmental factors. Future research should pay more attention to the integration of phenotypic analysis technology and genotypic technology, in order to screen crop varieties with stronger adaptability under specific environmental stresses.

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*Note: Figure translations are in progress. See original paper for figures.*