Phenotypic Diversity of Seeds and Fruits in Natural Populations of *Acer ginnala* (Postprint)

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Abstract

To investigate the degree and patterns of phenotypic variation in seeds and fruits of *Acer ginnala*, this study employed nested analysis of variance, principal component analysis, cluster analysis, and other methods to conduct a comparative investigation of 12 phenotypic traits of seeds and fruits from seven populations within the main distribution range of *Acer ginnala*, analyzing phenotypic diversity among and within populations and its relationship with geographical and ecological factors. The results indicated that, except for seed length/width (SLW), the remaining 11 phenotypic traits showed significant differences both among and within *Acer ginnala* populations. The average coefficient of variation (CV) across all traits was 13.90%, with a range of 8.14%-32.08%. The average CV of samara traits among populations (15.63%) was higher than that of seed traits (8.71%), demonstrating that seed traits possess greater stability than fruit traits. In principal component analysis, fruit morphological characteristics exhibited greater contribution rates to population phenotypic variation in *Acer ginnala* than seed characteristics. The phenotypic differentiation coefficient among populations (VST) was 35.47%, indicating that within-population variation (64.53%) exceeded among-population variation (35.47%), suggesting that variation primarily originates from within-population sources. Phenotypic traits of seeds and fruits in *Acer ginnala* were minimally influenced by geographical and ecological factors, being predominantly governed by intrinsic genetic factors. Cluster analysis based on Euclidean distances among populations classified the seven *Acer ginnala* populations into two major branches, which did not cluster strictly according to geographical distance, indicating discontinuity in phenotypic trait variation among populations. Different populations of *Acer ginnala* exhibit high phenotypic diversity in seeds and fruits, which is associated with their distribution range and biological characteristics. Multi-level phenotypic variation among and within populations establishes a foundation for the conservation and utilization of *Acer ginnala* germplasm resources.

Full Text

Preamble

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Abstract

To investigate the degree and patterns of phenotypic variation in seeds and fruits of *Acer ginnala*, we conducted a comparative study of 12 phenotypic traits across seven populations in the main distribution area using nested analysis of variance, principal component analysis, and cluster analysis. The relationships between phenotypic diversity and geographic-ecological factors were also examined. The results showed that, except for seed length/width ratio (SLW), the other 11 phenotypic traits exhibited significant differences both among and within populations. The average coefficient of variation (CV) across all traits was 13.90%, ranging from 8.14% to 32.08%. The mean CV for samara traits (15.63%) was higher than that for seed traits (8.71%), indicating greater stability in seed traits compared to fruit traits. Principal component analysis revealed that fruit morphological characteristics contributed more to population phenotypic variation than seed traits. The phenotypic differentiation coefficient among populations (VST) was 35.47%, indicating that variation within populations (64.53%) exceeded variation among populations, suggesting that the primary source of variation resides within populations. Phenotypic traits of *A. ginnala* seeds and fruits were less influenced by geographic-ecological factors and more strongly affected by genetic factors. Cluster analysis based on Euclidean distances divided the seven populations into two major groups that did not strictly follow geographic distance, indicating discontinuous variation in phenotypic traits. The high phenotypic diversity observed among different populations is associated with the species' distribution range and biological characteristics. This multilevel phenotypic variation among and within populations provides a foundation for the conservation and utilization of *A. ginnala* germplasm resources.

Keywords: *Acer ginnala*, population, fruit and seed traits, phenotypic diversity, variation, geographic-ecological factors

Introduction

Acer ginnala Maxim is a deciduous small tree or shrub belonging to the family Aceraceae and genus *Acer*. It is cold-resistant, shade-tolerant, and moistureloving, yet also drought-resistant and adaptable to poor soils, with strong disease resistance and adaptability. The species is widely distributed in mountainous areas and forest regions of Heilongjiang, Jilin, Liaoning, Shanxi, Shaanxi, Inner Mongolia, and other provinces in China, holding significant medicinal, ornamental, and economic value (Wang et al., 2010; Li, 2010). Natural forests of *A. ginnala* have been over-exploited, resulting in substantial loss of wild resources, scattered distribution, and unstable population structure. Therefore, understanding the distribution and genetic diversity of this resource is essential for its rational development and biodiversity conservation (Ma et al., 2005). Recent research on *A. ginnala* has primarily focused on physiological characteristics of leaf color changes in seedlings (Hong, 2008), extraction and separation techniques of gallic acid from leaves (Qiu, 2012), optimization of total phenol extraction and antioxidant activity (Zhao et al., 2016), phenotypic and genetic diversity studies (Wang et al., 2010; Lin, 2015; Yan et al., 2010), and cloning and bioinformatics analysis of the SDH gene (Cui et al., 2017).

Phenotypic diversity constitutes an important component of biodiversity research, primarily investigating phenotypic variation among populations under different environmental conditions while reflecting genetic variation (Yan & Chen, 1999). Plant phenotypic traits represent genotype adaptation to environmental changes, undergoing irreversible alterations under long-term selective pressure that become stably inherited to produce new phenotypes. Consequently, phenotypic variation often carries significant adaptive and evolutionary importance (Pigliucci et al., 2006). Plant phenotypic traits include leaf, fruit, and seed characteristics, among which seed and fruit traits are primary components of plant sexual reproduction systems, representing comprehensive expressions of reproductive and survival adaptations. Compared to other traits, seed and fruit variation serves as a fundamental basis for genetic diversity research with relatively high stability (Huang et al., 2016), revealing genetic patterns and variation magnitude among different populations while illuminating environmental and genetic influences on populations. Currently, domestic and international scholars have investigated seed and fruit variation in various species, including *Pinus albicaulis* (Garcia et al., 2009), *Pinus bungeana* (Li et al., 2002), *Keteleeria fortunei* var. *cyclolepis* (Liu et al., 2017), *Sapindus mukorossi* (Diao et al., 2014), *Xanthoceras sorbifolia* (Hou et al., 2013), *Acer truncatum* (Qiao et al., 2017), *Acer palmatum* (Huang et al., 2016), and *Gleditsia sinensis* (Li et al., 2013), providing foundations for germplasm resource selection and conservation.

This study examines seeds and fruits of *A. ginnala* populations to explore the degree of phenotypic variation among different populations and reveal patterns of phenotypic and eco-geographic variation, thereby providing reference for subsequent germplasm resource development, conservation strategies, and breeding programs.

Materials and Methods

1.1 Material Collection

Based on literature and records, seeds and fruits were collected from seven provinces across the main distribution areas of *A. ginnala* during October-November 2016, according to the species' primary distribution range and actual fruiting conditions (Table 1). Each sampling site was treated as a population. Within each selected site, 30 individuals without obvious defects or severe pests/diseases, exhibiting normal growth and spaced more than 30 m apart (to prevent maternal line effects), were randomly selected. Mature fruits were collected from the upper middle portion of each individual' s canopy in four directions and brought back to the laboratory for analysis. Geographic-ecological factors including longitude, latitude, and altitude were recorded for each population using a global positioning system (GPS) (Table 1).

1.2 Phenotypic Trait Measurement

Following the methods of Luo (2003), 12 phenotypic traits of *A. ginnala* seeds and fruits were measured: key fruit length (KFL), key fruit width (KFW), fruit length (FL), fruit width (FW), fruit stalk length (FSL), bearing mark (BM), seed width (SW), and seed length (SL) were measured using vernier calipers (precision 0.01 mm); samara connecting angle (SCA) was measured using a protractor (precision 0.1°); and ratios including key fruit length/width (KFLW), fruit length/width (FLW), and seed length/width (SLW) were calculated.

1.3 Statistical Analysis

Nested analysis of variance was performed on each trait using SPSS 21.0 software (Li et al., 2002) with the following linear model:

$$Y_{ijk} = L + S_i + T_{(i)j} + E_{(ij)k}$$

where Y_{ijk} is the kth observation of the jth family in the ith population, L is the overall mean, S_i is the population effect (fixed), $T_{(i)j}$ is the family effect within populations (random), and $E_{(ij)k}$ is the experimental error. This analysis characterized phenotypic variation patterns in *A. ginnala* populations.

The phenotypic differentiation coefficient was calculated according to Ge et al. (1988) as:

$$V_{st} = \frac{\delta^2_{t/s}}{\delta^2_{t/s} + \delta^2_t}$$

where $\delta^2_{t/s}$ is the variance component among populations and δ^2_t is the variance component within populations.

Principal component analysis was applied to phenotypic traits using SPSS 21.0 to identify primary traits contributing to phenotypic differences. Cluster analysis of populations was performed using the unweighted pair-group method with arithmetic means (UPGMA) in NTSYS v2.10 software (Ming & Gu, 2006). Other statistical analyses were conducted using standard procedures in Excel 2010 and SPSS 21.0.

Results and Analysis

2.1 Variation in Seed and Fruit Traits Among and Within Populations

Except for key fruit width (KFW), the other 11 seed and fruit traits showed extremely significant differences among populations ($P < 0.01$). Within populations, KFW exhibited significant differences ($P < 0.05$), seed length/width ratio (SLW) showed no significant difference, and the remaining traits were extremely significant (Table 2), indicating extensive genetic variation in *A. ginnala* seed and fruit traits at both among- and within-population levels.

2.2 Phenotypic Variation Characteristics

The coefficient of variation (CV) enables comparison of variation degrees among different phenotypic traits. Larger CV values indicate greater trait dispersion. The CV values for the 12 seed and fruit traits ranged from 8.14% to 32.08% (Table 3), with fruit stalk length showing the highest value (32.08%) and seed width the lowest (8.14%). The average CV across all traits was 13.90%, with seed traits (8.71%) lower than samara traits (15.63%), indicating relatively higher stability in seed phenotypic traits and richer variation in fruit phenotypic traits.

Variation amplitude for the same trait differed among populations, suggesting that environmental differences across regions lead to distinct population phenotypic variations. For example, key fruit width (KFW) showed the greatest variation in the Benxi (BX) population (20.14%), which was 1.91 times higher than the least variable Tongbai Mountain (TBS) population (10.54%). Different traits within the same population also showed substantial CV differences; in the BX population, KFW had the highest CV (20.14%), 2.89 times greater than the lowest CV for seed width (6.98%), demonstrating that differential adaptive processes of various traits to the environment ultimately result in population phenotypic variation differences.

2.3 Sources of Phenotypic Variation and Differentiation Among Populations

Nested analysis of variance further elucidated the sources of variation for 12 phenotypic traits, partitioning variation into three components: among populations, within populations, and random error, with each component' s percentage reflecting the source of variation in *A. ginnala* seeds and fruits (Zhang et al., 1999). The phenotypic differentiation coefficient reflects the magnitude of among-population differentiation; larger values indicate greater genetic differentiation and variation among populations, and vice versa. As shown in Table 4 , the average variance component percentage among populations was 30.68%, while within populations it was 54.03%, indicating greater differentiation within than among populations.

The phenotypic differentiation coefficient (Vst) for the 12 traits ranged from 15.99% to 82.35%, with fruit width showing the highest value (82.35%) and fruit length the lowest (15.99%). The mean Vst across all traits was 35.47%, with among-population variation (35.47%) lower than within-population variation (64.53%), confirming that the primary source of phenotypic variation in *A. ginnala* seeds and fruits resides within populations.

2.4 Principal Component Analysis of Seed and Fruit Phenotypic Traits

Principal component analysis of the 12 seed and fruit traits identified the degree of influence each trait exerts on phenotypic variation. The first three principal components cumulatively explained 71.65% of the total variance, essentially representing all information from the original variables. The first component contributed 37.044%, with decisive traits including fruit length/width ratio (FLW), bearing mark (BM), fruit length (FL), and key fruit width (KFW). The second component contributed 20.21%, dominated by fruit width (FW). The third component contributed 14.40%, primarily influenced by key fruit length/width ratio (KFLW) and key fruit width (KFW) (Table 5). Fruit traits contributed more than seed traits to population phenotypic diversity, consistent with previous analyses.

2.5 Correlation Between Phenotypic Traits and Geographic-Ecological Factors

Correlation analysis between the 12 seed and fruit traits and geographic-ecological factors revealed that only fruit length (FL) and fruit width (FW) were significantly negatively correlated with mean annual temperature and precipitation (correlation coefficients of 0.821 and 0.790, respectively), reflecting *A. ginnala*' s cold resistance, shade tolerance, and preference for moisture despite drought tolerance. The remaining 10 seed and fruit traits showed no significant relationships with geographic-ecological factors. Overall, fruit traits appeared slightly more influenced by geographic-ecological factors than seed traits.

2.6 Cluster Analysis of Population Seed and Fruit Phenotypes

UPGMA cluster analysis based on Euclidean distances of the 12 phenotypic traits divided populations into two groups at a distance of 0.10 (Figure 1 [Figure 1: see original paper]). The first group included populations from Inner Mongolia Manhan Mountain (MHS), Shaanxi Huanglong Mountain (HLS), Jilin

Laoyeling (JLLYL), Shanxi Qiliyu (QLY), and Henan Tongbai Mountain (TBS). The second group comprised Liaoning Benxi (BX) and Shandong Lao Mountain (LS). Populations did not cluster strictly according to geographic distance.

Discussion

3.1 Phenotypic Variation Diversity in *Acer ginnala* Seeds and Fruits

Phenotypic diversity results from the interaction between genetic diversity and environmental heterogeneity, with phenotypic variation necessarily containing genetic variation. Greater phenotypic variation suggests larger potential genetic variation (Zhang et al., 2017). Significant differences existed among and within *A. ginnala* populations for all seed and fruit traits, with greater diversity within than among populations, reflecting the complex interaction between genetic diversity and environmental heterogeneity as a product of differential environmental selection and a source of population differentiation (Li et al., 1998). Furthermore, the same trait differed among populations, and different traits varied within the same population, indicating that identical traits in the same species respond differently to environmental conditions or that different traits respond differently to the same environmental conditions (Liu et al., 2010; Eller & Brix, 2012).

The average CV for 12 seed and fruit traits was 13.90%, ranging from 8.14% to 32.08%, indicating high dispersion and rich variation. The mean CV for seed traits (8.71%) was lower than for fruit traits (15.63%), suggesting higher stability in seed traits and less environmental influence, consistent with findings for *Pinus bungeana* (Li et al., 2002), *Acer mono* (Zhang et al., 2015), *Acer truncatum* (Qiao et al., 2017), and *Gleditsia sinensis* (Li et al., 2013), but differing from studies on *Sapindus mukorossi* (Diao et al., 2014), *Picea crassifolia* (Wang & Li, 2008), and *Keteleeria fortunei* var. *cyclolepis* (Liu et al., 2017). The high phenotypic diversity in *A. ginnala* may result from its wide distribution range and evolutionary history. The species is broadly distributed across Heilongjiang, Jilin, Liaoning, Shanxi, Shaanxi, Inner Mongolia, and other regions with varying climatic conditions, leading to different morphological variations to adapt to diverse habitats (Garbutt & Bazza, 1987). Additionally, *A. ginnala* has an evolutionary history spanning hundreds of years, during which rich genetic variation has accumulated, with current populations retaining ancestral variation (Huang et al., 2009).

Among-population variation constitutes an important component of intraspecific diversity and reflects differences in reproductive and geographic isolation. Its magnitude indicates a species' adaptive capacity to different environments, with larger values suggesting stronger adaptability (Yan et al., 2010). The mean phenotypic differentiation coefficient among *A. ginnala* populations (35.47%) was higher than that of the tree species *Pinus bungeana* (22.86%) (Li et al., 2002), the grass *Miscanthus sinensis* (32.05%) (Xiao et al., 2013), *Michelia yunnanensis* (24.38%) (Song et al., 2013), *Gleditsia sinensis* (20.42%) (Li et al., 2013), and the endangered *Ulmus lamellosa* (28.102%) (Zheng et al., 2013), but lower than *Acer mono* (48.42%) (Zhang et al., 2015) and *Acer grosseri* (50.16%) (Meng et al., 2013), indicating broad environmental adaptability in *A. ginnala*. As a wind-pollinated species with wind-dispersed pollen and fruits, gene flow among populations is facilitated. Within-population variation represents the primary source of phenotypic variation; the species produces samaras with high natural fruit set but generally low seed maturity and physiological after-ripening, biological characteristics that increase potential for within-population variation (Liang et al., 2007), possibly also resulting from genetic differences and individual responses to microenvironmental conditions during development (Luo et al., 2003).

3.2 Geographic Variation Patterns of Seed and Fruit Phenotypic Traits

Geographic variation patterns in plant seed and fruit phenotypic traits are complex, with different species exhibiting distinct environmental adaptations. Studies on *Picea crassifolia* (Wang & Li, 2008) and *Acer truncatum* (Qiao et al., 2017) suggest longitudinal gradients as the primary pattern of seed and fruit trait variation. Luo et al. (2003) and Liu et al. (2017) identified altitude as an important environmental factor influencing seeds and fruits of spruce and *Keteleeria fortunei* var. *cyclolepis*. For *Sapindus mukorossi* (Diao et al., 2014), variation was significantly correlated with latitude and mean annual temperature, while for *Litsea cubeba* (Tian et al., 2012), annual precipitation and altitude were primary factors. In *A. ginnala*, correlation analysis indicated minimal environmental influence on seed and fruit phenotypic traits, with seeds slightly less affected than fruits, likely due to stronger genetic control—consistent with Lin (2015). Cluster analysis showed that *A. ginnala* populations did not group strictly by geographic distance, indicating discontinuous phenotypic variation, a pattern also observed in *Acer mono* (Zhang et al., 2015).

3.3 Conservation Strategies and Utilization of *Acer ginnala*

Acer ginnala is an important colorful foliage tree species in China with beautiful form and brilliant red autumn leaves. Its leaves are rich in gallic acid, making it a significant biomass energy species. Excessive human disturbance has caused substantial waste and destruction of genetic resources, placing the species in a vulnerable state (Ma et al., 2005). The rich phenotypic variation in *A. ginnala* seeds and fruits provides a foundation for selecting superior germplasm resources. Improvement efforts should combine between- and within-population variation, selecting superior populations while simultaneously identifying outstanding individuals within populations. These results provide a material basis for biodiversity conservation and are significant for further genetic diversity research, germplasm resource conservation, rational development of natural forests, and cultivation of plantations.

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