Analysis of the Genotype Interaction of Four-Year-Old Populus euramericana Using the BLUP-GGE Technique

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Abstract: Poplar is a commercially globalized tree species that provides energy and economic and ecological support. To evaluate the twelve hybrid Populus euramericana genotypes developed in China, a total of six locations were selected for the current study, comprising four climatic types and three kinds of soil. The objective of this study was to characterize the early stages of Populus euramericana growth and to test the locations; to identify good genotypes for stable and high yield; and to offer practical experience and technical assistance for further breeding of Populus euramericana. The main research methods included the statistical description of tree heights and diameter at breast height (DBH), the establishment of a mixed effect model to analyze the genotype and environmental interaction effect (G × E), the use of best linear unbiased prediction (BLUP) values as GGE biplots to achieve visual screening, and the calculation of genetic parameters. Results show that the genotype effect (G), the environmental effect (E), and the G × E interaction is significant; the BLUP value has a strong correspondence with the observed value; the goodness of fit of all biplots can explain more than 85% of the variation; the broad-sense heritability of tree height and DBH is 0.13 and 0.3, respectively; and the type-B correlation is 0.36 and 0.65. In addition, G5, G7, G4, and G9 are excellent genotypes with high yield and stability; using the tree height and DBH of these four genotypes can achieve genetic gains of 3.35% and 0.81%. The conclusions of this study are as follows: the rank-change and scale-effect interactions were distinct; G, E, and G × E all had a significant effect on the growth of poplar trees during their early stage; G4, G5, G7, and G9 genotypes have favorable development characteristics; and N146 is an excellent source of paternal genetics.

Keywords: genotype and environment interaction; multi-environment test; genotype selection; BLUP-GGE; Populus euramericana

1. Introduction

Forests are necessary for all lifeforms to thrive. They are essential for humans because they provide fresh air, nutrition, a habitat, energy, and a source of income for people who rely on them. Forests are habitat to 80% of the diversity of the Earth’s species and provide all of the fundamental needs for adjacent human habitations [1–4]. Poplar (Populus spp.) is China’s primary afforestation tree species, accounting for the largest planted area in the world. Populus deltoides and its hybrid offspring Populus euramericana are the predominant clones used in commercial production in China [5]. When growing poplar for commercial purposes, its high yield, stability of yield, and tolerance to a variety of conditions should be considered [6]. The phenotypic observation values of various genetic materials grown in various locations frequently vary. The statistical validity of the evaluation is low, and the evaluation performance is largely affected by genotype and environment. G × E interaction has a substantial effect on forest productivity. Prior to
the widespread promotion of genotypes, multi-environment tests (MET) are required to determine the yield level and adaptability of genotypes, which will serve as the foundation for their widespread promotion. G × E analysis was found to be an efficient approach for tree breeding research on Chinese fir (Cunninghamia lanceolata (Lamb.) Hook.), radiata pine (Pinus radiata D.Don), and poplar [6–11]. G × E analyses are used to screen out high-yielding varieties with a broad range of adaptability, which is an important trait for plant breeding scientists. Breeders and geneticists have long been interested in studying and integrating G and G × E in order to identify superior genotypes in crop production performance tests [12]. The GGE biplot may be used to analyze vast environments and potential genotypes. It is an effective tool for intuitive analysis of genotype performance, test environment representativeness, and identification ability [13–15]. The GGE biplot has been widely used to analyze the field performance of the world’s major agricultural crops [16]. However, the GGE biplot is rarely employed in tree breeding in China, despite its enormous potential [17]. As a result of the advance in computer technology, an increasing number of statistical models based on random effects are being employed. In comparison to the fixed effect model, the mixed linear model is capable of addressing issues associated with imbalanced data and inconsistent group conditions in experimental areas [10,14]. The best linear unbiased prediction [BLUP] technique is used to obtain the model’s predicted value, which may be more reliable than the observation value for the GGE biplot. When coupled with spatial analysis, it is capable of resolving not only data imbalances and fixed effect limitations in the data, but also the error homogeneity of the test site [18]. The BLUP-GGE technique was used in this work to examine MET data from Populus euramericana, to assess G, E, and G × E, and to select good genotypes.

2. Materials and Methods

2.1. Test Sites and Conditions

Zhucheng, Ningyang, Yuncheng, Shishou, Luannan, and Heishan were the six experimental locations used for this MET study (Figure 1). Zhucheng and Yuncheng have a continental warm temperate semi-arid climate, whereas Ningyang and Luannan have a continental warm temperate monsoon climate; Shishou has a subtropical monsoon climate, and Heishan has a continental temperate monsoon climate. Environmental variables varied significantly across locations (Table 1).

| Environmental Factor               | Zhucheng | Yuncheng | Ningyang | Luannan | Shishou | Heishan |
|-----------------------------------|----------|----------|----------|---------|---------|---------|
| Soil type                         | Loam     | Sand     | Sandy soil | Sand | Loam    | Sand    |
| Annual temperature range/°C       | 27.2     | 27.9     | 28       | 30.6    | 23.9    | 34.2    |
| Annual average of temperature/°C  | 13.1     | 13.9     | 13.7     | 11.4    | 16.9    | 9.0     |
| Annual average of daily temperature range/°C | 9.6 | 10.2 | 11.2 | 10.2 | 7.4 | 10.2 |
| Annual average of minimum temperature/°C | 8.9 | 9.4 | 8.7 | 6.8 | 13.7 | 4.2 |
| Daily precipitation in a year ≥0.1 mm days/d | 79.2 | 70.9 | 67.9 | 70.2 | 127.6 | 71.6 |
| Daily precipitation in a year ≥10.0 mm days/d | 20.9 | 16.7 | 18.2 | 15.9 | 36.1 | 16.8 |
| Daily minimum temperature in a year ≤2.0 °C days/d | 114.5 | 111.8 | 118 | 139.7 | 42.1 | 159.4 |
Table 1. Cont.

| Environmental Factor | Zhucheng | Yuncheng | Ningyang | Luannan | Shishou | Heishan |
|----------------------|----------|----------|----------|---------|---------|---------|
| Daily maximum temperature in a year ≥30.0 °C days/d | 52.7     | 73.4     | 75.7     | 46.4    | 79.9    | 22.9    |
| Annual average wind speed/(m·s⁻¹) | 3.1      | 1.9      | 1.8      | 2.2     | 2.2     | 3.4     |
| Average monthly sunshine duration/h | 200.5    | 181.8    | 160.6    | 201.7   | 141.8   | 229.1   |
| Annual average sunshine duration/h | 2405.7   | 2182.0   | 1927.2   | 2420.7  | 1702.0  | 2749.1  |

Figure 1. Locations of test sites.

2.2. Test Materials and Design

The MET employed 12 genotypes of *Populus euramericana* hybrid clones. Table 2 shows the genetic background of the clones. The trial was established in 2015 using cutting clones. The test design at each location was a single-factor balanced randomized complete block trial design. There are 3 blocks in each location, 12 plots per block, and each plot included 4–12 clones.
Table 2. Description of *Populus Euramericana* used in MET. The female parent of genotypes 1 to 9 is the Chinese poplar cultivar “Danhong”, and the remainder are Chinese poplar cultivar “Zhongshi8”.

| Genotype IDs | Clone IDs | Female Parent | Male Parent  | Male Parent’s Region       |
|--------------|-----------|---------------|--------------|----------------------------|
| G1           | 11-20-2   | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N46-2’ | Northern Russia            |
| G2           | 11-21-8   | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N50-8’ | Northern Russia            |
| G3           | 11-24-4   | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N120-4’ | Northern Germany           |
| G4           | 11-26-10  | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N146-10’ | Northern Netherlands       |
| G5           | 11-26-4   | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N146-4’ | Northern Netherlands       |
| G6           | 11-26-8   | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N146-8’ | Northern Netherlands       |
| G7           | 11-26-9   | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N146-9’ | Northern Netherlands       |
| G8           | 11-27-4   | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N150-4’ | Northern Netherlands       |
| G9           | 11-28-11  | *P. deltoides* ‘Danhong’ | *P. nigra* ‘N166-11’ | Northern Netherlands       |
| G10          | 11-32-1   | *P. deltoides* ‘Zhongshi8’ | *P. nigra* ‘N23-1’ | Northern Germany           |
| G11          | 11-36-26  | *P. deltoides* ‘Zhongshi8’ | *P. nigra* ‘N31-26’ | Southern Italy             |
| G12          | 11-40-2   | *P. deltoides* ‘Zhongshi8’ | *P. nigra* ‘N39-2’ | Southern Italy             |

2.3. Measurements

In the winter of 2019, the tree height and DBH were measured with a tower ruler and girth measure. Abnormal values in the data of each location were excluded. Abnormal values include those seedlings that do not have main branches (the top buds are damaged or broken by wind), do not grow well in the first year but sprout from the roots in the second year, and sprout from the original cut roots of the poplar in the test site.

2.4. Analysis

Asreml-R4.0, a statistical software package in the R environment, was used for analysis of the mixed linear models [16]. The variance components of random effects were determined by solving a given mixed linear model using the restricted maximum likelihood (REML) technique. The advantage of this technique is that missing values were able to be replaced with estimates, therefore resolving the data imbalance problem associated with missing values in MET [19]. The mixed linear model used in asreml-R4.0 was as follows:

\[ y_{ij} = \mu + \beta + \alpha_i + g_j + sg_{ij} + e_{ij} \]

where ‘i’ = 1, . . . , 6, represents the 6 sites; ‘j’ = 1, . . . , 12, represents the 12 genotypes; ‘y’ is the observed value; ‘\(\mu\)’ is the mean value of all observed values; ‘\(\beta\)’ is the effect of the block; ‘\(\alpha\)’ is the environmental effect; ‘\(g\)’ is the genotype effect; ‘\(sg\)’ is the G × E effect; ‘\(e\)’ is the random error. The fixed effects are \(\beta\) and \(\sigma\), and the random effects are \(g\) and \(sg\).

Broad-sense heritability is an important concept in quantitative genetics [20], used to explain which of heredity and the environment has a greater effect on a given trait [21]. Compared with sexual reproduction (family) forestry, the broad-sense heritability in clonal forestry has more guiding significance [22]. The formula for calculating broad-sense heritability [19] was as follows:

\[ H_B^2 = \frac{V_G}{V_G + V_e} \]

where ‘\(H_B^2\)’ is the broad-sense heritability of traits; \(V_G\) is the variance component of G; \(V_e\) is the variance component of random error.

The formula [21] for calculating genetic gain (\(\Delta G\)) was as follows:

\[ \Delta G = H_B^2 (\mu_s - \mu_p) \]

where \(\mu_s\) is the average of the genotypes selected; \(\mu_p\) is the average of all MET population.

The formula of type-B correlation (\(r_b\)) among the six sites was as follows:

\[ r_b = \frac{V_G}{V_G + V_{GE}} \]
At present, G × E statistical methods are mainly based on linear and mixed linear models, and an ordinary linear model cannot estimate population genetic parameters such as the square difference component and breeding value; the BLUP value can be extracted from the asreml model, enabling the GGE biplot to be used to effectively analyze MET data [14,15]. The GGEbiplotGUI software package is a visual data analysis tool for multiple environmental trials, facilitating plant breeders and geneticists to study the yield of genotypes and the G × E interaction [22]. The GGEbiplotGUI package in R was used to draw the GGE biplot. When setting the parameters, 0 (non-standardized) was selected for scaled, the centralized G + GE and SVP eigenvalue option were selected, and 1 or 2 were selected according to the type of graph [14,23,24].

3. Results
3.1. Statistics of Height and DBH Observations

The statistical results in Figure 2 show that there were significant rank changes and scale effects in the performance of 12 four-year-old hybrid poplar genotypes. In terms of tree height and DBH, NY had the best performance, while HS had the worst performance.

![Figure 2](image)

**Figure 2.** Descriptive statistics of DBH and height. “Mean Value ± Standard Deviation” is the data format in the figure. The position of the bottom horizontal axis represents six locations from left to right: “Heishan”, “Luannan”, “Ningyang”, “Shishou”, “Yuncheng”, and “Zhucheng”.

3.2. Six Sites

Table 3 showed that the block effect had a very significant influence on the tree height of poplar (p < 0.01), but not on the DBH. Site had a significant effect on height and DBH of poplar (p < 0.01).

| Trait   | Source | Degree of Freedom | Sum of Squares | F-Value | Significance |
|---------|--------|-------------------|----------------|---------|--------------|
| DBH     | Block  | 2                 | 10.46          | 4.67    | 0.10         |
|         | Site   | 5                 | 1880.33        | 839.28  | 0.00         |
|         | Residual | -                | 2.24           |         |              |
| Height  | Block  | 2                 | 35.70          | 32.40   | 0.00         |
|         | Site   | 5                 | 861.20         | 780.40  | 0.00         |
|         | Residual | -                | 1.10           |         |              |

Table 4 showed that G and G × E were significantly higher in both tree height and DBH (p < 0.01).
Table 4. The random effect.

| Source            | Height Variance Components | Height % of Variance Components | Height Significance | DBH Variance Components | DBH % of Variance Components | DBH Significance |
|-------------------|----------------------------|---------------------------------|--------------------|-------------------------|-------------------------------|------------------|
| Genotype          | 0.16                       | 10%                             | 0.00               | 0.95                    | 26%                           | 0.00             |
| Genotype × Site   | 0.29                       | 19%                             | 0.00               | 0.51                    | 14%                           | 0.00             |
| Error             | 1.10                       | 71%                             | -                  | 2.24                    | 61%                           | -                |

The comparison between the BLUP value and the observed value in Figure 3 shows an obvious correspondence, and the results are highly reliable.

![Figure 3](image-url)

**Figure 3.** The relationship between BLUP value and mean value.

### 3.3. Selection of 12 Genotypes by GGE Biplots

#### 3.3.1. DBH

The X-axis of Figure 4 represents the first principal component (PC1) (79.25%), whereas the Y-axis represents the second principal component (PC2) (11.49%). The goodness of fit is the sum of PC1 and PC2, and equal to 90.74%. For discriminativeness, HS is the worst, LN is poor, and the others are good. For representativeness, NY and YC are the strongest, and other locations are good (less than 45°). There is a positive correlation and representativeness among all test locations. HS, LN, and ZC have a strong correlation. NY and YC are highly correlated; the correlation between SS and any other sites is small. Good correlations between locations represent similar or identical rankings of excellent genotypes, and locations with this characteristic are collectively called a mega-environment (ME) [15]. The results in Figure 5 indicate that SS, NY, and YC were all located in the same ME, with G5 being the best genotype and G9 and G4 being good genotypes. HS, LN, and ZC were located in another ME, and the optimal genotype was G7. According to Figures 6 and 7, the genotypes that yielded more than the norm were G5, G7, G4, and G9. G5 exhibited the highest peak output but the lowest stability of the four genotypes. G5 manifests itself as significantly better in the ‘SS-NY-YC’ ME than in the ‘HS-LN-ZC’ ME. The remaining genotypes exhibited excellent consistent stability, in the following order: G7 > G4 > G9.
**Figure 4.** Discriminativeness and representativeness of tree height of 6 sites. The smaller the angle with the blue solid line, the greater the representativeness; the longer the length of the dashed line, the greater the discriminativeness. The angle between the dotted lines can reflect the correlation between the locations; the smaller the angle, the more positive the correlation.

**Figure 5.** Biplot of “which won where” of tree height. The area formed by two adjacent red lines is an ME, where the genotype of the quadrilateral vertex is the best for the ME, and the remainder are good.

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**Figure 5.** Biplot of “which won where” of DBH. The area formed by two adjacent red lines is an ME, where the genotype of the quadrilateral vertex is the best for the ME, and the remainder are good.
Figure 6. Ranking genotypes of tree height. The smaller the radius of the concentric circles, the higher the ranking of the genotypes for comprehensive high yield and stability.

Figure 7. Tree height mean and stability of 12 genotypes. The small blue circle on the horizontal axis represents the average environmental yield. The yield of genotype traits increases along the horizontal axis, and the stability decreases with the increase in the vertical distance.

Figure 6. Ranking genotypes of DBH. The smaller the radius of the concentric circles, the higher the ranking of the genotypes for comprehensive high yield and stability.

Figure 7. DBH mean and stability of 12 genotypes. The small blue circle on the horizontal axis represents the average environmental yield. The yield of genotype traits increases along the horizontal axis, and the stability decreases with the increase in the vertical distance.
3.3.2. Tree Height

Results showed in Figures 8–11. Figure 8 shows PC1 (66.71%) and PC2 (18.7%). The goodness of fit was 85.41%, which explains a high percentage of the observed variability in G and GE. The results in Figure 8 show that HS, LN, ZC, and YC are closely positively correlated. For the correlation with other sites, that of NY is slightly smaller and that of SS is low. The results in Figure 9 show that, in all regions, the best genotype was G5, and the good genotypes were G4, G9, and G7. Based on Figures 10 and 11, the genotypes with higher-than-average yield were G5, G7, G4, and G9. Among the four genotypes, G5 had the best yield but an unstable yield character. G5 manifests itself as better in the ‘SS-NY’ ME than in other sites. The stability and high yield of other genotypes were consistent with the DBH.

![Discriminiveness and representativeness of DBH of 6 sites.](image1)

**Figure 8.** Discriminiveness and representativeness of tree height of 6 sites.

![Biplot of “which won where” of tree height.](image2)

**Figure 9.** Biplot of “which won where” of tree height.
3.4. Heritability, Type B Correlation, and Genetic Gain of Selected Genotypes

Related genetic parameters calculated were showed in Table 5. The results of $H_B^2$ show that tree height and DBH are more affected by the environment, and tree height is more affected by the environment than DBH ($0.13 < 0.28$). The results of $H_B^2$ were similar to previous studies [25].

The type-B genetic correlation coefficient results showed that both tree height and DBH traits were affected by $G \times E$ ($r_b < 0.7$), and tree height was more affected.

The genotypes G5, G4, G7, and G9 were selected for the high yield and stable yield of tree height and DBH. The genetic gains were calculated for tree height as 6.84 cm (3.35%) and DBH as 0.34 cm (0.81%).
Table 5. Genetic parameters.

| Genetic Parameter | Height | DBH |
|-------------------|--------|-----|
| $H_b$             | 0.13   | 0.30|
| $r_b$             | 0.36   | 0.65|
| $\Delta G$/cm     | 6.84   | 0.34|
| % of $\Delta G$   | 3.35%  | 0.81%|

4. Discussion

The G × E interaction had a significant effect on the expression of tree height and DBH during the early growth stage of the *Populus euramericana* population. The G × E analysis enables breeders to pick trees that are suited to the unique environment of future generations [4,8]. The selected genotypes in this MET were G4, G5, and G7. G9 was not chosen because its yield was lower than usual at the time. For two years in a row, data screening findings were almost, but not fully, identical [26], indicating that the test is reliable. In addition, it shows the volatility of the early growth stage of *Populus euramericana*: different genotypes may exhibit variations in the rapid growth stage. In terms of cultivar selection strategies for specific regions with strong tree characteristics, the G4, G5, G7, and G9 genotypes are acceptable for planting on all sites. In terms of cultivars appropriate for certain places with DBH, it was discovered that G5 was more adaptable, whereas G7 thrived in northern regions with annual sunlight duration surpassing 2400 h and a short growing season. All female parents of the four good genotypes were *P. deltoides* ‘Danhong’, whereas 75% of male parents were *P. nigra* ‘N146’. The results indicated that N146 was most likely characterized by superior paternal material with superior genetic material. However, G10, G11, and G12 had the lowest DBH performance and the lowest tree height performance. This is most likely due to their female parent being *P. deltoides* ‘Zhongshi8’. Tree height exhibited a lower heritability and type-B correlation coefficient than DBH, whereas G × E had a stronger effect on tree height in a hybrid *Populus euramericana* population. Thus, tree height is more environment-dependent and less genetically determined than DBH. The results of broad-sense heritability calculations differ from those of [25]. Numerous studies have demonstrated that various characteristics are genetically controlled to varying degrees, that wood is less impacted by environmental factors, and that the impact of biomass is distinct from the impact of tree height or DBH [9,27]. Lin [22] summarized pertinent research and concluded that the G × E of many tree species would deteriorate as forest age increased, and that analysis of the initial development stage would not be reliable. In contrast, rapidly growing species, such as poplar, have a shorter building time and achieve population maturity sooner (usually less than 9 years). It is important to examine the hybrid poplar’s first development stage and to conduct experiments. Both growth and material stages require a comprehensive construction phase supported by expertise. The trial’s future performance cannot be entirely predicted. It is impossible to assume that the ranking of individual genotypes will not suddenly improve in the coming year, and the trend must be observed over time to determine this. Thus far, no distinction has been made between the third and fourth years. Although studies have demonstrated that tailored clone testing may yield higher yields than family tests, and clone forestry also provides a more powerful technique of detecting G × E, there are hazards associated with both methods [11,27]. This project will be conducted indefinitely to provide important experience and technical assistance for poplar clonal forestry.

5. Conclusions

The growth traits (DBH and tree height) of hybrid *Populus euramericana* at the early stage of growth showed significant rank change and scale effect interactions. This is because the growth was significantly affected by G, E and G × E. Compared with tree height, DBH was more affected by E and G × E in the MET. The selected G4, G5, G7, and G9 genotypes showed high yield and stability, and have broad promotion potential. In terms of regionally relevant varieties, the height of the selected genotypes was good in
all places; G7 has the potential to develop large-diameter materials in sandy soil regions, whereas G5 has the potential to develop large-diameter materials in loam and sandy soil areas. *P. nigra* ‘N146’ possesses considerable genetic potential and is an excellent source of male paternal material.

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