

Article



Application of BLUP-GGE in Growth Variation Analysis in Southern-Type *Populus deltoides* **Seedlings in Different Climatic Regions**

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Abstract: In the present study, using the BLUP-GGE approach, southern-type (ST) *Populus deltoides* genotypes were analyzed and evaluated, and variations in growth traits, seedling height (H), and ground diameter (GD) were studied in various climatic regions, which could facilitate the increase of the breeding range of ST. The test materials were 119 one-year-old ST genotypes, and the test sites were Ningyang (NY) and Hainan (HN). A linear mixed-effects model was constructed, and the BLUP values of H and GD were obtained using the linear unbiased prediction (BLUP) method. GGE-Biplots were generated. The H variation was greater than the GD variation. The effects of environment, block, and genotype–environment interaction ($G \times E$) were highly significant. The goodness of fit of the GGE-Biplots obtained by extracting the BLUP values was >95%. According to the GGE-biplot results, the performance of each genotype varied considerably. The genotype No. 13 had the highest average GD and the highest average H. In NY, the genotypes No. 93 and 115 had the highest H and GD, and in HN, the genotype No. 9 had the highest H and GD. ST had a better second-year survival rate in NY than in HN. The hybridization of tropical *Populus deltoides* can be performed using the No. 13 and 117 genotypes, which grow rapidly and have high yields.

Keywords: genotype and environment interaction; BLUP-GGE; genotype selection; southern-type *Populus deltoides*

1. Introduction

The eastern and central regions of North America represent the major distribution ranges of *Populus deltoides* [1]. *Populus deltoides* exhibits high intraspecific variation due to its vast distribution range and the variable climate over its natural range. The species lacks a natural distribution in China [2], and the *Populus deltoides* introduced in the country can be classified into two breeding types: the Southern Type (ST) and the Northern Type (NT). The NT is primarily found in the upper Mississippi River Basin in Iowa, the middle Mississippi River Basin in Missouri, the lower St. Lawrence River Basin in Canada and the Columbia River Basin in Washington State and Quebec Province. Conversely, the ST is primarily found in the lower Mississippi River Basin in the states of Louisiana (LA) and Tennessee (TN), in the US [2]. Every year, poplar plantations are increasingly established in China due to an increasing demand for industrial timber. Chen assessed the morphological and physiological characteristics of 258 *Populus deltoides* clones comprehensively [2]. In the



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). core germplasm bank of *Populus deltoides*, the proportion of ST was 57.89%. In addition, among the superior *Populus deltoides* clones screened, the proportion of ST was 77.78%, with excellent growth performance and high genetic diversity. The ST originates from a humid subtropical monsoon climate with high summer temperatures and copious precipitation [2]. The climate is similar to that of the temperate and tropical parts in the southern region in China. Therefore, ST has high breeding and promotion potential in the tropics. However, the adaptability of ST in the temperate and tropical parts of China remains unclear, which hampers the broad exploitation of *Populus deltoides* in many places. To facilitate the meeting of the demand for industrial wood and the rapid introduction and cultivation of *Populus deltoides* in the temperate and in other regions [3], it is crucial to study the genetic diversity of ST germplasm resources comprehensively and to continuously develop new genotypes that are adapted to different climatic and soil types [4].

Tree growth is often influenced by diverse factors; genetics and genotype–environment interactions ($G \times E$) often have significant effects [5]. Consequently, in multi-environment testing, $G \times E$ is frequently used to assess the fitness of various genotypes. GGE-biplots, which are essential for the selection and promotion of novel cultivars [6,7], have been adopted in numerous agricultural regionalization studies in recent years to assess the performance of crops at various experimental sites. Currently, the BLUP-GGE approach is not widely used in forestry [8], especially when it comes to poplar, and it is hardly ever used during the seedling stage of a forest. It is possible to significantly minimize the impacts of unfavorable factors, such as variations in growing conditions and unbalanced data across experimental sites, by analyzing $G \times E$ based on a mixed-effects model [9,10]. In addition, using GGE-biplots could improve tree breeding timelines substantially and the selection, evaluation, and promotion of novel tree types, in general.

The exploration of the genetic resources of *Populus deltoides* that are adaptable to harsh conditions requires a comprehensive assessment of the growth of the plant in the two locations. To examine $G \times E$ in *Populus deltoides*, in the present study, a mixed linear model was built based on growth data collected from Hainan (HN) and Ningyang (NY), China. The GGE-Biplots were used for visualization.

2. Materials and Methods

2.1. Test Sites and Design

The growth experiments were carried out at the Gaoqiao Forest Farm in NY, Shandong, and at the Jinjiling Forest Farm in Hainan, HN (Table 1). According to Liu, NY is an area that is very suitable for the growth of black poplar trees [8]. The HN environment has high temperature and high humidity and is slightly unfavorable for ST plants.

Table 1. Test site environmental conditions. Ningyang (NY) has a temperate monsoon climate, and Hainan (HN) has a tropical monsoon climate. Shown are the average climate data for the past two decades.

Environmental Factors	HN	NY
Longitude	E: 110°32	E: 116°80
Latitude	N: 19°69	N: 35°76
Annual temperature range (°C)	11.4~38.9	$-18.1 \sim 38.1$
Annual average temperature (°C)	25.9	15.8
Annual average minimum temperature (°C)	23.1	11.6
Annual daily precipitation ≥ 0.1 mm days	124	106
Annual daily precipitation ≥ 10.0 mm days	39	21
Number of days with a daily minimum temperature ≤ 2.0 °C	0	146
Average number of days with an annual maximum temperature \geq 30.0 °C	202	102
Average monthly daylight duration (h)	166.6	202.1
Average annual daylight duration (h)	1999.2	2424.8

The test material consisted of seedlings of 187 ST genotypes, which were planted at each site in April 2019; there were 119 ST genotypes after selection (Table 2). A random block design was adopted in the two experimental plots, with 2–6 plants randomized within the block, three blocks, and a plant–row spacing of 60 cm.

Table 2. Genetic background. Numbers 1–50 are from Louisiana (LA) provenance, and numbers 51–119 are from Tennessee (TN) provenance. The samples used were all clones; they were all cuttings directly imported from the United States. The numbers after LA and TN do not represent the half-sib relationship and are only used to distinguish the clones.

Introduction Site	Genotype Number	Amount	Introduction Site	Genotype Number	Amount
LA01	1–5	5	TN01	51–67	17
LA04	6-10	5	TN02	68–76	9
LA05	11–22	12	TN03	77–90	14
LA06	23-27	5	TN04	91-103	13
LA07	28-33	6	TN05	104–119	16
LA08	34–39	6			
LA09	40-50	11			

2.2. Test Method and Analysis

The ASReml-R v4.0 package in R software resolves mixed linear equations using the restricted maximum likelihood (REML) method and yields the variance components of fixed and random effects [11]. The method can resolve the issue of missing data caused by seedling death by filling in the missing values based on estimates, which is essential in the present study, considering the significant imbalance in the data.

The equation is as follows:

$$y_{ij} = \mu + \beta_i + s_i + g_i + sg_{ij} + e_{ij}$$

In the equation, y_{ij} represents the genotype j's observed value at the ith site, μ represents the mean of all observations, β_i represents the block effect, s_i represents the environment effect, g_i represents the clones' genotype effect, and sg_{ij} represents the G × E effect. The block effect and the environment effect are fixed effects, whereas the genotype and the G × E effects are random effects.

The predict() function in the GGEBiplotGUI package was used to extract the BLUP values (trait prediction values), which were then used for plotting [10,12]. Plant breeders and geneticists can study genotype yield and $G \times E$ using the GGE-Biplot, a tool for visualization and data analysis in multi-environmental experiments [13]. The parameter settings for GGE-Biplot plotting were 0 (non-normalized), G + GE, and SVP1.

3. Results

3.1. Survival Rate

The survival rate results are shown in Table 3. Since there were too few data from HN in 2020, the growth data of 2019 were selected for the analysis. After elimination, there were 119 genotypes shared by the two places with more than 3 seedlings, as shown in Table 2. The survival rate in Hainan (HN) in 2020 was too low, and for most of the surviving genotype, the remaining trees were less than three.

Provonanco	Н	N	Ν	Y
rrovenance –	2019	2020	2019	2020
LA01	97.78%	20.00%	89.74%	76.92%
LA04	90.74%	27.78%	83.78%	75.68%
LA05	92.47%	19.35%	85.59%	84.68%
LA06	95.92%	30.61%	89.13%	82.61%
LA07	96.61%	30.51%	80.49%	75.61%
LA08	89.55%	23.88%	81.36%	72.88%
LA09	96.61%	16.95%	90.14%	88.73%
LA	93.90%	23.71%	85.89%	80.94%
TN01	96.70%	9.89%	90.06%	88.82%
TN02	95.45%	6.06%	96.34%	95.12%
TN03	83.87%	6.45%	97.25%	95.41%
TN04	89.71%	20.59%	93.70%	92.13%
TN05	93.51%	10.39%	97.48%	96.86%
TN	93.09%	11.11%	94.67%	93.42%
Total	88.58%	91.27%	93.54%	18.18%

Table 3. Average survival rate.

3.2. Growth Variation

The seedling height (H), ground diameter (GD), and Coefficient of Variation (CV] results for the 119 ST poplars are listed in Table 4. The mean values of H and GD for the NY site were 148.41 cm and 1.21 cm, respectively, and the mean values of H and GD for the HN site were 46.01 cm and 0.51 cm, respectively. The GD and H values of seedlings in NY were larger than those of seedlings in HN. The CV values of H were 65.61%, 48.51%, and 77.41% in HN, NY, and overall, respectively. The CV values of GD were 47.91%, 43.71%, and 62.61%, in HN, NY, and overall, respectively.

Sites	Traits (cm)	$\mathbf{Mean} \pm \mathbf{SD}$	CV(%)
LINI	Н	46.01 ± 30.21	65.61
HN	GD	0.51 ± 0.31	47.91
	Н	148.41 ± 72.01	48.51
IN Y	GD	1.21 ± 0.51	43.71
TT (1	Н	97.21 ± 75.31	77.41
Iotal	GD	0.91 ± 0.61	62.61

Table 4. Descriptive statistics of for height (H) and ground diameter (GD).

3.3. $G \times E$ and Its Visualization

3.3.1. $G \times E$

Mixed linear models for H and GD of ST were assessed using the ASReml-R package. The fixed effects in the model were block and environment, and the random effects were genotype and $G \times E$. The variance components and significance of block, environment, genotype, and $G \times E$ were obtained following the analysis. The results are shown in Tables 5 and 6. The 'predict()' function was used to extract the BLUP values in the model. By comparison, BLUP values were significantly correlated with the observed values of H and GD (Figure 1), and BLUP values were used to visualize the $G \times E$. According to the results, the H and GD of ST were affected significantly by the environment and the $G \times E$ (p < 0.01), and the effect of the genotype was not significant.

Trait	Source	Degree of Freedom	Sum of Squares	F Value	Significance
	Block	2	3.1197	75.41	< 0.001 ***
Ground	Site	1	3.9201	189.51	< 0.001 ***
Diameter	Residual	-	0.0207		
	Block	2	56235	178.21	< 0.001 ***
Height	Site	1	36836	233.47	< 0.001 ***
	Residual	-	158		

Table 5. Fixed-effects analysis. '***' means extremely significant (p < 0.01).

Table 6. Random-effects analysis. '***' means extremely significant (p < 0.01).

	GD Variance % Variance Components Components		Height			
Source			Significance	Variance Components	% Variance Components	Significance
Genotype	0.0018	1%	0.45	207.6891	7%	0.22
$G \times E$	0.1554	87%	0.00 ***	2620.8155	88%	0.00 ***
Error	0.0207	12%	-	157.7734	5%	-



Figure 1. Comparison of the observed and linear unbiased prediction (BLUP) values. The BLUP value has an obvious correlation with the observed value and can reflect the real growth status.

3.3.2. Ground Diameter and Height Visualization

The first principal component (PC1) explained 85.88% of the variance, the second principal component (PC2) explained 14.12% of the variance, and the sum of PC1 and PC2 was the goodness of fit, surpassing 95%, based on the BLUP value of H as a GGE-Biplot.

Figures 2–4 display the H GGE-Biplots. According to Figure 2, NY had better representativeness and discrimination than HN, and the angle between the environmental vectors of the two sites was approximately 90°, indicating that the two environments were almost completely unrelated.

According to Figure 3, the genotype with the highest H was No. 13, followed by the genotypes No. 90, 39, 89, 14, 82, 68, etc. The genotype with the lowest H was No. 19, followed by the genotypes No. 117, 22, 116, 93, 87, 9, 107, 106, etc. The output of the genotype No. 13 was the highest; however, this genotype was not stable and performed better in HN than in NY. The top 10 genotypes in terms of seedling height varied greatly, with the exception of the genotypes No. 34, 107, 87, and 93, which exhibited good stability. The genotype No. 9 in HN performed much better than in NY, in terms of seedling height, and both plants were ranked within the top 10. Therefore, the genotype No. 9 appeared better suited for planting in the south.

Figure 4 displays the top genotypes and the excellent genotypes in the two environments. The top genotype in HN was No. 9, while the excellent genotypes were the Nos. 116, 103, 13, 7, 63, 30, 117, 31, 10, and 17. The top genotype in NY was No. 93, while the excellent genotypes were the Nos. 13, 106, 87, 100, 26, 75, 107, 22, 118, and 117.



Figure 2. Discrimination and representativeness of H. The average environment axis is the blue solid line through the origin, and the dotted line is the environment vector of the two sites. The test area discrimination was measured based on the environment vector length. Site discrimination improves with longer dotted lines. The test site is represented by the angle between the dotted and the solid blue lines. The representativeness increases as the angle decreases. The angle between the two dotted lines—the site angles—can indicate a correlation [6].



Figure 3. Height (H) mean and stability. The average environment axis, with a single arrow, points to the genotype's approximate average yield in all environments. The $G \times E$ interaction is shown by the vertical dashed line between the genotype and the mean environment axes. Longer dashes indicate a less stable seedling H (yield) [14].

In the GGE-Biplot based on the BLUP value of GD, PC1 explained 83.47% of the variance, PC2 explained 16.52% of the variance, and the fit was >95%. GD had good discrimination and representation in both environments (Figure 5), with higher representation

in NY than in HN. The genotype with the highest GD was the No. 13; other genotypes with high GDs were the Nos. 117, 116, 107, 28, 9, 113, 98, 77, and 93. The genotype No. 19 had the lowest GD, and other genotypes with low GDs were the Nos. 90, 39, 6, 89, 82, etc. Among the top 10 genotypes based on GD, the Nos. 9 and 116 were both unstable, and their performance in HN was better than that in NY, whereas the genotypes Nos. 107 and 28 were stable (Figure 6). In HN, the No. 9 had the best GD genotype, and the genotypes Nos. 116, 36, 7, 103, 30, 13, 117, 63, 31, 17, and 10 were excellent. In NY, the best GD genotype was No. 13, and the Nos. 93, 106, 87, 100, 26, 75, 107, 22, 118, 117, and 115 were excellent genotypes (Figure 7).



Figure 4. Suitable genotypes for the Mega–environment (ME) with respect to height. ME is the location sandwiched within the two neared red lines, and the genotype of the polygon vertex in the ME is the best genotype for this location [9].



Figure 5. Ground diameter discrimination and representativeness.



Figure 6. Ground diameter mean and stability.



Figure 7. Suitable ground diameter genotypes for the mega–environment.

3.4. Genotype Selection

Based on the intersections of the zero scores and the rankings of the visualization results, the top performing genotypes in HN in 2020 were the Nos. 7, 9, 13, 17, 31, 36, and 117, all of which had a survival rate >33%. The excellent HN genotypes were almost exclusively from LA provenance, and seedling No. 9 performed the best. Based on the 2020 survival rates, the NY top performers were the genotypes No. 5, 13, 26, 34, 50, 54, 75, 93, 96, 98, 99, 100, 106, 107, 115, 117, and 118, with TN accounting for the majority. All survival rates were >83%. The genotypes No. 13 and No. 117 had high and stable yields, in addition to high survival rates at both study sites.

4. Discussion

The climate in regions of ST origin is a subtropical humid climate, similar to the weather in southern China. The distribution area of *Populus deltoides* is primarily between 29°N and 36°N latitude [1,2]. In the present study, the survival rates of ST genotypes in NY were high and stable, whereas in HN, they dropped dramatically in the second year. The results indicate that ST is more suitable for the NY conditions than for the HN conditions. The external environment has a significant impact on the growth of seedlings [15–17]. Seedlings from dry tropics that have been exposed to low-temperature dry winters have better root systems and higher water-use efficiency than seedlings from humid tropics exposed to low temperatures and adverse conditions that limit water availability [18]. Following adversity, plants exhibit improved environmental adaptability and growth, which may be passed on to their offspring [19]. Therefore, selecting ST genotypes is crucial for the expansion of poplar hybrid breeding resources and the cultivation of adaptable poplar varieties.

In the present study, the CV of ST was high, indicating that its growth was relatively unstable. HN had a higher CV for H than NY, potentially due to the more complex weather conditions in HN. The GD was more stable than the H because HN and NY had a similar CV. Most of the excellent HN genotypes originated from LA, and LA had a higher local survival rate. The survival rates of LA06 and LA07 in HN were relatively high, >30%. The two genotypes may be more tolerant to the tropical environment and can be considered breeding materials for tropical regions. Most of the excellent genotypes in NY originated from TN, and TN had a higher local survival rate, indicating that TN genotypes grow better in NY and are suitable as female parents for cultivating a high-growth hybrid progeny in temperate zones.

According to the mixed linear model analysis results, block and environment as fixed effects had significant impacts on ST. The block effect was significant because the experimental area of the seedlings was large, and it was difficult to overcome the homogeneity of errors in the test field [20]. In random effects, the genotype had no significant effect on growth, while the $G \times E$ had a very significant effect, indicating that the genetic influence on growth in ST was much less than that of the environment, and seedling growth was affected by the environmental conditions.

The initial growth status of ST in NY and HN was visually represented using the BLUP value as a GGE biplot. The BLUP values of different traits can typically be selected as a GGE-biplot depending on the breeding objectives. Forestry breeders primarily use the BLUP-GGE approach to assess large trees or agricultural plants because their growth traits are generally stable [13,21–23]. However, in early seedling experiments, the external environment had a significant impact on the seedlings, and their growth traits frequently displayed significant variation. A genotype can be selected by combining the biplot data. The best ST genotypes for the two sites, Nos. 13 and 117, were determined by comparing biplots, combining sites, and survival rates. Additionally, the genotype No. 9 performed extremely well in HN and appeared suitable for ST promotion in the NY region.

Poplars are primarily found in the temperate zones of the northern hemisphere; they hardly adapt to tropical environments; currently, only *Populus ilicifolia* naturally grows in tropical environments [24]. ST is adapted to the subtropics, and the results of studies conducted in temperate and tropical environments suggest that it may be more suited to the former than the latter environment. However, the present study identified some excellent genotypes that can be adopted for hybridization and re-cultivation activities, as well as for initial local promotion efforts. Crosses with native poplars may yield better results and superior genotypes, and we will continue to observe them in later stage. Further studies are required to enhance our understanding of plant stress physiology, in addition to the influence of genome-level factors.

5. Conclusions

At the seedling stage, the southern-type *Populus deltoides* exhibited greater growth variation, with H exhibiting greater variation than GD, and greater variation in HN than in NY. The environment and $G \times E$ effects had a significant impact on ST genotype development. The ST genotypes performed better in the NY region, but were not adapted to the HN region, and the second-year survival rate decreased markedly. The genotypes of LA06 and LA07 provenance were relative heat-resistant compared with those of other provenances, while the TN genotypes are suited for temperate climates. The excellent genotypes for NY were the Nos. 13, 26, 93, 100, 106, 107, and 117, whereas the excellent genotypes for HN were the Nos. 7,9 31, 36, 17, 13, and 117. The No. 13 and 117 genotypes are fast-growing and high-yielding; therefore, they can be adopted as hybrid parents for tropical *Populus deltoides* breeding activities.

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