doi:10.20944/preprints202111.0505.v1

Article

Analysis of Genotype-Interaction of a 4-Year-Old *Populus euramericana* Using BLUP-GGE Technique

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Abstract: Poplar is a globalized commercial tree species that supports humanity's economy, energy, and ecology. To evaluate the twelve hybrid *Populus euramericana* genotypes developed in China, a total of six locations were selected for the test, comprising four climatic types and three soil kinds. The objective of this study is to characterize the early stages of *Populus euramericana* growth and test locations; to identify good genotypes for stable and high yield that may be encouraged; and to offer practical experience and technical assistance for further breeding of *Populus euramericana*. Main research methods include the statistical description of tree heights and diameter at breast heights[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 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; broad-sense heritability of tree height and DBH is 0.13 and 0.3, type-B correlation is 0.36 and 0.65; G5, G7, G4 and G9 are excellent genotypes with high yield and stability; using these four genotypes tree height and DBH can get 3.35% and 0.81% genetic gains.The study concludes as follows: Rank-change interaction and scale-effect interaction were distinctly occurred. The 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. N146 is a great source of paternal genetics.

Keywords: Genotype and environment interaction，Muti-environment test，Genotype selection，BLUP-GGE，*Populus euramericana*

1. Introduction

Forests are necessary for all lifeforms to thrive. They are essential for human because they give fresh air, nutrition, habitat, energy, and a source of income for the various people that rely on forest. Forests are habitat to 80% of the planet's species diversity and provide all of the fundamental needs for nearby human habitation[1-4]. Poplar (*Populus spp*.) is China's primary afforestation tree species, with the largest planted area in the world. *Populus deltoides* and its hybrid offspring *Populus euramericana* are the predominant clones used in commercial production across 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 locales frequently vary. The statistical validity of the evaluation is low, and its performance is largely affected by genotype and environment. G×E has a substantial effect on forest productivity. Prior to widespread promotion of genotypes, Muti-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 study on Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.), Radiata pine (*Pinups radiata* D.Don), and Poplar[6-11]. G×E analysis are always used to screen out high-yielding varieties with a broad range of adaptability, which are important results 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, genotypes, and test environments. 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, GGE biplot is rarely employed in tree breeding in China, despite its enormous potential[17]. As computer technology advances, an increasing number of statistical models based on random effects are 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[14,19]. The best linear unbiased prediction [BLUP] technique is used to obtain the model's predicted value, which may be more trustworthy than the observation value for GGE biplot: When coupled with spatial analysis, it is capable of resolving not only data imbalance and fixed effect limitations on the data, but also the error homogeneity of the test site[18]. The BLUP-GGE technique will be utilized 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 are 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).

Table 1. Environmental Conditions at Six Site. The listed factors mainly include light, temperature and water, which mainly affect growth indicators. The extreme value of temperature related to adaptability is also included. The meteorological data are the annual average values of environmental indicators in the past two decades, and the sunshine duration is the average value in 2017 and 2018.

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

doi:10.20944/preprints202111.0505.v1

Daily precipitation in a year ≥0.1mm days /d	79.2	70.9	67.9	70.2	127.6	71.6
Daily precipitation in a year ≥10.0mm 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
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

2.2 Test Materials and Design

The MET employed 12 genotypes of *Populus euramericana* hybrid clones. Table 2 summarized the genetic background. The trial was established in 2015 by using cutting clones. The test design at each location is 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.

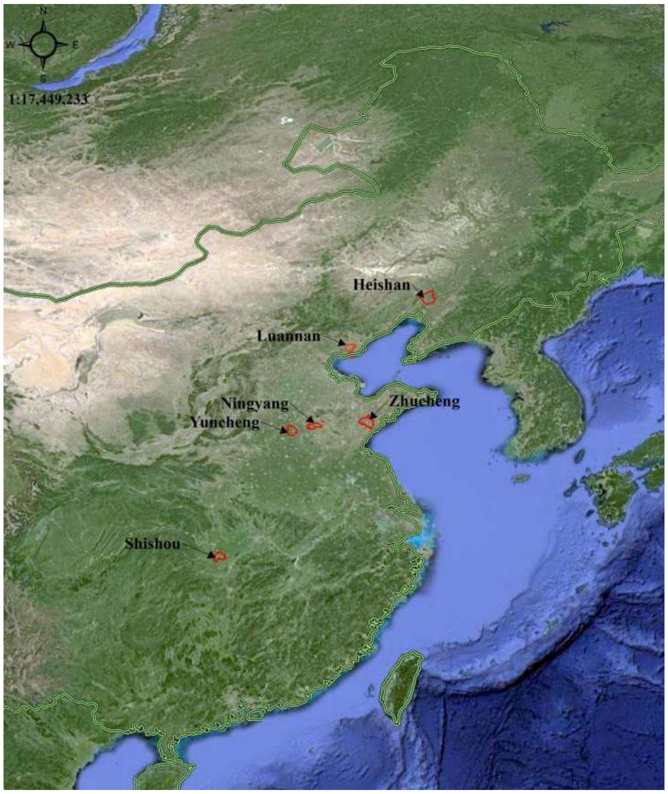


Figure 1. Locations of test sites.

Table 2. Description of *Populus Euramericana* in Test. The female parent of genotypes 1 to 9 is the Chinese poplar cultivar "Danhong", and the rest is 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 tower ruler and girth. Exclude the abnormal values in the data from various places, including those without main branches (the top bud is damaged or broken by the wind), the seedlings that did not grow well in the first year but sprouted from the roots in the second year, germinated from the original cut roots of the poplar tree in the test site .

2.4 Analysis

Asreml-R4.0 is a statistical software package that enables the analysis of mixed linear models in the R environment[16]. The variance components of random effects can be determined by solving a given mixed linear model using the restricted maximum likelihood (REML) technique. The advantage of this technique is that missing values may be replaced with estimate, 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:

$$\mathbf{y_{ij} = \mu + \beta_i + \sigma_i + g_j + sg_{ij} + e_{ij}}$$

'i'= 1,...,6, representing 6 sites respectively; 'j' = 1,...,12, representing 12 genotypes respectively.'Y' is the observed value. 'μ' is the mean value of all observed values, 'β' is the effect of block, 'σ' is the E, 'g' is the G, 'sg' is the G×E, 'e' is the random error. The fixed effects are β and σ, 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 plants[21]. Compared with sexual re-production (family) forestry, the broad-sense heritability in clonal forestry has more guiding significance [22]. The formula[19] was as follows:

$$\mathbf{H_B^2 = V_G/[V_G+V_e]}$$

'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(ΔG) was as follows:

$$\mathbf{\Delta G = H_B^2\,(\mu_s - \mu_P)}$$

μ_s is the average of the genotypes selected, μ_P is the average of the experimental population.

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

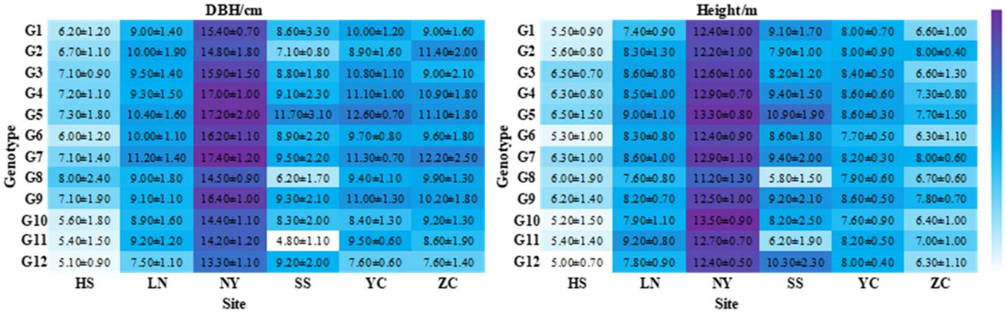
$$\mathbf{r_b=V_G/(V_G+V_{GE})}$$

At present, G × E statistical methods are mainly based on linear model and mixed linear model, and ordinary linear model can not estimate population genetic parameters such as square difference component and breeding value; extracting BLUP value from asreml model and making GGE biplot can 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[22]. The GGEbiplotGUI package in R is used to draw the GGE biplot. When setting the parameters, select 0 (non standardized) for scaled, and select the centralized G + GE, SVP eigenvalue option, and select 1 or 2 according to the type of graph[14,24].

3. Results

3.1 Statistics of Height and DBH Observations



DBH/cm

Genotype	HS	LN	NY	SS	YC	ZC
G1	6.20±1.20	9.00±1.40	15.40±0.70	8.60±3.30	10.00±1.20	9.00±1.60
G2	6.70±1.10	10.00±1.90	14.80±1.80	7.10±0.80	8.90±1.60	11.40±2.00
G3	7.10±0.90	9.50±1.40	15.90±1.50	8.80±1.80	10.80±1.10	9.00±2.10
G4	7.20±1.10	9.30±1.50	17.00±1.00	9.10±2.30	11.10±1.00	10.90±1.80
G5	7.30±1.80	10.40±1.60	17.20±2.00	11.70±3.10	12.60±0.70	11.10±1.80
G6	6.00±1.20	10.00±1.10	16.20±1.10	8.90±2.20	9.70±0.80	9.60±1.80
G7	7.10±1.40	11.20±1.40	17.40±1.20	9.50±2.20	11.30±0.70	12.20±2.50
G8	8.00±2.40	9.00±1.80	14.50±0.90	6.20±1.70	9.40±1.10	9.90±1.30
G9	7.10±1.90	9.10±1.10	16.40±1.00	9.30±2.10	11.00±1.30	10.20±1.80
G10	5.60±1.80	8.90±1.60	14.40±1.10	8.30±2.00	8.40±1.30	9.20±1.30
G11	5.40±1.50	9.20±1.20	14.20±1.20	4.80±1.10	9.50±0.60	8.60±1.90
G12	5.10±0.90	7.50±1.10	13.30±1.10	9.20±2.00	7.60±0.60	7.60±1.40

Site

Height/m

Genotype	HS	LN	NY	SS	YC	ZC
G1	5.50±0.90	7.40±0.90	12.40±1.00	9.10±1.70	8.00±0.70	6.60±1.00
G2	5.60±0.80	8.30±1.30	12.20±1.00	7.90±1.00	8.00±0.90	8.00±0.40
G3	6.50±0.70	8.60±0.80	12.60±1.00	8.20±1.20	8.40±0.50	6.60±1.30
G4	6.30±0.80	8.50±1.00	12.90±0.70	9.40±1.50	8.60±0.60	7.30±0.80
G5	6.50±1.50	9.00±1.10	13.30±0.80	10.90±1.90	8.60±0.30	7.70±1.50
G6	5.30±1.00	8.30±0.80	12.40±0.90	8.60±1.80	7.70±0.50	6.30±1.10
G7	6.30±1.00	8.60±1.00	12.90±1.10	9.40±2.00	8.20±0.30	8.00±0.60
G8	6.00±1.90	7.60±0.80	11.20±1.30	5.80±1.50	7.90±0.60	6.70±0.60
G9	6.20±1.40	8.20±0.70	12.50±1.00	9.20±2.10	8.60±0.50	7.80±0.70
G10	5.20±1.50	7.90±1.10	13.50±0.90	8.20±2.50	7.60±0.90	6.40±1.00
G11	5.40±1.40	9.20±0.80	12.70±0.70	6.20±1.90	8.20±0.50	7.00±1.00
G12	5.00±0.70	7.80±0.90	12.40±0.50	10.30±2.30	8.00±0.40	6.30±1.10

Site

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".

The statistical results in Figure 2 showed 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.

3.2 Six Sites

Table 3. The Fixed Effect. The statistical results showed that the block effect had a very significant influence on the tree height of Poplar ($P < 0.01$), but not on the DBH. And site had a significant effect on height 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. The Random Effect. The statistical results showed that G and G × E were significantly higher in both tree height and DBH ($P < 0.01$).

Source	Height			DBH		
	Variance components	% of Variance components	Significance	Variance components	% of Variance components	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%	-

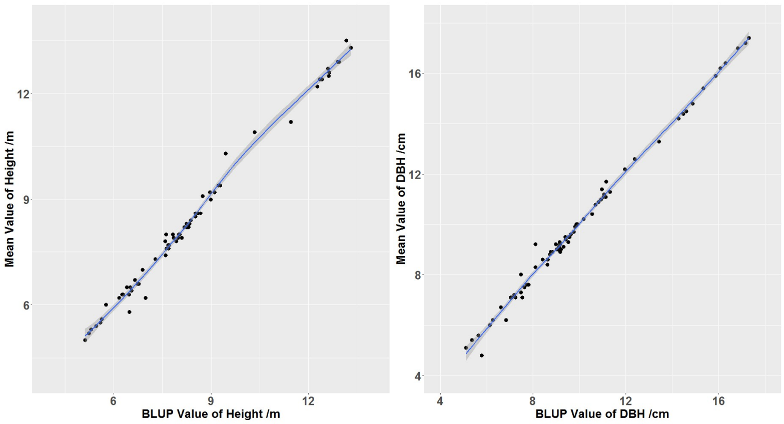


Figure 3. The relationship between BLUP value and mean value.

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

3.3 Selecton of 12 Genotypes by GGE Biplot

3.3.1 DBH

The X-axis represents the first principal component [PC1] (79.25 %), while the Y-axis represents the second principal component [PC2] (11.49 %). The Goodness of fit is, the sum of PC1 and PC2, 90.74 %.For Discrimitiveness, 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 a same ME, with G5 being the best genotype and G9 and G4 being good genotypes. HS, LN, and ZC were located in an another ME, and the optimal genotype was G7. According to Figure 6&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, with the following order: G7 > G4 > G9.

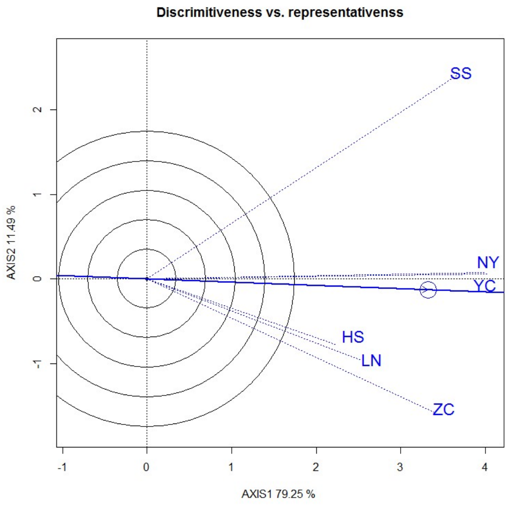


Figure 4. Discrimitiveness and representativenss of 6 Sites. The smaller the angle with the blue solid line, the greater the representativeness; the longer the length of the dashed line, the greater the discrimitiveness. The angle between the dotted lines can reflect the correlation between the locations, the smaller the angle, the more positive the correlation.

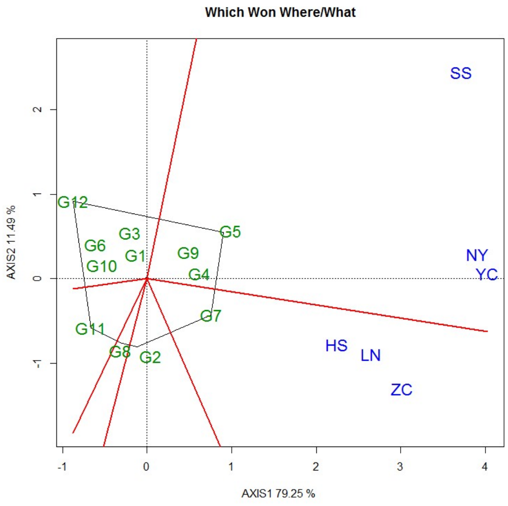


Figure 5. Biplot of "which won where". The area formed by two adjacent red lines is an ME, where the genotype of the quadrilateral vertex is the best for the ME, and the rest are good.

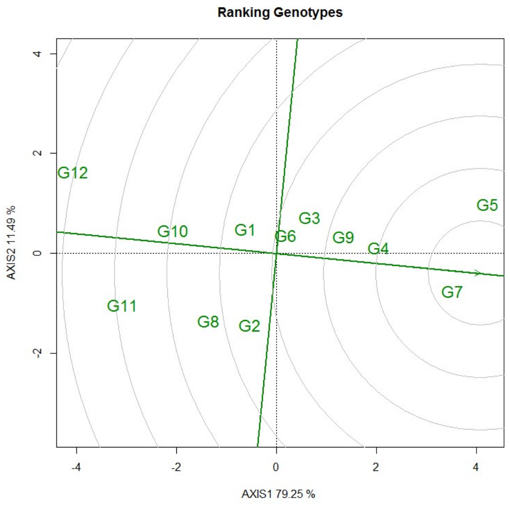


Figure 6. Ranking genotypes. The smaller the radius of the concentric circles, the higher the ranking of the genotypes for comprehensive high yield and stability.

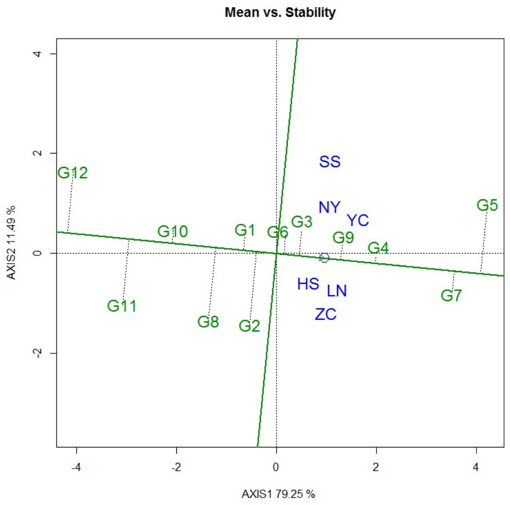


Figure 7. Mean and stability of 12 genotypes. The small blue circle on the horizontal axis represents the average environmental yield. The yield of genotype traits increases along the horizontal axis, and the stability decreases with the increase in the vertical distance.

3.3.2 Tree height

The Figure 8 shows the PC1(66.71%) and the PC2(18.7%). The Goodness of fit was 85.41%,which explains high percentage of observed variability in G and GE.The results in Figure 8 show that: HS, LN, ZC and YC are closely positively correlated. NY and the correlation of each place point slightly small; SS has little correlation with the various points.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 Figure 10&11, the genotypes with higher than average yield were G5, G7, G4 and G9. Among the four genotypes, G5 had the best high yield but the unstable yield character. G5 manifests itself as better in 'SS-NY' ME than in other sites. The stability and high yield of other genotypes were consistent with DBH.

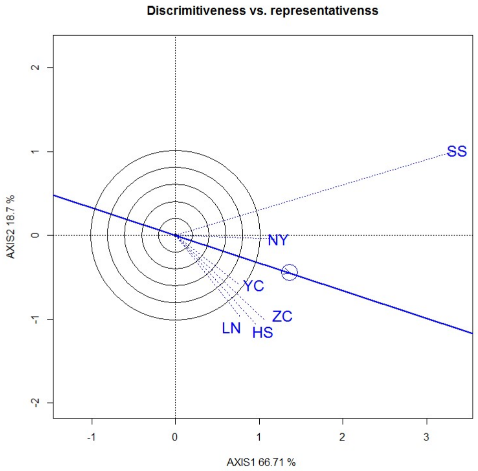


Figure 8. Discrimitiveness and representativenss of 6 Sites.

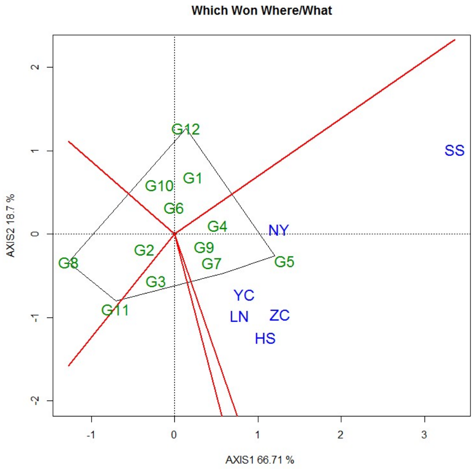


Figure 9. Biplot of “which won where”.

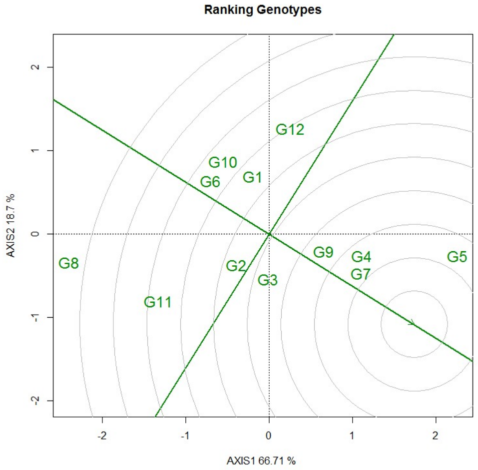


Figure 10. Ranking genotypes.

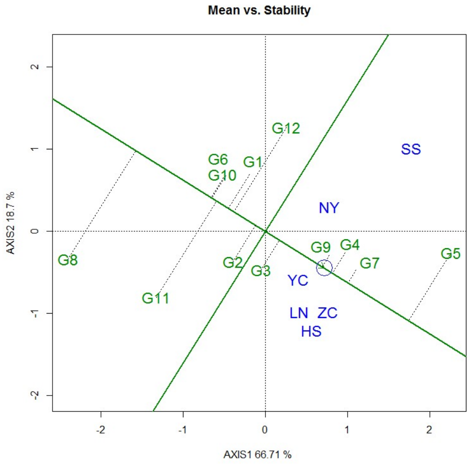


Figure 11. Mean and stability of 12 genotypes.

3.4 Heritability&Type B correlation&Genetic gain of selected genotypes

The results of H_B^2 show that growth traits are more affected by the environment, and tree height is more affected by the environment than DBH(0.13<0.28). The Type-B genetic correlation coefficient results showed that both tree height and DBH traits were affected by G×E (r_b <0.7), and tree height was more affected.

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

Table 5. Genetic parameters.

Genetic Parameter	Height	DBH
H_B^2	0.13	0.30
r_b	0.36	0.65
ΔG/cm	6.84	0.34
% of ΔG	3.35%	0.81%

4. Discussion

The G×E 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 since its yield was lower than usual at the time. Two years in a row, data screening findings are almost, but not fully identical[26], indicating that the test is trustworthy. At the same time, it shows the volatility of *Populus euramericana*'s early growth stage: 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 2400h 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 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 owing to their female parent being P. deltoides 'Zhongshi8'. Tree height exhibited a lower heritability and type-B correlation coefficient than DBH, while G×E had a stronger effect on tree height in a hybrid Populus euramericana population. In other words, 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 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 trustworthy. Rapidly growing species, on the other hand, like 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 the experiment. Whether it is growth or material, it requires a comprehensive construction phase supported by expertise. The trial's future performance cannot be predicted entirely. It is impossible to assume that the ranking of individual genotypes will not suddenly improve in the coming year, and we will need to observe the trend over time to determine this. Thus far, no distinction has been made between the third and fourth years. While studies have demonstrated that tailored clone testing may yield higher yields than family tests, and clone forestry also gives a more powerful technique of detecting G×E, there are hazards associated with both methods[11,27]. This project will be conducted indefinitely to give important experience and technical assistance for poplar clonal forestry.

5. Conclusions

1. In the experiment, a significant rank change interaction and a scale effect interaction were observed.

2. The G, E, and G×E all had a significant effect on the early growth traits (DBH and tree height) of *Populus euramericana*; E and G×E have a greater effect on tree height than DBH.

3. The genotypes G4, G5, G7, and G9 were chosen due to their high and steady production. They are all capable of developing into great *Populus euramericana*.

4. 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, while G5 has the potential to develop large-diameter materials in loam and sandy soil areas.

5. *P. nigra* 'N146' possesses considerable genetic potential and is a great source of male paternal material.

6. Patents

Author Contributions:

N.L. and C.D. contributed equally to this paper.Conceptualization, N.L. and C.D.; methodology, N.L.; software, C.D.; validation, B.L., M.D.; formal analysis, N.L.; investigation, N.L.; resources, Q.H.; data curation, C.D.; writing—original draft preparation, N.L.; writing—review and editing, C.D.; visualization, N.L. and C.D.; supervision, X.S. and Q.H.; project administration, Q.H.; funding acquisition, X.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Basic Research Fund of RIF (Grant No. CAFYBB2020SZ002) and the National Key Research and Development program of China (Grant No. 2016YFD0600400).

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