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Genetic Variation of Growth Traits and Genotype-by-Environment Interactions in Clones of *Catalpa bungei* and *Catalpa fargesii* f. *duclouxii*

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Abstract: Clones of *Catalpa bungei* and *Catalpa fargesii* f. *duclouxii* were studied over several years in central China to explore genetic variation in growth traits and to identify clones of high wood yield and high stability. The genetic parameters for height, diameter at breast height (DBH), and stem volume of clones, were estimated. The effect of clone × year on the increment of stem volume in the two species was analyzed by genotype and genotype × environment (GGE) biplot methods. Significant differences in growth traits among clones and between species were found. The growth of *C. bungei* exceeded that of *C. fargesii* f. *duclouxii* after 4 years. Furthermore, from the 5th year, the repeatability and genetic variation coefficient (GCV) of the *C. bungei* clones were higher than those of the *C. fargesii* f. *duclouxii* clones in most cases. The phenotypic variation coefficient (PCV) of the *C. fargesii* f. *duclouxii* clones was significantly lower than that of the *C. bungei* clones. The repeatability of stem volume was intermediate or high in the two species. ANOVA revealed significant effects of the clone by year interaction in these two species. GGE biplot analysis revealed that wood yield and stability were largely independent in *C. bungei*; clones 22-03, 19-27, and 20-01 were the optimal clones in this species. In contrast, the optimal clones 63 and 128 of *C. fargesii* f. *duclouxii* combined the desired characteristics of high yield and high stability. In conclusion, our results indicated that the height and stem volume of *C. bungei* was under strong genetic control, whereas that of *C. fargesii* f. *duclouxii* was influenced by the environment more than by genetic effects. Genetic improvement by clone selection can be expected to be effective, as the repeatability of stem volume was high. Francis and Kannenberg's method and GGE biplot analysis were used in combination to evaluate the clones. *C. bungei* clone 22-03 and *C. fargesii* f. *duclouxii* clones 63 and 128 were identified as the optimal clones, which exhibited both a high increment of stem volume and high stability.

Keywords: genetic variation; stability of performance; clones; *Catalpa bungei*; *Catalpa fargesii* f. *duclouxii*

1. Introduction

Manchurian catalpa (*Catalpa bungei*) and *Catalpa fargesii* f. *duclouxii* belong to the *Catalpa* genus of the Bignoniaceae family and are native to China. *C. bungei* is mainly distributed in the Yellow River and Yangtze River regions. *C. fargesii* f. *duclouxii* is distributed within the Yunnan-Guizhou plateau. They are recognized for their straight stems and high quality timber, which is of high density and has high bending strength and hardness. These characteristics make them valuable material for furniture production [1,2]. However, their natural germplasm resources are becoming scarce due to hercogamy and deforestation [3]. Thus, the selection of fast-growing varieties is urgently needed to alleviate the shortage of *Catalpa* wood.

Tree breeding is the application of genetic, reproductive biology and economics principles to the genetic improvement and management of forest trees. Significant genetic variations among families or clones suggest a strong foundation for genetic improvement of *Catalpa* trees [4,5]. Clonal forestry has become increasingly important for forestry development [6–9]. In breeding work, the heritability of a target trait refers to the degree of variation in a phenotypic trait in a population that is due to genetic variation among individuals in that population [10]. Because clones of a single individual have the same genotype, we cannot estimate heritability. However, repeatability can be estimated. Repeatability is a measure of the stability of a trait expressed in a fluctuating environment. The higher the heritability or repeatability is, the greater the genetic control of the trait and the lower the influences of environmental effects [11]. Furthermore, the genetic gain of a selected population can be estimated by heritability or repeatability. Genetic gain can be improved more rapidly with appropriate genetic testing and selection of clones than of families or provenances. However, phenotypic variation arises from variation in individual genetic background and environmental effects [12]. Clones can have stronger genotype-by-environment interactions (GEIs) than families or provenances as a result of their specific genotypes [13]. Environmental effects can be divided into site and year effects. Environmental factors such as temperature, rainfall, atmospheric conditions, soil conditions and biotic factors vary among different sites and among years within sites. For perennial species, year effects should be seriously considered.

The systematic study of GEIs can reduce risk in variety selection and improve production [14,15]. Previously, regression coefficients were frequently used to study GEIs and evaluate trait stability [16,17]. However, this method ignores genetic effects among species and clones. The genotype and genotype × environment (GGE) model overcomes this defect by considering the effects of both genotype and genotype × environment. To date, GGE has been widely used to evaluate the growth stability in crop yield [18–20]. However, as the majority of crops are therophytes or biennials, GEI studies of crops mostly focused on site effects. Trees are perennials, and a year represents one growth cycle of a tree. To enhance genetic improvement and maximize clone potential, it is important to analyze the stability of plant growth over years. In our study, clones of *C. bungei* and *C. fargesii* f. *duclouxii* were investigated, and several years of data on clone growth were collected (1) to estimate and compare genetic parameters of growth traits in clones and evaluate the variation in growth traits, (2) to evaluate the stability of clone stem volume across years, and (3) to identify clones with high and stable yield as optimal clones.

2. Materials and Methods

2.1. Site and Materials

Ramets of 32 clones of *C. bungei* and 20 clones of *C. fargesii* f. *duclouxii* were planted in Laodong Village of Henan Province (32.93° N, 112.41° E) in 2009. Detailed information on the clones is shown in Table 1. A randomized block design was applied, with 2 ramets in each clone plot and 5 replications. The height and DBH (diameter at breast height) of the clones were measured at the end of each yearfrom 2009 to 2014.Information on the distribution of materials is provided in Figure 1.

Table 1. Experimental materials.

Species	Origin	Climate of Origin	Clones
C. bungei	Yellow River and Yangtze River regions	mean temperature:12–14 °C, annual precipitation: 500–900 mm	22-03, 17-05, 19-27, 16-05, 16-10, 13-05, 16-04, 9-05, 18-09, 17-06, 16-01, 9-1, 12-09, 20-02, 20-06, 1-1, 22-08, 21-03, 22-05, 22-01, 22-07, 20-01, 23-05, 22-10, 21-02, 6-05, 19-12, 7-01, 12-13, 16-07, 19-01, 13-06
C. fargesii f. *duclouxii*	Yunnan-Guizhou plateau	mean temperature:5–24 °C, annual precipitation: 600–2000 mm	1, 7, 15, 26, 31, 38, 43, 48, 52, 60, 63, 74, 77, 79, 110, 111, 118, 120, 128, 137

Figure 1. Natural distribution of the *C. bungei* and *C. fargesii* f. *duclouxii*.

The experimental field was in a region with a humid and subhumid continental monsoon climate. The mean annual temperature ranges from 14.4 °C to 15.7 °C, the mean annual precipitation ranges from 703.6 mm to 1173.4 mm, and the annual frost-free period is 220 days to 245 days. The elevation is 145 m, and the soil of the experimental field is yellow brown loam and has high natural fertility.

2.2. Data Analysis

Variation and the genetic parameters (repeatability, clonal variance) were estimated of growth traits among clones for *C. bungei* and *C. fargesii* f. *duclouxii* were analyzed. ASReml-R 3.0 [21] and SAS 9.4 [22] software was used to perform ANOVA, *F*-tests and evaluation of genetic parameters.

2.2.1. Analysis at a Species Level for Each Year

A multifactor linear model was followed for each individual trait per year:

$$y_{ijkl} = \mu + S_i + C(S)_{ij} + B_k + (SB)_{ik} + e_{ijkl} \quad (1)$$

where y_{ijkl} is the observed value of clone j in species i in block k; μ is the mean value of the population; S_i is the fixed effect of species i = 1, 2; $C(S)_{ij}$ is the fixed effect of clone j within species i, j = 1, 2, . . . , 20 for *C. fargesii* f. *duclouxii*, j = 1, 2, . . . , 32 for *C. bungei*; B_k is the fixed effect of block k = 1, . . . , 5; $(SB)_{ik}$ is the fixed effect of the interaction of species i and block k; and e_{ijkl} is the random error, NID (Normally and independently distributed) (0, σ_e^2).

2.2.2. Analysis at a Clonal Level for Each Individual Year

An ANOVA to evaluate the clone effect in each species and year was carried out using the following model:

$$y_{ijk} = \mu + C_i + B_j + e_{ijk} \quad (2)$$

where y_{ijk} is the observed value of clone i in block j; μ is the mean value of the population; C_i is the random effect of clone i = 1, 2, . . . , 20 for *C. fargesii* f. *duclouxii*, i = 1, 2, . . . , 32 for *C. bungei*, NID(0, σ_C^2); B_j is the fixed effect of block j = 1, 2, . . . , 5; and e_{ijk} is the random error, NID(0, σ_e^2). The formula of repeatability within years was as follows:

$$R' = \frac{\sigma_C^2}{\sigma_C^2 + \sigma_e^2 / B} \quad (3)$$

where R' is repeatability; σ_C^2 and σ_e^2 are the estimates of between-clone and within-clone variance, respectively, as obtained from the analysis of variance; and B is the number of blocks.

The formula of phenotypic variation coefficient was as follows:

$$PCV = \frac{\sigma}{\overline{X}} \times 100 \quad (4)$$

where σ is the standard deviation of the phenotypic variation, and $\overline{X}$ is the trait mean.

The formula of genetic variation coefficient is expressed as follows:

$$GCV = \frac{\sqrt{\sigma_C^2}}{\overline{X}} \times 100 \quad (5)$$

where σ_C^2 is the clonal variance, and $\overline{X}$ is the trait mean. The genetic variation was estimated by Equation (2).

2.2.3. Analysis at a Clonal Level across Years

A second multifactor linear model was followed for each trait across years:

$$y_{ijkl} = \mu + Y_i + C_j + B_k + (YC)_{ij} + (YB)_{ik} + (CB)_{jk} + e_{ijkl} \quad (6)$$

where y_{ijkl} is the observed value of clone j in year i in block k; μ is the mean value of the population; Y_i is the effect of year i, NID(0, σ_Y^2); C_j is the effect of clone j, NID(0, σ_C^2); B_k is the effect of block k; $(YC)_{ij}$ is the effect of the interaction of year i and clone j, NID(0, σ_{YC}^2); $(YB)_{ik}$ is the effect of the interaction of year i and block k, NID(0, σ_B^2); $(CB)_{jk}$ is the effect of the interaction of clone j and block k; e_{ijkl} is the random error. B_k was treated as a fixed effect, and Y_i, C_j, $(YC)_{ij}$, $(YB)_{ik}$ and $(CB)_{jk}$ were random effects, NID(0, σ_e^2). In this model, year and block were 6 and 5, respectively, and the number of clones for *C. bungei* and *C. fargesii* f. *duclouxii* were 32 and 20, respectively.

The formula of repeatability across years was as follows:

$$R = \frac{\sigma_C^2}{\sigma_C^2 + \sigma_{BC}^2/B + \sigma_{YC}^2/Y + \sigma_e^2/NYC} = \frac{MS_c - MS_{YC} - MS_{BC} + MS_e}{MS_C} = 1 - \frac{1}{F} \quad (7)$$

where R is repeatability; σ_C^2 is the clone variance; σ_{BC}^2 is the interaction of block and clone variance; σ_{YC}^2 is the interaction of year and clone variance; σ_e^2 is the environmental variance; and B is the number of blocks. The parameters were estimated using Equation (1). N, Y and C were the number of individuals, years and clones; MS: mean square; F: F statistic

To interpret genotype × environment, a GGE biplot model was used and performed by R software 3.5.1 [23]. GGE biplots were constructed from the first two principal components (PC1 and PC2) derived by subjecting the environment-centered increment of stem volume means to singular-value decomposition. In this study, the weather conditions of different years were considered the environmental effect. The equation was as follows:

$$Y_{ij} - \overline{Y}_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} \quad (8)$$

where Y_{ij} is the mean stem volume increment of clone i in year j; $\overline{Y}_j$ is the mean stem volume increment of all clones in year j; λ_1 and λ_2 are the singular value decomposition for PC1 and PC2. ξ_{i1} and ξ_{i2} are the eigenvector of PC1 and PC2, respectively, for genotype i. η_{j1} and η_{j2} are the eigenvector of PC1 and PC2, respectively, for year j. ε_{ij} is the random error.

The formula of genetic gain was as follows:

$$\Delta G = \frac{S \times R'}{\overline{X}} \times 100 \quad (9)$$

where R' is repeatability; S is the selection differential; and $\overline{X}$ is the population mean.

3. Results

3.1. Growth Differences between the Two Species in Different Years

The ANOVA results showed that the height of *C. fargesii* f. *duclouxii* was significantly greater than that of *C. bungei* in 2009 (Table 2 and Figure 2). However, in the fourth year, the height of *C. bungei* was consistently higher than that of *C. fargesii* f. *duclouxii* (Figure 2a). Stem volume showed patterns similar to that of height (Figure 2c). From 2009 to 2011, the DBH of *C. fargesii* f. *duclouxii* was significantly higher than that of *C. bungei*. However, after 2012, the DBH of *C. bungei* exceeded that of *C. fargesii* f. *duclouxii*. This latter difference is likely the result of a genetic effect: *C. bungei* adapted more readily to the environment, as it is native to the Yellow River region.

Table 2. ANOVA of growth traits of two species in different years.

	Year	Mean Square					*F*-Value			
		Species	Clone (Species)	Block	Species × Block	Error	Species	Clone (Species)	Block	Species × Block
Height	2009	1.363	0.264	0.530	0.120	0.228	5.97 *	1.16	2.32	0.53
	2010	0.020	0.347	0.360	0.668	0.162	0.12	2.14 **	2.22	4.11 **
	2011	0.006	0.429	0.264	1.395	0.186	0.03	2.31 **	1.42	7.52 **
	2012	5.198	0.503	1.121	3.015	0.271	19.15 **	1.85 **	4.13 **	11.11 **
	2013	78.229	0.610	1.398	4.661	0.240	325.91 **	2.54 **	5.83 **	19.42 **
	2014	33.076	3.014	8.036	8.674	1.300	25.44 **	2.32 **	6.18 **	6.67 **
DBH	2009	3.055	0.492	0.755	0.535	0.297	10.29 **	1.66 **	2.54 *	1.8
	2010	4.156	0.769	1.492	2.377	0.361	11.5 **	2.13 **	4.13 **	6.58 **
	2011	3.642	1.626	1.213	13.237	0.586	6.21 *	2.77 **	2.07	22.58 **
	2012	3.825	2.931	2.168	38.914	0.895	4.27 *	3.27 **	2.42 *	43.48 **
	2013	84.589	7.126	9.182	63.773	3.261	25.94 **	2.19 **	2.82 *	19.56 **
	2014	69.107	18.496	36.565	64.318	6.838	10.11 **	2.7 **	5.35 **	9.41 **

Table 2. *Cont.*

	Year	Mean Square					*F*-Value			
		Species	Clone (Species)	Block	Species × Block	Error	Species	Clone (Species)	Block	Species × Block
Stem volume	2009	0.00019	0.00006	0.00009	0.00011	0.00004	4.47 *	1.35	2.04	2.53 *
	2010	0.00005	0.00019	0.00019	0.00045	0.00009	0.58	2.06 **	2.02	4.78 **
	2011	0.00007	0.00051	0.00033	0.00160	0.00025	0.29	2.04 **	1.32	6.42 **
	2012	0.00322	0.00074	0.00031	0.00614	0.00027	11.88 **	2.72 **	1.13	22.69 **
	2013	0.04994	0.00127	0.00077	0.01220	0.00045	110.19 **	2.8 **	1.7	26.93 **
	2014	0.03561	0.00192	0.00351	0.02064	0.00065	54.82 **	2.95 **	5.4 **	31.78 **

** $p < 0.01$; * $p < 0.05$. DBH: Diameter at breast height.

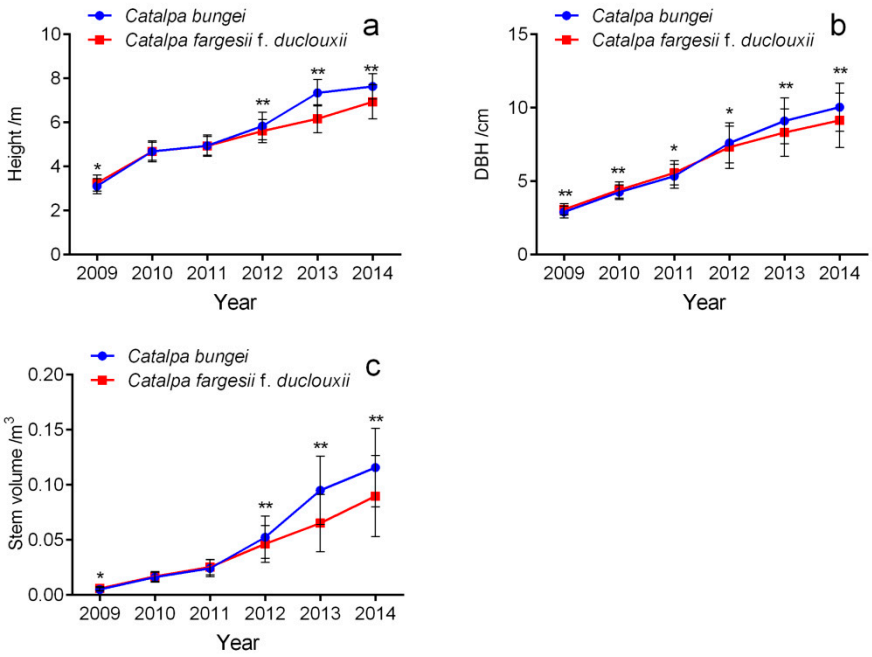

Figure 2. Comparisons of growth traits of two species. (**a**–**c**) represent height, DBH (diameter at breast height) and stem volume, respectively; ** $p < 0.01$; * $p < 0.05$.

3.2. Repeatability of Height, DBH and Stem Volume in the Two Species

The variance analysis of clones growth traits in different years was performed (Tables S1 and S2) It showed that most traits in 2009–2014 of two species were significantly different at the 0.05 or 0.01 level among clones. And the repeatability of traits was eatimated. The repeatability of height was consistently higher in *C. bungei* than in *C. fargesii* f. *duclouxii* (Figure 3a). The range of DBH repeatability in *C. fargesii* f. *duclouxii* was 0.65 to 0.72, which indicated a strong genetic effect on DBH in these clones (Figure 3b). The repeatability of DBH in *C. bungei* was stable from 2010 to 2014 (Figure 3b). The trends of repeatability in stem volume were largely identical between the two species: repeatability increased sharply from 2009 to 2010 and then remained largely stable. The repeatability of most of the traits in 2009 was very low. These might reflect the unstable statement of the plantlet, which was still taking root.

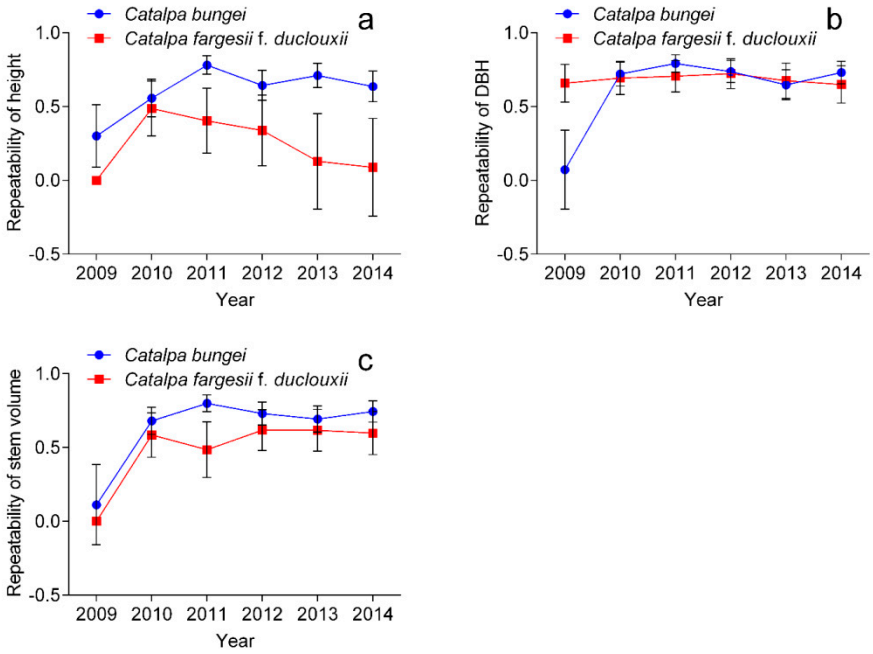

Figure 3. Growth traits repeatability of two species in each year. (**a**), (**b**) and (**c**) represent height, DBH (diameter at breast height) and stem volume, respectively.

3.3. Variation Coefficients of Height, DBH and Stem Volume in the Two Species

The phenotypic variation coefficient (PCV) indicates the total degree of variation. The PCV of height was only approximately 10% for both species. The PCV of height in *C. fargesii* f. *duclouxii* increased in 2013 and 2014, whereas that in *C. bungei* decreased in 2013 and 2014 (Figure 4a). Similar patterns were observed for the PCVs of DBH and stem volume (Figure 4b,c). These results indicated that the environmental responses of the two species changed in 2012. In addition, the PCV of stem volume in *C. bungei* and *C. fargesii* f. *duclouxii* ranged from 27.95%–36.50% and 26.48%–40.95%, respectively. The average PCV of stem volume was over 30% in both species. This result suggested there was abundant genetic variation, representing a strong foundation for improvement in stem volume in the two species.

The genetic variation coefficient (GCV) indicates the degree of variation due to genetic effects. The patterns of GCV for all traits were similar to those of repeatability. The GCV of height in *C. fargesii* f. *duclouxii* decreased continuously from the third year while the PCV of height in this species continuously increased (Figure 4a). These findings implied the environmental effect became more significant with increasing year in *C. fargesii* f. *duclouxii*. The GCVs of DBH and stem volume in *C. fargesii* f. *duclouxii* were approximately stable, but the PCVs of these two parameters continuously increased (Figure 4b,c). These data further suggested that the environmental effect played a leading role in the growth variation of *C. fargesii* f. *duclouxii*. In contrast, for *C. bungei*, the PCVs of height, DBH and stem volume continuously decreased from 2012, whereas the GCVs of DBH and stem volume remained largely stable (Figure 4b,c). These results suggested that the growth of *C. bungei* was under stronger genetic control than was that of *C. fargesii* f. *duclouxii* and that *C. bungei* exhibited stronger environmental adaptation than did *C. fargesii* f. *duclouxii*.

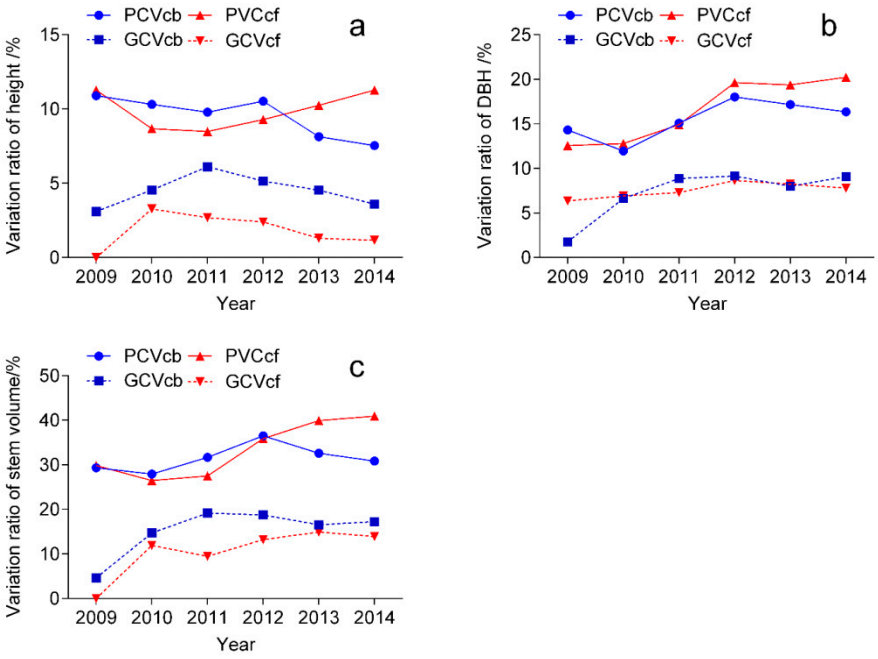

Figure 4. Genetic variation coefficient and phenotypic variation coefficient of two species. (**a**), (**b**) and (**c**) represent height, DBH (diameter at breast height) and stem volume, respectively; PCVcb represents phenotypic variation coefficient of *C. bungei*, PCVcf represents phenotypic variation coefficient of *C. fargesii* f. *duclouxii*, GCVcb represent genetic variation coefficient of *C. bungei*, GCVcf represents genetic variation coefficient of *C. fargesii* f. *duclouxii*.

3.4. Analyses of Growth Traits in the Two Species

The ANOVA showed that the height, DBH and stem volume of the two species were significantly different at the 0.01 level among clones and blocks and that year × clone had a significant effect at the 0.01 level on all these traits except height in *C. fargesii* f. *duclouxii*, where the interaction effect was significant at the 0.05 level (Table 3). These findings indicated that (1) the clones of the two species showed significant variation, which indicated the selection of clones could be performed with high reliability, and (2) GEIs were significant in the two species. Thus, an assessment of the stability of clone growth was necessary.

The variance components analysis indicated (Figure 5) that the DBH had the highest proportion of genetic variance among the three traits and that height had the smallest for *C. fargesii* f. *duclouxii*. This result implied that the variation due to genetic effects was greater for DBH than for height. The proportions of genetic variance in height, DBH and stem volume were higher in *C. bungei* than in *C. fargesii* f. *duclouxii*. In addition, the variation in the year×clone effect on the three traits was greater for *C. bungei* than for *C. fargesii* f. *duclouxii*, indicating that the GEI of *C. bungei* may be greater than that of *C. fargesii* f. *duclouxii*. The results of the broad-sense repeatability estimation showed that the height repeatability of *C. fargesii* f. *duclouxii* was only 0.223 (Figure 6), indicating a low degree of genetic control. The repeatability of stem volume for the two species was high, suggesting that genetic improvements in volume are possible.

Table 3. ANOVA of growth traits of two species.

Species	Source of Variation	Df	Mean Square			*F*-Value		
			Height	DBH	Stem Volume	Height	DBH	Stem Volume
C. bungei	Year	5	463.289	1204.700	0.310	1095.246 **	286.833 **	155.000 **
	Clone	31	2.392	10.735	0.003	3.441 **	3.565 **	2.859 **
	Block	4	4.290	44.273	0.012	4.783 **	7.406 **	4.000 *
	Clone × Year	155	0.160	0.808	0.000	1.622 **	2.114 **	3.013 **
	Block × Year	20	0.362	3.774	0.002	3.675 **	9.876 **	11.208 **
	Clone × block	122	0.634	2.586	0.001	6.430 **	6.766 **	5.002 **
	Error	598	0.099	0.382	0.000			
C. fargesii f. *duclouxii*	Year	5	159.337	520.887	0.097	106.296 **	63.000 **	32.333 **
	Clone	19	0.781	9.209	0.001	1.287 **	3.751 **	2.367 **
	Block	4	7.883	55.697	0.014	4.080 **	5.667 **	4.667 **
	Clone × Year	95	0.141	0.622	0.000	1.293 **	1.777 **	2.069 **
	Block × Year	20	1.467	7.996	0.003	13.410 *	22.850 **	25.660 **
	Clone × Block	76	0.574	2.183	0.000	5.248 **	6.237 **	4.155 **
	Error	369	0.109	0.350	0.000			

** $p < 0.01$; * $p < 0.05$. Df: Degrees of freedom; DBH: diameter at breast height.

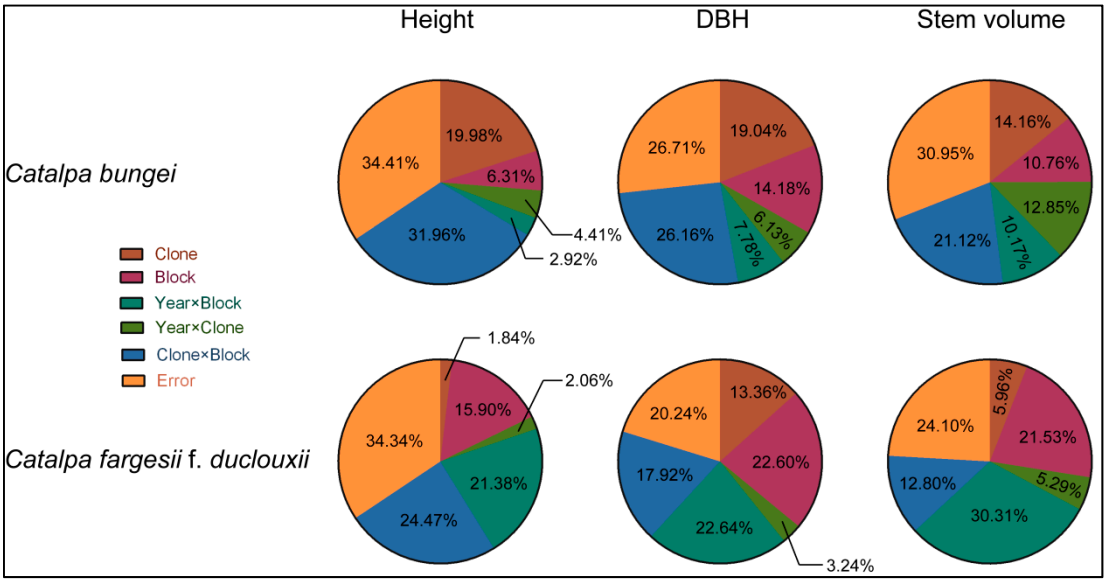

Figure 5. Variance components of growth characters from different variation sources.

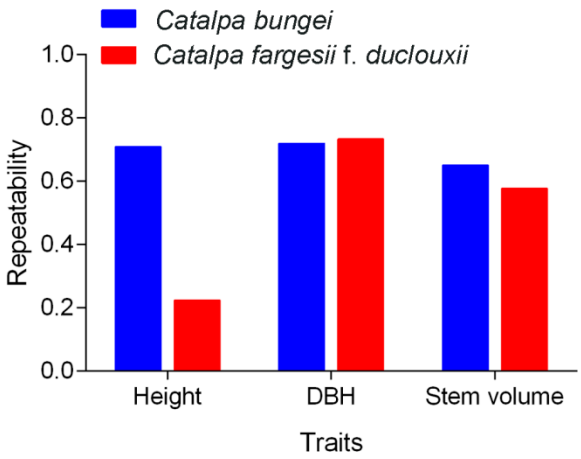

Figure 6. Repeatability of height, DBH (diameter at breast height) and stem volume.

3.5. Analysis of Increment of Stem Volume in the Two Species

The *C. bungei* clone 22-03 had the maximum increment of stem volume (0.0319 m^3) among the *C. bungei* clones, with a value 164.28% higher than the minimum increment, exhibited by clone 7-01 (Table 4). The variation coefficient of clone 16-04 (51.18%) was the smallest among all of the *C. bungei* clones. However, its mean increment of stem volume (0.0209 m^3) was lower than the population value (0.0222 m^3). This result suggested that for clone 16-04, the increment of stem volume was stable but was associated with a very low growth rate. For *C. fargesii* f. *duclouxii* (Table 5), the largest increment of stem volume (0.0248 m^3) was found in clone 63 and was 82.35% higher than the minimum increment (in clone 110). Clone 74 had the minimum variation coefficient (28.48%). However, its increment of stem volume (0.0137 m^3) was very low. We found that the mean increment of stem volume of *C. bungei* was 32.30% higher than that of *C. fargesii* f. *duclouxii*. In contrast, the mean variation coefficient of *C. fargesii* f. *duclouxii* (48.66%) was lower than that of *C. bungei* (65.57%). The multiple comparison tests of stem volume of clones was also performed (Tables S3 and S4). The results showed that the 22-03 had the highest stem volume in 2009 to 2014 for *C. bungeii.* And the 63 had the highest stem volume in 2010 to 2014 for *C. fargesii* f. *duclouxii*. It indicated that the two clones maybe the optimal clones, but their yield stability still need to be evaluated.

Table 4. Increment and variable coefficient of clones on *C. bungei.*

Clones	Increment of Stem Volume/m^3					Mean/m^3	Standard Deviation/m^3	Variable Coefficient/%	Minimum/m^3	Maximum/m^3	Range/m^3
	2009–2010	2010–2011	2011–2012	2012–2013	2013–2014						
22-03	0.017	0.011	0.042	0.052	0.038	0.032	0.017	53.74	0.011	0.052	0.041
17-05	0.007	0.003	0.013	0.034	0.014	0.014	0.012	82.73	0.003	0.034	0.031
19-27	0.011	0.013	0.033	0.058	0.028	0.028	0.019	66.60	0.011	0.058	0.047
16-05	0.013	0.015	0.030	0.051	0.018	0.025	0.016	62.74	0.013	0.051	0.038
16-10	0.010	0.010	0.032	0.038	0.018	0.022	0.013	58.37	0.010	0.038	0.027
13-05	0.011	0.009	0.026	0.046	0.021	0.023	0.015	66.17	0.009	0.046	0.037
16-04	0.013	0.010	0.025	0.037	0.021	0.021	0.011	51.18	0.010	0.037	0.027
9-05	0.011	0.009	0.025	0.048	0.024	0.023	0.016	67.06	0.009	0.048	0.039
18-09	0.010	0.007	0.022	0.035	0.021	0.019	0.011	59.16	0.007	0.035	0.028
17-06	0.010	0.006	0.026	0.033	0.013	0.018	0.011	64.35	0.006	0.033	0.027
16-01	0.013	0.009	0.030	0.040	0.021	0.023	0.013	56.08	0.009	0.040	0.032
9-1	0.012	0.006	0.030	0.041	0.014	0.021	0.014	70.10	0.006	0.041	0.035
12-09	0.007	0.005	0.025	0.041	0.026	0.021	0.015	73.16	0.005	0.041	0.036
20-02	0.010	0.008	0.028	0.039	0.022	0.021	0.013	59.64	0.008	0.039	0.031
20-06	0.015	0.011	0.034	0.047	0.023	0.026	0.015	56.22	0.011	0.047	0.036
1-1	0.015	0.008	0.028	0.046	0.039	0.027	0.016	59.14	0.008	0.046	0.038
22-08	0.012	0.009	0.037	0.039	0.013	0.022	0.015	66.87	0.009	0.039	0.030
21-03	0.008	0.006	0.020	0.032	0.020	0.017	0.010	59.53	0.006	0.032	0.025
22-05	0.011	0.008	0.036	0.049	0.018	0.025	0.017	70.68	0.008	0.049	0.040
22-01	0.013	0.006	0.035	0.052	0.021	0.025	0.018	72.21	0.006	0.052	0.045
22-07	0.012	0.011	0.029	0.050	0.031	0.027	0.016	59.51	0.011	0.050	0.038
20-01	0.015	0.010	0.033	0.058	0.029	0.029	0.019	65.83	0.010	0.058	0.048
23-05	0.011	0.006	0.019	0.039	0.016	0.018	0.013	69.84	0.006	0.039	0.033
22-10	0.012	0.006	0.030	0.028	0.012	0.018	0.011	60.30	0.006	0.030	0.024
21-02	0.011	0.002	0.027	0.040	0.013	0.019	0.015	82.09	0.002	0.040	0.039
6-05	0.010	0.010	0.036	0.058	0.018	0.026	0.021	78.07	0.010	0.058	0.048
19-12	0.011	0.007	0.026	0.043	0.019	0.021	0.014	67.32	0.007	0.043	0.036
7-01	0.006	0.004	0.011	0.025	0.015	0.012	0.009	70.46	0.004	0.025	0.022
12-13	0.007	0.005	0.020	0.035	0.013	0.016	0.012	74.80	0.005	0.035	0.030
16-07	0.012	0.010	0.029	0.035	0.019	0.021	0.011	51.58	0.010	0.035	0.025
19-01	0.012	0.011	0.043	0.055	0.016	0.027	0.020	74.90	0.011	0.055	0.044
13-06	0.012	0.006	0.030	0.050	0.027	0.025	0.017	67.92	0.006	0.050	0.044
Mean	0.011	0.008	0.028	0.043	0.021	0.022	0.015	65.57	0.008	0.043	0.035

Table 5. Increment and variable coefficient of clones on *C. fargesii* f. *duclouxii.*

Clones	Increment of Stem Volume/m^3					Mean/m^3	Standard Deviation/m^3	Variable Coefficient/%	Minimum/m^3	Maximum/m^3	Range/m^3
	2009–2010	2010–2011	2011–2012	2012–2013	2013–2014						
1	0.012	0.005	0.018	0.008	0.026	0.014	0.009	62.02	0.005	0.026	0.022
7	0.012	0.007	0.019	0.015	0.016	0.014	0.005	34.16	0.007	0.019	0.012
15	0.012	0.006	0.017	0.013	0.041	0.018	0.014	76.14	0.006	0.041	0.035
26	0.013	0.010	0.029	0.019	0.028	0.020	0.009	42.84	0.010	0.029	0.019
31	0.013	0.007	0.018	0.012	0.024	0.015	0.007	44.95	0.007	0.024	0.017
38	0.009	0.007	0.021	0.014	0.018	0.014	0.006	41.95	0.007	0.021	0.013
43	0.010	0.011	0.026	0.016	0.027	0.018	0.008	45.35	0.010	0.027	0.017
48	0.010	0.009	0.021	0.029	0.025	0.019	0.009	47.77	0.009	0.029	0.020
52	0.010	0.013	0.019	0.018	0.023	0.016	0.005	30.82	0.010	0.023	0.013
60	0.008	0.011	0.025	0.025	0.018	0.017	0.008	45.16	0.008	0.025	0.017
63	0.014	0.014	0.033	0.026	0.037	0.025	0.011	43.38	0.014	0.037	0.024
74	0.011	0.009	0.018	0.013	0.017	0.014	0.004	28.48	0.009	0.018	0.009
77	0.009	0.008	0.023	0.021	0.020	0.016	0.007	43.04	0.008	0.023	0.014
79	0.011	0.009	0.019	0.017	0.036	0.018	0.011	57.72	0.009	0.036	0.027
110	0.010	0.007	0.016	0.033	0.003	0.014	0.012	85.69	0.003	0.033	0.030
111	0.016	0.004	0.025	0.026	0.029	0.020	0.011	52.24	0.004	0.029	0.026
118	0.009	0.007	0.018	0.021	0.019	0.015	0.007	43.94	0.007	0.021	0.014
120	0.011	0.009	0.016	0.009	0.025	0.014	0.007	47.69	0.009	0.025	0.016
128	0.014	0.010	0.026	0.028	0.032	0.022	0.010	43.47	0.010	0.032	0.023
137	0.009	0.005	0.015	0.017	0.026	0.015	0.008	56.43	0.005	0.026	0.022
Mean	0.011	0.008	0.021	0.019	0.025	0.017	0.008	48.66	0.008	0.027	0.019

According to Francis and Kannenberg's [24] method, the variation coefficient of increment of stem volume was used as the abscissa, the increment of stem volume was used as the ordinate, and their means were used as boundaries to create a scatterplot to define clone yield and stability. Four groups were established for each species (Figure 7): Group I had a high increment but low stability, Group II had a high increment and high stability, Group III had a low increment but high stability, and Group IV had a low increment and low stability. Accordingly, 22-03, 1-1, 20-06, 20-07, and 16-05 of *C. bungei* and 63, 128, 26, 48, 43, and 60 of *C. fargesii* f. *duclouxii* were selected as high-increment and high-stability clones.

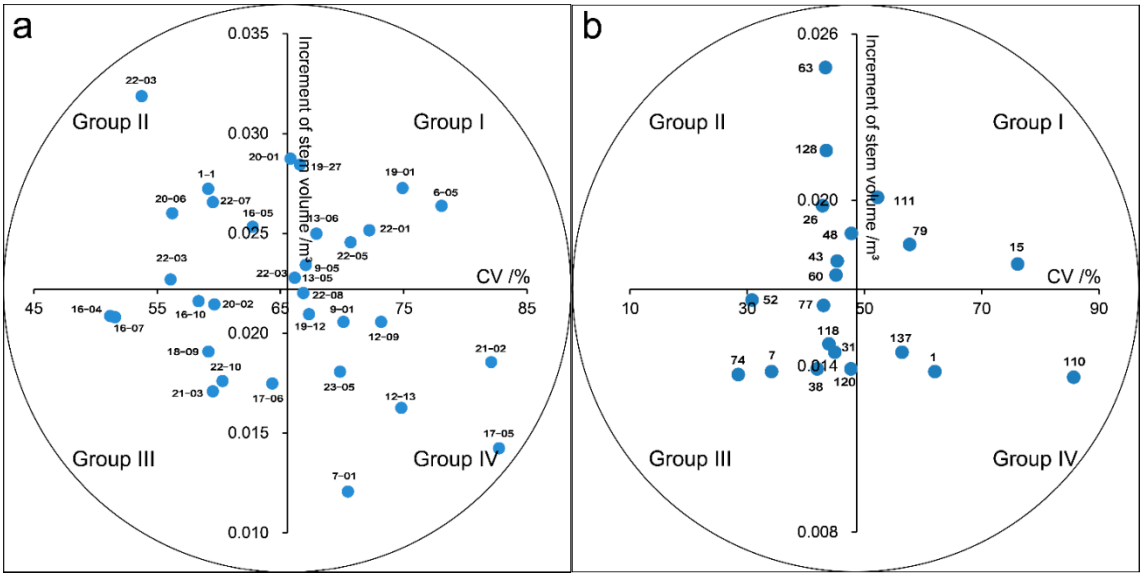

Figure 7. Comparisons of clones for the mean stem volume and coefficient of variation by Francis and kannenberg method. (**a**) represents *C. bungei*, (**b**) represents *C. fargesii* f. *duclouxii*. Each blue point represents a clone.

3.6. Stability and Increment of Stem Volume of Clones Analyzed by GGE Biplots

It was of interest to identify those genotypes for which a significant GEI was found, as these represent genotypes that adapted to the environment. A GGE biplot model was used to identify the clone that performed best in each year. All vertex clones were connected form a polygon; then, starting from the origin, vertical lines to the sides of the polygons were drawn, and the polygons were divided into multiple sectors. Each sector contained some clones and years or only clones. The vertex clone in each sector represented the highest-yielding clone in the years that fell within that particular sector. According to this rule, clone 1-1 was found to exhibit the highest increment of stem volume in 2014, and 22-03 had the highest increment of stem volume in 2010, 2011 and 2013. These data implied that 22-03 was an excellent clone with high increment of stem volume and stability. The sector containing Y2012 had two vertex clones, 19-01 and 22-08, indicating that these two clones had unique adaptability to the weather conditions in 2012. No year fell into the sector in which 7-01 and 22-10 were the vertex clones, indicating that these clones had the lowest increment of stem volume in all years tested (Figure 8a). In *C. fargesii* f. *duclouxii*, clone 63 was found to have the highest increment of stem volume in 2010-2012, and clones 110 and 15 were had the highest increments in 2013 and 2014, respectively.

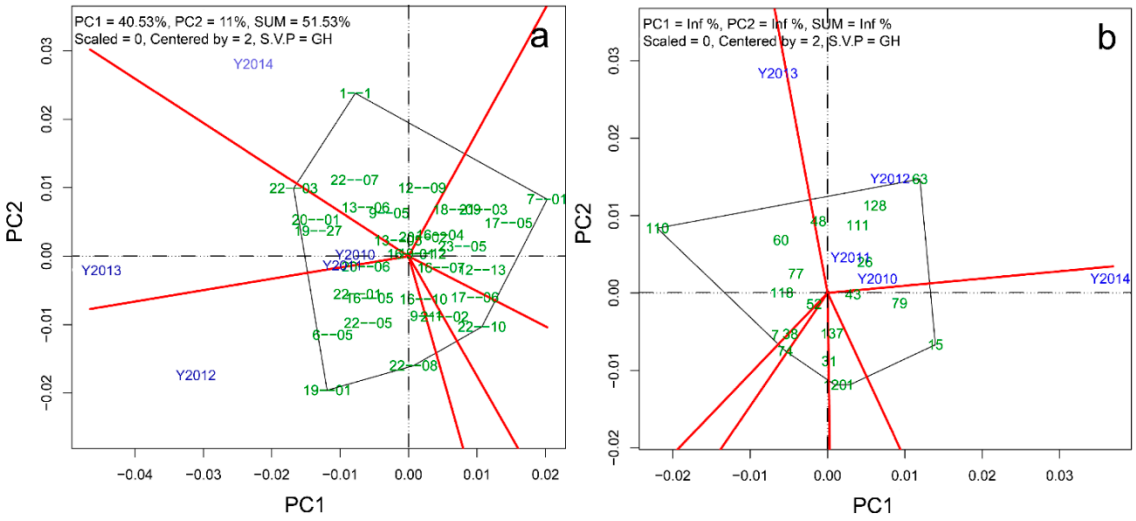

Figure 8. The "which-won-where" based on genotype × environment of two species clones evaluated in different years. (**a**) represents *C. bungei*, (**b**) represents *C. fargesii* f. *duclouxii*. PC1: Principal component 1; PC2: Principal component 2; Blue numbers: Environment effect in different years; Green number: Clone numbers.

The GGE biplot incorporated the AEC (Average Environment Coordinate) to analyze genotype effects and environmental effects; the arrow points to the largest value according to the mean performance of genotypes across all environments. The mean increment of stem volume of the clones was approximated by the projections of their markers on the average environment axis. The stability of the hybrids was measured by their projection onto the average environment coordinate *y*-axis. The greater the absolute length of the projection of a clone, the less stable the hybrid. The top 5 *C. bungei* clones for increment of stem volume were 22-03 > 20-01 > 19-27 > 19-01 > 6-05, and those for stability were 16-01 > 19-12 > 16-07 > 12-13 > 20-06. 19-01 was a high-yield clone but with very low stability (Figure 9a). The stability and yield of 19-27 and 20-01 were both high. The top 5 *C. fargesii* f. *duclouxii* clones for increment of stem volume were 63 > 128 > 111 > 48 > 26, with clone 63 showing the highest increment of stem volume and stability.

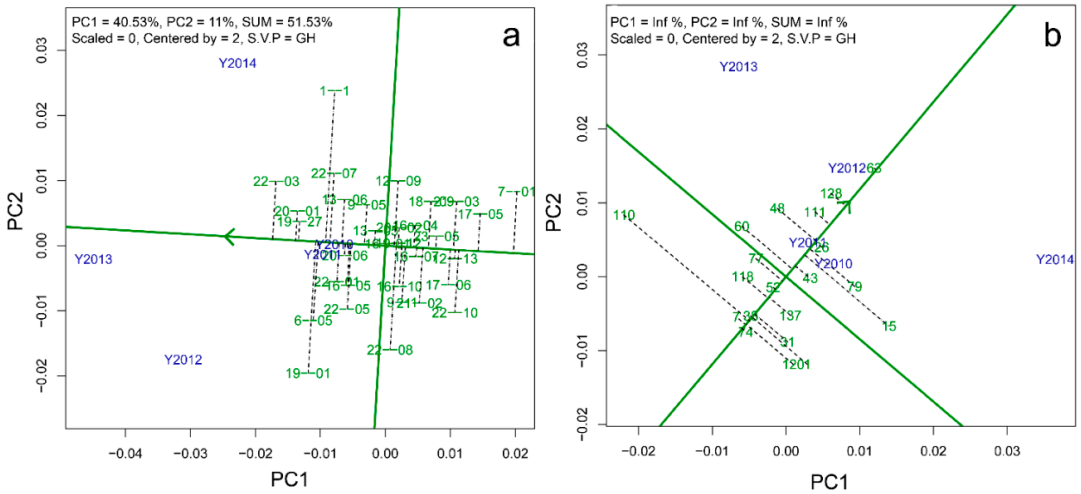

Figure 9. The "mean vs. stability" view showing the mean stem increment performance and stability of different clones in different years. (**a**) represents *C. bungei*, (**b**) represents *C. fargesii* f. *duclouxii*. PC1: Principal component 1; PC2: Principal component 2; Blue numbers: Environment effect in different years; Green number: Clone numbers.

There was no overall consistency between-clone yield and stability. To address this problem, the GGE biplot was used to predict an ideal variety. The center of the multiple concentric circles represented the ideal variety (Figure 10). The closer to the smallest concentric circle, the better is the clone. The top 5 clones were 19-27, 20-01, 22-03, 20-06, and 22-01 for *C. bungei* (Figure 10a) and 63, 128, 111, 26, and 48 for *C. fargesii* f. *duclouxii* (Figure 10b).

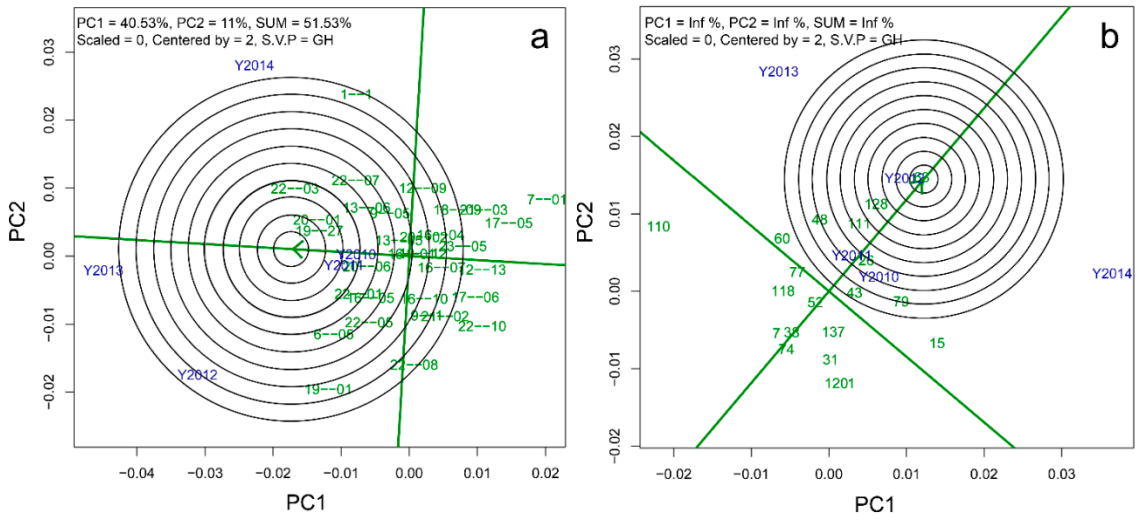

Figure 10. Comparisons of clones with the ideal clone for both mean stem volume and its stability. (**a**) represents *C. bungei*, (**b**) represents *C. fargesii* f. *duclouxii*. PC1: Principal component 1; PC2: Principal component 2; Blue numbers: Environment effect in different years; Green number: Clone numbers.

3.7. Identification of Optimal Clones

Using the Francis and Kannenberg method in combination with the GGE biplot, we identified 1 optimal clone for *C. bungei* and 2 optimal clones for *C. fargesii* f. *duclouxii* (Table 6). The mean height of the optimal clones of *C. bungei* and *C. fargesii* f. *duclouxii* in the 6th year were 8.10 m and 7.39 m, respectively. The genetic gain, which was 3.81% and 0.57% for *C. bungei* and *C. fargesii* f. *duclouxii*, respectively, was low for this trait. The genetic gain of DBH was 14.32% and 11.13% for *C. bungei* and *C. fargesii* f. *duclouxii*, respectively, which was much higher than that for height. The genetic gain of stem volume can potentially reach 31.55% and 22.67% for *C. bungei* and *C. fargesii* f. *duclouxii*, respectively. Thus, stem volume has the potential for large genetic improvement via the selection of suitable clones.

Table 6. Selection of optimal clones.

Species	Clones	Height/m	DBH/cm	Stem Volume/m3
C. bungei	22-03	8.10	12.01	0.1647
	mean	8.10	12.01	0.1647
	Population mean	7.64	10.04	0.1157
	Genetic gain	3.81%	14.32%	31.55%
C. fargesii f. *duclouxii*	63	7.49	11.16	0.132
	128	7.28	10.28	0.1157
	mean	7.39	10.72	0.1239
	Population mean	6.94	9.15	0.0898
	Genetic gain	0.57%	11.13%	22.67%

The height, DBH (diameter at breast height) and stem volume were the data in 2014.

4. Discussion

4.1. Genetic Variation of C. bungei and C. fargesii f. duclouxii Clones

This study aimed to evaluate the genetic parameters of growth traits in *C. bungei* and *C. fargesii* f. *duclouxii* in Henan Province in China and to explore the effect of genotype on growth patterns over years. The height and DBH of the clones were measured annually. The results showed that growth pattern and environmental adaptive ability differed between *C. bungei* and *C. fargesii* f. *duclouxii*. The growth of *C. bungei* exceeded that of *C. fargesii* f. *duclouxii* from the 4th year as represented by all traits. *C. bungei* showed stronger growth potential than *C. fargesii* f. *duclouxii*. As *C. bungei* is native to the Yellow River basin, it is understandable that *C. bungei* had a better response than *C. fargesii* f. *duclouxii* to the weather and soil conditions of the study area. Furthemore, *C. fargesii* f. *duclouxii* was distributing in environments with a much greater range of variation (Table 1) and it forced the species to be more plastic and thus exhibit potentially lower heritability values. Some reports also showed that fluctuations in the environment have major impact on the response of a population to environmental change and the potential for plasticity to evolve is facilitated after exposure to environmental fluctuations [25]. The mean repeatability of stem volume of *C. bungei* and *C. fargesii* f. *duclouxii* from 2010 to 2014 was high (0.72) and intermediate (0.58), respectively. A high repeatability estimate indicates that the selection of the trait in question would be effective and minimally influenced by environmental effects [11]. These findings suggest that stem volume in the two species can be improved by artificial selection.

In addition, the PCVs of growth traits in *C. fargesii* f. *duclouxii* were higher than those in *C. bungei*, whereas the GCVs of growth traits in *C. fargesii* f. *duclouxii* decreased or remained stable. The GCVs of height and stem volume were generally higher in *C. bungei* than in *C. fargesii* f. *duclouxii*. All of these findings provided further evidence to support that the influence of environment each year on *C. fargesii* f. *duclouxii* growth was strong, whereas the growth of *C. bungei* was more under genetic control than under environmental control. No consistent pattern in the genetic parameters of the 1-year-old trees was observed. The most likely reason for this finding was that the ramets were at the rooting stage in the first year. The unstable growth stage significantly limited the accuracy of genetic parameter estimation. Overall, our results indicated that there were significant differences in growth traits between species and among clones. These data provide a good foundation for genetic improvement.

4.2. Genotype Effect and Genotype and Environment Interaction

Plant growth is highly dependent on environmental conditions [26], and each species occupies a unique ecological niche in time and space; that is, it forms a unique, stable relationship with the environment [27]. For example, annual rainfall can affect the plant distribution [28,29], and effective temperature affects physiological functions [30,31]. The environment varies, even in the same place among years. Plants can perceive environmental changes and respond to them. Differences between species in their response to environmental fluctuations cause asynchronized growth series and within-species variability of responses also may impact the stabilizing effect of growth asynchrony [32]. In this study we already found that the two kinds of trees have different growth responses to the same environment. The genetic effect is the main cause of this phenomenon, the *C. bungei* native the test site, its genetic factors regulate the body to adapt to the special environment. So a good genotype is crucial for breeding. However, except genectic effect, GEI can't also be ignored. Revealing the mechanisms underlying genotype and environment interactions can greatly benefit forest breeding and selection. To do so, it is necessary to study the responses of clones to different environments and select clones with steady yields [15,33,34]. The GEI model can help tree breeders design effective breeding programs and select suitable genotypes for a given environment [4]. In trees, GEIs are widespread. Meier et al. [35] found that annual variation in the environment significantly impacted wood formation in Douglas fir (*Pseudotsuga menziesii*) clones. Studies of clones of white poplar [36], *Michelia chapensis* [37] and River red gum (*Eucalyptus camaldulensis*) [38] have also indicated significant GEI effects. In this study, we

examined the GEIs of *C. bungei* and *C. fargesii* f. *duclouxii* clones. We found significant year and clone effects. A GGE biplot allows the visual interpretation of GEI [23,39,40]. We used GGE biplots to readily identify differences in the increment of stem volume and stability among clones and a GGE model to further analyze the GEI effect. According to the analyses, among *C. bungei* clones, clone 22-03 had the highest mean increment of stem volume and the highest values at 1, 2 and 4 years old. These results indicated that 22-03 was a high stability clone. In *C. fargesii* f. *duclouxii*, clones 63 and 128 had both high yield and high stability when we evaluated wood yield and stability independently.

5. Conclusions

Genetic variation is the precondition for genetic improvement. In this study, growth traits were significantly different between species and among clones. The *C. bungei* clones had greater growth potential than the *C. fargesii* f. *duclouxii* clones. Height, DBH and stem volume were all significantly larger in *C. bungei* than in *C. fargesii* f. *duclouxii* after 4 years of age. Moreover, the stem volume repeatability was intermediate or high in the two species, indicating that clone selection would be effective. The comparison of the genetic parameters between the two species showed that the growth of *C. bungei* was controlled more by genetic effects than environmental effects.

GEI is a very important factor for selecting breeding strategies. Our analysis indicates the two *Catalpa* species both have significant GEIs for increment of stem volume. Using GGE biplots, we found that wood yield and stability are largely independent in the *C. bungei* clones. However, clones 63 and 128 of *C. fargesii* f. *duclouxii* had both high wood yield and high stability. As each model has limitations, we combined Francis and Kannenberg's method with GGE biplot analysis to minimize error. *C. bungei* clones 22-03 and *C. fargesii* f. *duclouxii* clones 63 and 128, which adapted to the diverse climatic conditions in the experimental site and presented high yield, were identified as optimal clones.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/1/57/s1, Table S1. ANOVA of growth traits of clones for *C. Bungei*, Table S2. ANOVA of growth traits of clones for *C. fargesii* f. *duclouxii*, Table S3. Multiple comparison of stem volume of clones for *C. Bungei*, Table S4. Multiple comparison of stem volume of clones for *C. fargesii* f. *duclouxii*.

Author Contributions: This study was carried out with collaboration among all authors. J.W. and W.M. conceived and designed the experiments; Y.X., N.W., W.Z. and Q.W. performed the experiments; G.Q. and N.L. carried out data correction; L.K. and Z.W. carried out manuscript revision; and Y.X. wrote the paper.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

GEI—Genotype and environment interaction;
GCV—Coefficient of genetic variation;
PCV—Coefficient of phenotypic variation;
GGE—Genotype and genotype × environment.

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