

Article

Screening and Site Adaptability Evaluation of Qi-Nan Clones (*Aquilaria sinensis*) in Southern China

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Abstract: In recent years, plantations of *Aquilaria sinensis* in China have been dominated by Qi-nan, yet there remains limited research on the growth evaluation and breeding of these clones. In this study, a multi-point joint variance analysis, an additive main effect and multiplicative interaction (AMMI) model, a weighted average of absolute scores (WAASB) stability index, and a genotype main effect plus a genotype-by-environment interaction (GGE) biplot were used to comprehensively analyze the yield, stability, and suitable environment of 25 3-year-old Qi-Nan clones from five sites in southern China. The results showed that all the growth traits exhibited significant differences in the clones, test sites, and interactions between the clones and test sites. The phenotypic variation coefficient (PCV) and genetic variation coefficient (GCV) of the clones' growth traits at the different sites ranged from 16.56% to 32.09% and 5.24% to 27.06%, respectively, showing moderate variation. The medium–high repeatability (R) of tree height and ground diameter ranged from 0.50 to 0.96 and 0.69 to 0.98, respectively. Among the clones, Clones G04, G05, G10, G11 and G13 showed good growth performance and could be good candidates for breeding. Environmental effects were found to be the primary source of variation, with temperature and light primarily affecting growth, while rainfall influenced survival and preservation rates. Yangjiang (YJ) was found to be the most suitable experimental site for screening high-yield and stable clones across the different sites, whereas the tree height and ground diameter at the Chengmai (CM) site were significantly higher than at the other sites, and the Pingxiang (PX) and Zhangzhou (ZZ) sites showed poor growth performance. The findings suggest that Qi-nan clones are suitable for planting in southern China. There were also abundant genetic variations in germplasm resources for the Qi-nan clones. The five selected clones could be suitable for extensive planting. Therefore, large-scale testing is necessary for determining genetic improvements in Qi-nan clones, which will be conducive to the precise localization of their promotion areas.

Keywords: *Aquilaria sinensis*; Qi-nan; excellent clones; AMMI model; GGE biplot



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1. Introduction

Agarwood is a type of resin-containing wood that forms after *Aquilaria*, *Gonystylus*, and *Gyrinops* species are injured [1]. It is frequently used as a precious medicine and natural spice [2], causing it to have extremely high medical and economic value. Therefore, many countries and regions have attached great importance to the cultivation and breeding of easily formed agarwood and high-yield agarwood varieties [3]. In China, researchers and farmers have been breeding for many years to develop a novel special germplasm of

A. sinensis (Lour.) Spreng [4], which is cultivated by grafting from a specific wild *A. sinensis* population that possesses outstanding induced agarwood potential [5,6]. This special clone germplasm, called “Qi-nan” [4], has the advantage of being more likely to induce agarwood and high-quality agarwood [6–8]. Studies have shown that Qi-Nan can be distinguished from ordinary *A. sinensis* through SSR and the composition of agarwood [5,9]. After one year of induction, the alcohol extract content of Qi-Nan will exceed 40% [6,10], accompanied by a richer and more diverse range of sesquiterpenes [5].

In recent years, plantations of *A. sinensis* in China have been dominated by Qi-nan, which is mainly planted in Hainan, Guangdong, Guang xi, Fujian and Yunnan in the south of China [4]. Compared to ordinary *A. sinensis*, the biggest advantage of Qi-nan is its ability to maintain the excellent traits of mother trees [11]. However, with the increased demand for its planting, the species source of many Qi-Nan clone germplasms remains unclear, and they are widely planted without systematic breeding [4], resulting in significant differences between the clones, with coefficients of variation reaching 19% for tree height, 23% for ground diameter, and 17% for agarwood yield, as has been observed in a single test site [7]. Therefore, we carried out a trial in multiple environments to study the variation in the important traits of Qi-nan clones, which is of great significance for their successful planting.

Multi-environmental trials, as an important part of the commercial breeding process, are a prerequisite for varieties promotion [12]. In research, they are often used to evaluate the productivity, stability, and adaptability of species genotypes, as well as to explain the interaction effects between the genotype and the environment [13–15]. Additionally, they have also been applied to *Larix olgensis* [16], *Pinus koraiensis* [17], poplar clones [18–20], and European beech [21]. Through these multi-environmental trials, the growth traits and genetic variation patterns of the tested varieties (lines) have been analyzed [22], the interaction effect between genotypes and the environment has been explained [23], the causes of variation have been revealed, and improved varieties have been screened out [24]. This confirms the necessity and validity of multi-environmental trials in the breeding of improved varieties.

Generally, univariate, multivariate, mixed, and non-parametric methods are usually used to study the interactions between genotypes and the environment in multi-site identification trials [25]. Variance analysis and linear regression are not comprehensive and intuitive enough for analyzing the effects of genotype $\times$ environment interactions (GEI) [26]. The AMMI model and GGE biplot are effective statistical methods in multivariate analyses which are commonly used in the analysis of multi-environmental trials [27,28]. The AMMI model combines factor analysis with variance analysis to separate the model error and interference from the residuals of the additive model [29,30]; it draws a biplot to describe the yield and interaction effect of the variety and location, and it intuitively analyzes the stability of the varieties [31]. Compared to the AMMI model, the GGE biplot is a principal component analysis of environmental centralization, and it has a strong regional genotype evaluation function [32]. It can be used to combine the main effect of the genotype with the interaction of the GE at the same time, cluster the environment, and divide the suitable area of the varieties [18,33]. Moreover, a new variety evaluation system has been introduced: the WAASB (Weighted Average of Absolute Scores) stability index, which is a mixed model incorporating a best linear unbiased prediction (BLUP) framework that takes into account all the IPCAs for stability analysis [31,34]. Through the WAASB stability index and the AMMI model, the stability of various crops and the discrimination of the experimental environment were reasonably explained [13,15], which effectively shortened the breeding process [35,36]. Combining the AMMI model with the GGE biplot can help in better understanding the effects of GEI identifying ideal test conditions, and ensuring cultivar adaptation regions [16].

By the end of 2023, it was roughly estimated that there were more than 100 varieties of Qi-nan clones in China, with a planting area of more than 35,000 hectares. However, there is a lack of excellent Qi-nan clones adapted to different regions, which hinders the development of the agarwood industry. In this study, the growth performances of 25 Qi-nan

clones from five sites were investigated, the effect of the GEI was examined via variance analysis, the AMMI model, the WAASB stability index, and the GGE biplot to understand the process of tree breeding. Our results can provide a scientific basis for the selection and evaluation of Qi-nan clones in southern China.

2. Materials and Methods

2.1. Study Area and Materials

In November 2020, scions from twenty-five Qi-nan clones (G01 to G25) were collected from the first generation of grafted mother trees in Maoming, Huizhou, Shenzhen, and Shanwei, and were grafted onto seedlings of *A. sinensis* as rootstocks in Maoming, Guangdong Province. After six months of growth, the ground diameters and tree heights of the grafted seedlings ranged from 0.8 cm to 1.3 cm and from 1.0 m to 1.3 m. In April 2021, these seedlings were transported to various test sites, and a randomized block design with 3 blocks and 15–20 replicates was implemented across five test sites (Figure 1 and Table 1), with a row spacing of 2.0 m × 1.5 m. Consistent management was applied to each site.

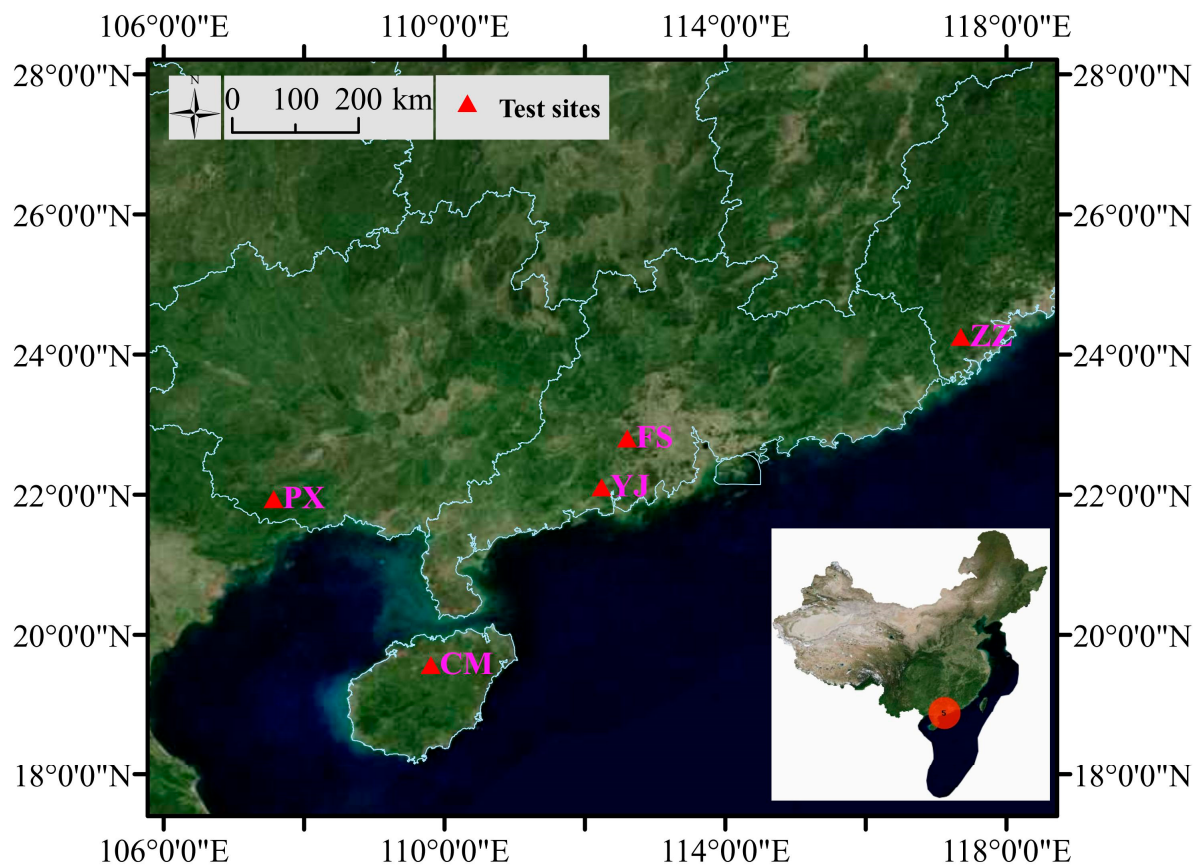


Figure 1. Map showing positions at five different sites in South China. The red triangle mark represents the test site. CM, FS, PX, YJ and ZZ denote the Chengmai site, Foshan site, Pingxiang site, Yangjiang site, and Zhangzhou site, respectively.

Table 1. Main geographical and environmental characteristics of test sites.

Test Site	Longitude	Latitude	Altitude (m)	Frost-Free Season (days)	Mean Annual Precipitation (mm)	Mean Annual Temperature (°C)	Mean Annual Sunshine Duration (h)
Yangjiang (YJ)	112°09′24″ E	21°59′23″ N	16	358	1886	22.3	2012
Foshan (FS)	112°44′58″ E	22°43′24″ N	85	356	1572	21.6	1629

Table 1. Cont.

Test Site	Longitude	Latitude	Altitude (m)	Frost-Free Season (days)	Mean Annual Precipitation (mm)	Mean Annual Temperature (°C)	Mean Annual Sunshine Duration (h)
Chenmai (CM)	110°01'58" E	19°52'13" N	38.66	365	1786	23.8	2059
Pingxiang (PX)	106°55'12" E	22°03'20" N	251	344	1500	21.5	1614
Zhangzhou (ZZ)	117°21'03" E	24°21'37" N	446	319	1736	21.3	1764

Notes: Climate information comes from China Meteorological Data Network (<https://data.cma.cn/>), accessed on 6 April 2024).

2.2. Measurement of Growth Characters

In June 2022, the number of surviving trees of all the sample trees in the experimental plot was investigated. In December 2023, the growth traits, such as the preserved ratio (%), ground diameter (cm), and tree height (m) of all the sample trees in each experimental plot were measured.

2.3. Statistical Analysis

In this study, data entry was performed using Excel 2021. An analysis of variance (ANOVA), Duncan's multiple-comparison analysis, and an estimation analysis of genetic parameters were performed using SPSS 28.0 (International Business Machines—IBM Corporation, Chicago, IL, USA).

A linear mixed model for a single site analysis of variance is as follows [37]:

$$Y_{jkl} = \mu + B_j + G_k + BG_{jk} + \varepsilon_{jkl} \quad (1)$$

where B_j is the block effect ($j = 1, 2, 3$), G_k is the clone effect ($k = 1, 2, 3, 25$), BG_{jk} is the interaction effect between k th clone and j th block, and ε_{jkl} is the random error.

A variance linear model for multi-site joint analysis is as follows [38]:

$$Y_{ijkl} = \mu + S_i + B_{ij} + G_k + SG_{ik} + BG_{ijk} + \varepsilon_{ijkl} \quad (2)$$

where μ is the overall mean, S_i is the site effect ($i = 1, 2, 3, 4, 5$), B_{ij} is the block effect, G_k is the clone effect, SG_{ik} is the interaction effect of k th clones at i th site, BG_{ijk} is the interaction effect between k th clones and j th blocks in i th site, and ε_{ijkl} is the random error.

2.4. Estimate the Genetic Parameters

The calculation formulas of the phenotypic and genotypic coefficient of variation (PCV and GCV) are as follows [39]:

$$PCV(\%) = \frac{\sqrt{\delta_p^2}}{\bar{X}} \times 100 \quad (3)$$

$$GCV(\%) = \frac{\sqrt{\delta_g^2}}{\bar{X}} \times 100 \quad (4)$$

where δ_p^2 and δ_g^2 are the phenotypic variance and genetic variance of a trait, and $\bar{X}$ is the phenotypic mean of the trait.

The formula of clone repeatability (R) is as follows [16,19]:

$$R = \frac{\sigma_A^2 + \sigma_b^2}{\sigma_A^2 + \sigma_b^2 + \sigma_e^2} = 1 - \frac{1}{F} \quad (5)$$

where σ_A^2 , σ_b^2 and σ_e^2 are the genetic variance of clones, the interaction variance between clones and environment, and the environmental random error variance, respectively. F is the F value of variance analysis.

2.5. The AMMI Model, WAASB Stability Index, and GGE Biplot

The “Agricolae” package, “metan” package, and “GGE Biplot GUI” package in the R 4.2.1 software were used to analyze the AMMI model, WAASB stability index, and GGE biplot, respectively, as well as to conduct correlation analysis.

The AMMI model is as follows [40]:

$$Y_{ikl} = \mu + S_i + G_k + \sum_{r=1}^n \lambda_r \Psi_{kr} b_{ir} + P_{kr} + \varepsilon_{ikl} \quad (6)$$

where μ is the overall mean, S_i is the average deviation of the environment, G_k is the average deviation of clones, λ_r is the eigenvalue of the r principal component analysis, and Ψ_{kr} and b_{ir} are the clone and environmental scores of the r principal component analysis, respectively. n is the total number of principal component factor axes in model analysis, P_{kr} is the interactive remaining items, and ε_{ikl} is the residual error.

The GGE biplot is as follows [41]:

$$Y_{ij} - \mu - B_j = \lambda_1 r_{i1} \delta_{j1} + \lambda_2 r_{i2} \delta_{j2} + BG_{ijk} + \varepsilon_{ij} \quad (7)$$

where μ is the overall mean, and B_j is the mean value of all clones in site j . λ_1 and λ_2 are the eigenvalues of the first and second principal components, respectively. r_{i1} and r_{i2} are the eigenvectors of the first and second principal components of the i clone, respectively. δ_{j1} and δ_{j2} are the eigenvectors of the first and second principal components at the j location, respectively. ε_{ij} is the residual error.

3. Results

3.1. Analysis of Variance for Growth Traits

The effects of the clones, test sites, and interactions between the clones and test sites on the tree height and the ground diameter were extremely significant across multiple environments ($p < 0.01$) (Table 2). The proportion of the sum of squares to the sum of squares of the total variation of the clone effect, the test sites effect, and the interactions between the clones and test sites for tree height were 19.7%, 63.3%, and 17.0%, respectively. The proportion of each effect on the ground diameter to the sum of squares of the total variation were 35.1%, 47.8%, and 17.1%, respectively. This indicated that the environmental effect was the main source of variation, followed by the clone effect. In each test site, there were significant differences in the tree height and the ground diameter between the different clones ($p < 0.01$). It was possible to select a variety of excellent Qi-nan clones as there were abundant variations in the Qi-nan germplasm.

Table 2. Results of ANOVA analysis of tree height and ground diameter within each site.

Sites	Source of Variations	Tree Height				Ground Diameter		
		DF	SS	MS	F	SS	MS	F
ME	Clone	24	194.00	8.08	65.743 **	1617.02	67.40	92.497 **
	Site	4	622.90	155.73	1266.871 **	2203.53	550.90	756.299 **
	Clone: Site	96	167.70	1.75	14.208 **	786.50	8.20	11.248 **
	Residuals	4329	518.31	0.12		2998.19	0.70	
CM	Clone	24	205.05	8.54	73.566 **	1010.72	42.11	54.700 **
	Residuals	983	111.03	0.11		746.91	0.77	
FS	Clone	24	35.24	1.47	22.432 **	355.33	14.81	29.414 **
	Residuals	665	43.53	0.07		334.74	0.50	
PX	Clone	24	15.79	0.66	4.819 **	155.20	6.47	6.702 **
	Residuals	571	77.94	0.13		551.11	0.96	
YJ	Clone	24	111.96	4.66	40.446 **	859.90	35.83	59.674 **
	Residuals	1174	135.41	0.12		704.93	0.60	

Table 2. Cont.

Sites	Source of Variations	Tree Height				Ground Diameter		
		DF	SS	MS	F	SS	MS	F
ZZ	Clone	24	15.86	0.66	4.133 **	125.71	5.24	5.969 **
	Residuals	562	89.87	0.15		493.20	0.87	

Notes: ME denotes the joint multi-environments. DF, SS, MS, and F denote the source of the variations, sum of squares, mean square, and F-test, respectively. **: significant difference at the 0.01 level.

3.2. Growth Performance of Different Clones and in Different Test Sites

The average tree height, ground diameter, 1-year average survival rate, and 3-year average preservation rate were 2.44 m, 5.18 cm, 86.03%, and 72.62%, respectively (Table 3). The tree height and ground diameter of G11, which exceeded the average by 14.89% and 29.65%, respectively, were found to be the largest. Conversely, G18 was found to have the smallest averages with respect to tree height and ground diameter, which were 22.95% and 25.19% smaller than the average values, respectively. The average tree height, ground diameter, 1-year average survival rate, and 3-year average preservation rate were the highest on the CM site, with values of 3.23 m, 6.57 cm, 94.42%, and 90.82%, respectively, which are significantly higher than those of other sites.

Table 3. Tree height (m), ground diameter (cm), 1-year survival rate (%), and 3-year preservation rate (%) of 25 clones and at five different test sites.

Clones/Sites	Tree Height (m)	Ground Diameter (cm)	1-Year Survival Rate (%)	3-Year Preservation Rate (%)
G01	2.11 ± 0.42 k	4.06 ± 0.83 l	87.11 ± 17.77 abc	68.01 ± 25.51 cde
G02	2.60 ± 0.56 cdef	5.29 ± 1.21 efg	91.57 ± 12.04 abc	82.02 ± 17.55 abc
G03	2.47 ± 0.55 fghi	5.63 ± 1.19 d	90.57 ± 10.81 abc	78.59 ± 21.51 abcd
G04	2.64 ± 0.58 bcde	5.74 ± 1.36 cd	89.14 ± 14.21 abc	81.43 ± 15.54 abc
G05	2.72 ± 0.58 abc	6.06 ± 1.31 b	90.35 ± 12.25 abc	83.13 ± 14.67 abc
G06	2.47 ± 0.38 fghi	4.98 ± 0.93 hi	85.22 ± 15.23 abc	79.78 ± 20.19 abcd
G07	2.53 ± 0.63 defgh	5.18 ± 1.04 fgh	88.67 ± 16.02 abc	75.67 ± 26.95 abcde
G08	2.55 ± 0.56 defg	4.85 ± 1.12 ij	94.67 ± 9.07 ab	87.56 ± 13.75 ab
G09	2.53 ± 0.61 defgh	5.5 ± 1.52 def	89.22 ± 13.77 abc	77.33 ± 23.42 abcd
G10	2.68 ± 0.56 abcd	5.94 ± 1.32 bc	95.44 ± 6.19 a	88.56 ± 15.96 a
G11	2.80 ± 0.78 a	6.7 ± 1.69 a	88.56 ± 18.6 abc	82.67 ± 21.68 abc
G12	2.39 ± 0.54 hij	4.87 ± 1.11 ij	83.89 ± 19.87 abc	71.44 ± 32.51 abcde
G13	2.76 ± 0.80 ab	5.58 ± 1.46 de	87.67 ± 12.15 abc	76.22 ± 15.25 abcd
G14	2.41 ± 0.54 ghij	5.31 ± 1.22 efg	83.89 ± 16.7 abc	74.33 ± 21.50 abcde
G15	2.59 ± 0.66 cdef	5.31 ± 1.56 efg	81.88 ± 19.62 abcd	62.03 ± 21.24 defg
G16	2.45 ± 0.52 fghij	4.68 ± 0.98 j	85.48 ± 16.14 abc	70.81 ± 21.56 abcde
G17	2.31 ± 0.41 j	4.61 ± 0.99 jk	82.56 ± 23.51 abc	69.44 ± 27.06 bcde
G18	1.88 ± 0.33 l	3.86 ± 0.88 l	78.31 ± 23.48 cd	49.94 ± 18.55 fg
G19	1.93 ± 0.40 l	3.95 ± 0.73 l	84.39 ± 16.9 abc	57.90 ± 23.7 efg
G20	2.50 ± 0.51 efghi	5.23 ± 1.06 fgh	87.89 ± 11.05 abc	76.31 ± 13.66 abcd
G21	2.65 ± 0.76 bcde	5.74 ± 1.37 cd	84.67 ± 14.72 abc	75.44 ± 16.57 abcde
G22	2.31 ± 0.52 j	5.25 ± 1.09 fgh	85.07 ± 22.24 abc	70.38 ± 23.93 abcde
G23	2.06 ± 0.34 k	4.36 ± 0.86 k	69.75 ± 23.45 d	48.22 ± 19.95 g
G24	2.36 ± 0.70 ij	5.30 ± 1.38 efg	80.86 ± 19.80 bcd	62.57 ± 25.02 defg
G25	2.38 ± 0.49 ij	4.99 ± 1.26 ghi	83.81 ± 18.05 abc	65.78 ± 23.86 cdef
CM	3.23 ± 0.68 a	6.57 ± 1.54 a	94.42 ± 5.13 a	90.82 ± 8.08 a
FS	2.14 ± 0.34 d	4.66 ± 0.99 c	74.49 ± 24.20 c	58.67 ± 26.96 d
PX	2.10 ± 0.39 d	4.6 ± 1.07 cd	77.82 ± 16.41 c	51.36 ± 33.43 d
YJ	2.61 ± 0.46 b	5.57 ± 1.14 b	90.40 ± 13.25 b	81.93 ± 15.27 b
ZZ	2.20 ± 0.44 c	4.50 ± 1.05 d	90.00 ± 14.82 b	71.33 ± 24.01 c

Notes: The data in the table are means ± standard deviation. Different lowercase letters indicate significant differences at the 0.05 level.

3.3. Estimating the Genetic Parameters

The PCV of the tree height and ground diameter ranged from 16.56% to 32.09% and 20.48% to 24.58%, respectively (Table 4). The GCVs of the tree height and the ground diameter ranged from 5.24% to 27.06% and 8.51% to 20.66%, respectively. The PCVs of the clone tree height and the ground diameter at each test site were greater than the GCVs, indicating abundant variations in the growth traits of the Qi-nan clones. The PCV and GCV values of the tree height at the PX site showed the highest values, 32.09% and 27.06%, respectively. Moreover, the GCVs of the tree height and the ground diameter on the ZZ site had the lowest values, 5.24% and 8.51%, respectively. The repeatability of the tree height and the ground diameter of the clones at different test sites ranged from 0.50 to 0.96 and 0.69 to 0.98, respectively. Except for the ZZ site, the repeatability of the height and the ground diameter of the clones at the other four sites was greater than 0.8. The ground diameter repeatability of the different sites was greater than the tree height except for at the FS site, indicating that the ground diameter was less affected by the environment than the tree height.

Table 4. Results of the phenotypic variation coefficient (PCV), genetic variation coefficient (GCV), and repeatability (R) of tree height and ground diameter at five different test sites.

Sites	Traits	PCV (%)	GCV (%)	R
CM	Tree height	21.94	18.71	0.96
	Ground diameter	24.58	20.66	0.98
FS	Tree height	16.56	10.76	0.90
	Ground diameter	21.39	14.67	0.85
PX	Tree height	32.09	27.06	0.80
	Ground diameter	23.29	10.33	0.85
YJ	Tree height	18.03	11.74	0.93
	Ground diameter	20.48	14.99	0.98
ZZ	Tree height	19.97	5.24	0.50
	Ground diameter	23.40	8.51	0.69

Notes: PCV—phenotypic coefficient of variation; GCV—genotypic coefficient of variation. R: the repeatability of the clone.

3.4. Correlation Analysis between Growth Traits and Environmental Factors

The tree height and the ground diameter had a highly significant positive correlation with the mean annual temperature (Table 5). Additionally, they also had a significant positive correlation with the mean annual sunshine duration. The 1-year survival rate had a significant positive correlation with the mean annual precipitation, and the mean annual sunshine duration. Similarly, the 3-year preservation rate had a highly significant positive correlation with the mean annual sunshine duration and a significant positive correlation with the mean annual precipitation.

Table 5. Results of the correlation analysis between the tree height, the ground diameter, the 1-year survival rate, and the 3-year preservation rate, and geographical and environmental factors.

Traits	Longitude	Latitude	Altitude	Frost-Free Season	Mean Annual Precipitation	Mean Annual Temperature	Mean Annual Sunshine Duration
Tree height	−0.18	−0.81	−0.57	0.60	0.65	0.98 **	0.9 *
Ground diameter	−0.28	−0.87	−0.68	0.71	0.62	0.99 **	0.89 *
1-year survival rate	0.28	−0.3	−0.06	0.02	0.88 *	0.64	0.89 *
3-year preservation rate	0.22	−0.49	−0.4	0.36	0.91 *	0.81	0.97 **

Notes: ** and * indicate the highly significant and significant difference at 0.01 or 0.05 levels, respectively.

3.5. AMMI Model Analysis

The AMMI biplots effectively explained the interaction information of the tree height and ground diameter (Figure 2). The AMMI biplots based on tree height and ground diameter explained 91% (PC1: 80.2%, PC2: 10.8%) and 78.1% (PC1: 58.4%, PC2: 19.7%) of the interaction effect between the clones and test sites, respectively. In the AMMI biplot, the closer the value to the coordinate origin, the more stable the clone was, and the farther away the value from the coordinate origin, the better the ability to distinguish the environment [42]. According to the selection rate of 20%, the excellent clones G14, G10, G03, G08, and G4 performed best in tree height stability. CM and YJ demonstrated the strongest ability to distinguish the tree height. G16, G21, G08, G10, and G05 performed best with respect to the stability of the ground diameter, and CM had the strongest ability to distinguish the ground diameter. In conclusion, G08 and G10 had the best stability in terms of tree height and ground diameter, and CM had the strongest ability to distinguish tree height and ground diameter, followed by YJ.

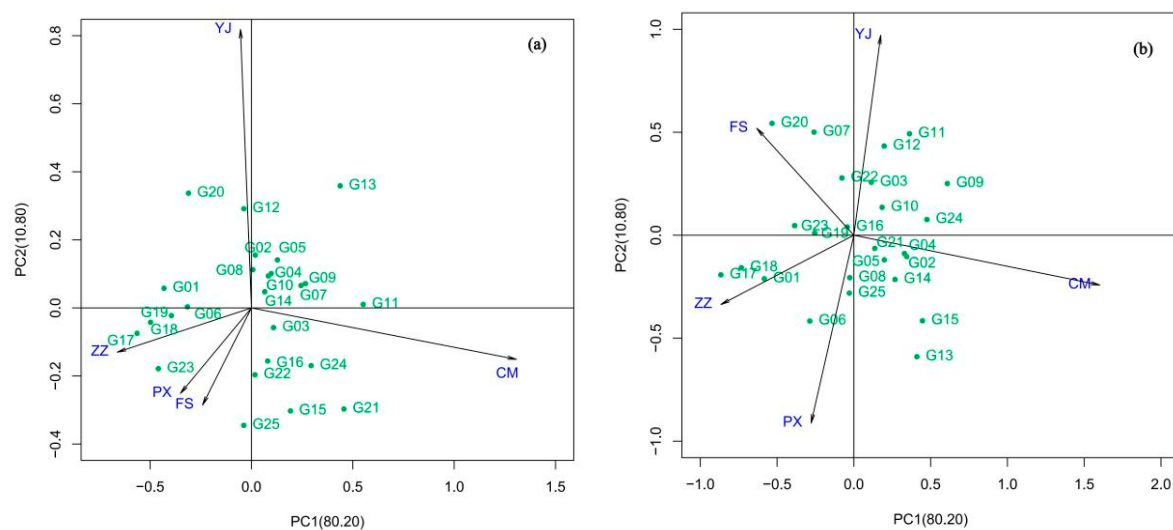


Figure 2. Tree height analysis based on AMMI biplot (a); ground diameter analysis based on AMMI biplot (b). The green numbers stand for clones and the blue characters stand for test sites.

3.6. WAASB Stability Index Analysis

Based on the WAASB stability index scores (Table 6), it is apparent that the clones G08, G15, G12, G02, and G18 exhibit exceptional stability in tree height, whereas the clones G24, G08, G19, G18, and G02 demonstrate robust ground diameter stability. Notably, the clones G08, G02, and G18 stand out as having remarkable stability in both tree height and ground diameter.

Table 6. The WAASB stability index score and ranking of tree height and ground diameter.

Clones	Tree Height		Ground Diameter	
	WAASB Value	Ranking	WAASB Value	Ranking
G01	0.40	24	0.37	22
G02	0.08	4	0.16	5
G03	0.22	15	0.31	16
G04	0.11	9	0.31	15
G05	0.25	18	0.34	20
G06	0.10	7	0.18	8
G07	0.30	21	0.32	17
G08	0.06	1	0.11	2
G09	0.45	25	0.61	25

Table 6. *Cont.*

Clones	Tree Height		Ground Diameter	
	WAASB Value	Ranking	WAASB Value	Ranking
G10	0.23	17	0.32	18
G11	0.26	19	0.21	10
G12	0.08	3	0.24	11
G13	0.33	22	0.38	23
G14	0.21	14	0.18	7
G15	0.08	2	0.20	9
G16	0.30	20	0.32	19
G17	0.34	23	0.35	21
G18	0.09	5	0.15	4
G19	0.11	10	0.15	3
G20	0.09	6	0.29	14
G21	0.14	11	0.16	6
G22	0.19	13	0.27	12
G23	0.16	12	0.27	13
G24	0.11	8	0.09	1
G25	0.22	16	0.53	24

3.7. GGE Biplot Analysis

The GGE biplot of the tree height and the ground diameter showed that AXIS1 and AXIS2 explained 92.20% and 90.56% of the total variation (the sum of the clone effect and the interaction effect between the clones and test sites), indicating that the inference results were reliable (Figure 3).

In the GGE biplot of “Mean vs. Stability” (Figure 3a,b), a green line with an arrow shows the Average Environmental Coordinate (AEC), and a line perpendicular to AEC shows the Average Environmental Axis (AEA) [43]. The intersection of the AEC and AEA represents the average value of the clones, with a higher yield being associated with a longer vertical distance from AEA in the direction of an arrow and a better stability being represented with a shorter vertical distance from the AEC. The clones G11, G13, G05, G10, and G04 exhibited high yield and good stability in tree height. The clones G11, G05, G10, G21, and G04 demonstrated high yield and good stability in ground diameter. The YJ site had good stability and a high yield in both tree height and ground diameter. The ZZ site had a poor yield and poor stability, while the CM site had the highest yield, but poor stability.

The outermost clones in the GGE biplot of “Which won where” formed a polygon shown by green straight lines, with red vertical lines from the origin dividing it into sectors, and test sites into groups (Figure 3c,d). Based on the tree height, the five sites divided the test points into two groups, with CM, PX, FS, and YJ as one group and ZZ as another group. G11 and G13 produced good tree height adaptability in CM, PX, FS, and YJ, whereas G20, G10, G05, G02, G04, G06, and G08 produced good tree height adaptability in ZZ. All of the test sites were concentrated in one area based on base diameter, which shows that G11, G10, G05, G04, G21, and G03 had strong adaptability in these test sites (Figure 3d). Overall, clone G11 had the strongest adaptability of growth traits among all the sites.

In the GGE biplot of tree height and ground diameter, the vectors of all the test sites form acute angles with the AEC, indicating that all the test sites are representative and suitable for Qi-nan clones (Figure 4a,b). It can be seen that PX, FS, and YJ have smaller angles with the AEC and stronger representativeness, while CM and ZZ have larger angles with the AEC and weaker representativeness. The length of the vector indicates the distinguishing force of the biplot; the longer the vector is, the stronger the distinguishing force. It can be seen that the CM and YJ vectors are longer and have strong discriminative ability, while the PX and ZZ vectors are shorter and have weaker discriminative ability. YJ is a representative and distinguishing test site, and it is the most suitable test site for breeding clones with good growth and stable yield.

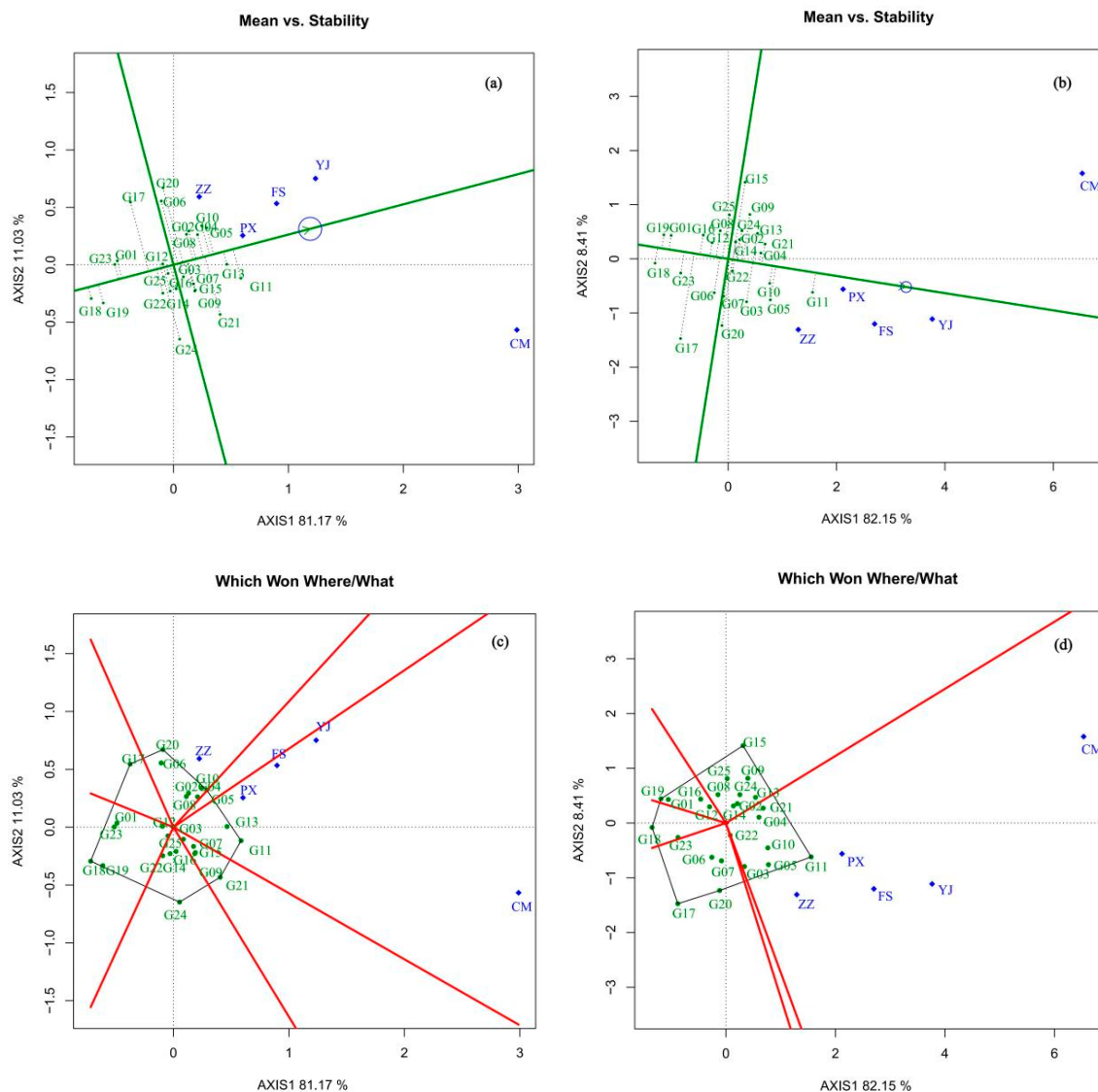


Figure 3. GGE biplot of “Mean vs. Stability” analysis based on tree height (a) and ground diameter (b); GGE biplot of “Which won where” analysis based on tree height (c); GGE biplot of “Which won where” analysis based on ground diameter (d).

In the ranking GGE biplot (Figure 5), the center represents the ideal test site or clone, and the closer a test site or clone is to the center, the better its overall performance. The ranking of the ideal test sites and the ideal clones in the biplot based on tree height was $CM > YJ > FS > PX > ZZ$ and $G13 > G11 > G05 > G10 > G04$, respectively (Figure 5a,b). The ranking of the ideal test sites and the ideal clones in the biplot based on the ground diameter was $CM > YJ > FS > PX > ZZ$ and $G11 > G10 > G05 > G04 > G21$, respectively (Figure 5c,d). Overall, CM and YJ were found to be the ideal test sites, and G11, G13, G05, G10, and G04 were the ideal clones.

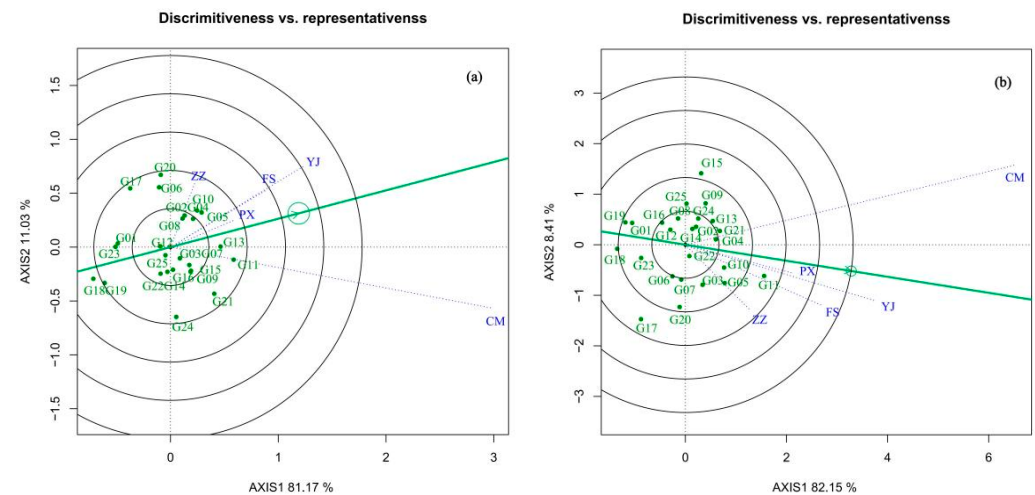


Figure 4. GGE biplot of “Discriminativeness vs. Representativeness” analysis based on tree height (a) and ground diameter (b).

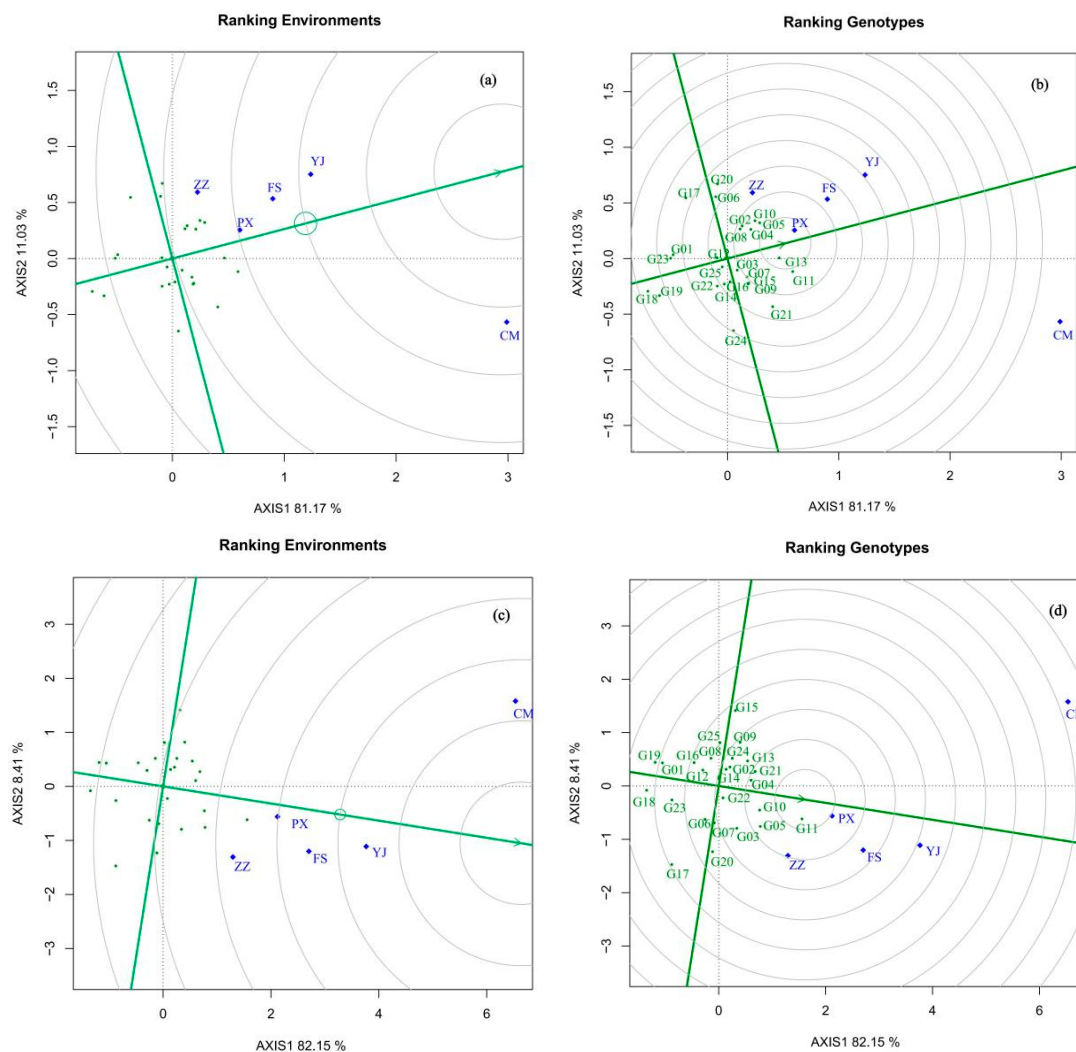


Figure 5. GGE biplot of “Ranking Environments” analysis and GGE biplot of “Ranking Genotype” analysis based on tree height (a,b); GGE biplot of “Ranking Environments” analysis and GGE biplot of “Ranking Genotypes” analysis based on ground diameter (c,d).

4. Discussion

4.1. Growth of Qi-Nan Clones

Excellent varieties should be high-yield, stable, and have certain adaptability [44]. Generally, the growth performance of different germplasms in different test sites is not consistent, which is not only related to the stability of the germplasm genotype itself, but also to its adaptability [45]. In this study, the growth performance of Qi-nan clones in multiple environments showed extremely significant differences ($p < 0.01$) among the clones, the test sites, and the interactions between the clones and the test sites (Table 2). Regarding the differences among the sites, the obvious coefficient of variation in the tree height and the ground diameter may be related to the environment conditions [46]. Similar phenomena were also reported for woody species, including *Eucalyptus* clones, *pinus pinaster*, and polar clones [18,26,47], in which the different results were considered related to the varying adaptability of the clones. The difference among the clones can be attributed to their substantial genetic variations [5], and different provenances [48]. The variance difference between the clones and the sites in the interaction regarding the growth performance of the Qi-nan clones indicated that environmental conditions comprised the most important growth-limiting factor [19]. Therefore, it is necessary to study the multi-site breeding of Qi-nan clones.

There was a significant positive correlation between the height and ground diameter of the Qi-nan clones and the mean annual temperature and mean annual sunshine duration. These correlations may be related to the traits of *A. sinensis*, as the original species demonstrated good growth in tropical and subtropical zones [49]. This evidence reveals why the growth performance of the CM and YJ clones showed an outyield effect in comparison to the others. Further research showed significant variations in the 1-year survival and 3-year preservation rates of the Qi-nan clones across environments. Notably, the CM, YZ, and ZZ sites with abundant mean annual precipitation have significantly higher rates, suggesting that the growth of Qi-nan clones is regulated by multiple factors, with yearly rainfall being a key influence [50]. Thus, it is recommended that Qi-nan clones be planted in tropical–subtropical regions with ample light, heat, and rain for optimal growth. Additionally, multi-environment breeding should be implemented to further enhance their adaptability.

4.2. Genetic Parameters for the Growth of Qi-Nan Clones

The ranges of the PCV and GCV values of growth traits among the clones were from 16.56% to 32.09% and from 5.24% to 27.06%, respectively, with both reaching a moderate degree of variation [18,51], suggesting that there are genetically excellent clones within the experimental group [52]. Moreover, the PCVs of the growth traits of the clones in each test site were found to be greater than the GCVs, indicating that the traits of the Qi-nan clones were not only controlled by heredity, but also affected by the environment [53]. Among the growth traits, the PCV and GCV values of the ground diameter were generally higher than those of tree height, which may be due to stronger environmental influence or larger differences in genetic control and environmental stress among the clones, resulting in increased phenotypic variations in the ground diameter [54].

The repeatability values of the tree height and ground diameter of the Qi-nan clones in the CM, YJ, PX, and FS sites were all greater than 0.80, which indicates that these test sites could effectively maintain the genetic stability of the clones [55]. Meanwhile, the repeatability values of the clones at the ZZ site were only 0.50 and 0.69, respectively, which was consistent with the low GCV and high PCV values, indicating that the clones in this area were greatly affected by the environment, which limited the normal growth of some of them [56]. In addition, it was found that the repeatability of the ground diameter was generally greater than that of the tree height, and the higher the repeatability, the more credible the test results [57]. Therefore, the selection of excellent Qi-nan clones should be based on ground diameter and supplemented by tree height.

4.3. Evaluation of Qi-Nan Clones

In this study, the AMMI model and GGE biplot were used to clarify the sums of the squares of interactions, which produced more than 78% and 90% of growth traits, respectively, indicating that it was feasible and reliable to use these two methods to evaluate the clones and adaptation areas of the Qi-nan clones in this experiment [15,58].

Regarding high yield, in the GGE biplot, clones G11, G13, G05, G10, and G04 were selected in the high-yield group for tree height, while clones G11, G05, G10, G21, and G04 were selected in the high-yield group for ground diameter. This consistency with the results of multiple comparisons (Table 3) suggests that the GGE biplot can be used to effectively evaluate the high yield potential of clones [59], and this is supported by the fact that it has already been utilized in several studies [19,60].

In terms of stability, the results of the WAASB stability index differ significantly from the other two models, possibly due to the large differences in three-year preservation rates among different clones, while the WAASB stability index is based on absolute bias, resulting in insensitivity to outliers [61]. The AMMI model and the GGE biplot were used to jointly screen the clones with good tree height stability, including G03 and G04, and the clones with good ground diameter stability, including G16, G21, and G10. However, there were differences in the identification of certain clones, such as the fact that the tree height stability of clones G01, G13, G18, and G23 in the GGE biplot were higher than that of G04, whereas their stability in the AMMI model was inferior to that of G04. Similar differences in the evaluation between the AMMI and the GGE were also found in previous studies [62,63]. The coefficient variation of tree height in different test sites further revealed that G01 (with a coefficient of variation of 18.6%), G13 (15.7%), G18 (16.9%), and G23 (15.3%) were all higher than G04 (14.7%), which was stable under both models. Therefore, the stability of clones G01, G13, G18, and G23 is worse than G04, indicating that the stability of the AMMI model is more reliable [28,64]. Clone G10, with its stable ground diameter in the two models, also had similar results when compared to G01, G11, and G18. The reason for this was because the GGE biplot evaluated stability while taking into account yield [63], while the AMMI evaluated stability more holistically because stability is not related to yield [28].

In terms of adaptability, clones G11 and G13 in the GGE biplot had better tree height adaptability in the CM, PX, FS, and YJ sites. Clones G20, G10, G05, G02, G04, G06, and G08 had better tree height adaptability in the ZZ site. Clones G11, G10, G05, G04, G21, and G03 had good ground diameter adaptability at the five test sites, indicating that these clones had specific adaptability in the corresponding test sites [65]. However, 64% and 72% of the clones in the GGE biplot of the tree height and the ground diameter, respectively, were not in the corresponding sector area of the test site, and they were considered unsuitable for the test environment [59]. Therefore, more test sites with different environmental types need to be carried out in future breeding tests.

In summary, clone G04 produced the best comprehensive performance in terms of yield, stability, and adaptability. Clones G05 and G10 also showed good growth, albeit with moderate tree height stability in certain areas; notably, despite having average stability, clone G11 excelled in both yield and adaptability, making it an excellent candidate for breeding. Meanwhile, clone G13 was grown well in all of the sites except the ZZ site. This is consistent with the ideal clone results (G11, G13, G05, G10, and G04) in the GGE biplot of “Ranking Genotypes”. Moreover, we noticed that G11 showed moderate stability while having a high ranking in the GGE biplot. We believe that the excellent yield and adaptability of G11 led to this contrast, indicating the comprehensiveness of the GGE biplot in clone evaluation [66].

4.4. Evaluations of Site Adaptability for Qi-Nan Clones

Multi-environment trials were carried out to scientifically evaluate the site adaptability of the germplasm, which was not only conducive to providing a basis for the scientific promotion of germplasm [16], effectively controlling the application risk of germplasm, but also conducive to determining the adaptation range of the germplasm [19]. In our study,

the AMMI model and the GGE biplot demonstrated that the Qi-nan clones at the YJ site exhibited the best comprehensive growth performance, displaying good discrimination and representativeness. The growth performance at the CM site was outstanding, featuring good discrimination, but lacking representativeness, which could potentially be attributed to the slightly lower mean annual precipitation recorded when compared to the YJ site and poor stability. The comprehensive growth performance at the FS site was general. The stability at the PX site was remarkable, but growth was hindered by limited rainfall, resulting in poor yield. Due to its high latitude and altitude, the ZZ site had the smallest frost-free period, resulting in poor stability and a poor yield, but it also had a certain degree of differentiation, which might aid in identifying cold-tolerant clone species.

Upon comprehensive evaluation, the ranking of ideal test sites in the GGE biplot was CM > YJ > FS > PX > ZZ, which is consistent with the gradient order of the mean annual temperature and mean annual sunshine duration (Table 5). This indicates that within a certain range, the higher the temperature and the more sufficient the light, the more conducive it is to tree growth [67]. Therefore, the YJ site is the most suitable experimental site for screening high-yield and stable clones. The CM site stands out for its high-yield potential but lacks stability; however, with timely irrigation during later maintenance, it could become the best-performing experimental site. The FS site is a suitable location for the growth of Qinan clones, though their performance is generally average. The PX site requires long-term watering for good growth. The temperature of the ZZ site is relatively low, and it is necessary to breed cold-resistant clones to promote growth.

5. Conclusions

There exist significant differences in the clones and sites, and the interactions between the clones and test sites. Genetic breeding is an efficient pathway for the potential selection of Qi-nan germplasms in southern China. The phenotypic variation coefficient (PCV), the genetic variation coefficient (GCV), and the repeatability of the ground diameter are generally greater than the tree height, which may indicate that the selection of excellent Qi-nan clones should focus on ground diameter. In the comprehensive evaluation of clones and site adaptability for Qi-nan clones, the AMMI model was better than the GGE biplot in screening the stability of clones, but the GGE biplot was more comprehensive in evaluating varieties. The southern region of China is suitable for the growth of Qi-nan agarwood, and different varieties have different growth performances in different regions. According to climate conditions. Targeted management measures should also be implemented in different growing regions according to the climate conditions. This study focused on the early growth performance of Qi-nan clones; thus, long-term observations are needed in the future. Additionally, a comprehensive evaluation of growth and agarwood traits should be carried out to verify the accuracy of clone selection. Furthermore, the evaluation of different environments is needed to improve the accuracy of genetic improvements.

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