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Seed Distribution and Phenotypic Variation in Different Layers of a Cunninghamia Lanceolata Seed Orchard

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Abstract: The phenotypic characteristics of forest seeds are the basis of germplasm innovation, genetic improvement, and biological research, and they also are the reference for the development of seed orchards. In this study, we analyzed seed quantity characteristics, phenotypic differentiation, and variation patterns in three seed-bearing clones from different crown layers of the Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) orchard located in Fujian Province, China. We divided the clones into six layers according to crown height and the sunny and shady sides, 14 phenotypic characteristics, and five quality indexes, and we measured the germination rate of seeds. The rate of seeds filled with tannin-like substance in the upper sunny layer was low, but it was high in the lower shady layer. The germination rate was highest in the upper sunny layer and lower in the middle and lower shady sides. Values of most of the 14 phenotypic traits tested differed significantly among clones and layers. The average value of the phenotypic differentiation coefficient was 81.16%, indicating that variation among clones explained most of the total phenotypic variation. The repeatability of the 14 phenotypic traits was high (R > 0.80), indicating that these traits are highly heritable. The phenotypic characteristics of cones and seeds varied from 6.86% to 129.51%. The 14 phenotypic traits exhibited different degrees of correlation, and seed weight, seed circumference, seed width, and seed area can be used to predict other seed traits. However, the correlations between cone traits were not strong. Our results show that when establishing a dwarfing Chinese fir seed orchard, the distribution and variation of seeds in different crown layers of clones should be considered, and clones with more cones in the lower crown layer should be selected as parents.

Keywords: *Cunninghamia lanceolata* (Lamb.) Hook; seed orchard; seed type; germination percentage; phenotypic traits; phenotypic differentiation

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1. Introduction

The Chinese fir, *Cunninghamia lanceolata* (Lamb.) Hook, is the main afforestation tree species in China, and it has good wood properties, high yield, and abundant germplasm resources [1]. The seed types of Cunninghamia lanceolata can be divided into three types: good seed, seeds filled with tannin-like substance, and empty seed. The seeds filled with tannin-like substance mainly occurs in the middle and late stage of embryo abortion, accompanied by the filling of tannin substances, which may be related to many factors such as clonal heredity, self-pollination infertility, bad climatic conditions, etc. [2]. High quality of cones and seeds in Chinese fir seed orchards ensures good seeds, strong seedlings, and high yield. Therefore, improving the utilization rate of germplasm and promoting good seed generation is essential for improving the afforestation process. The number of cones and seeds of Chinese firs determines the seed yield of the seed orchard [3]. The seed yield is also related to the cone and seed phenotype, so the number, germination characteristics, and phenotypic characteristics of Chinese fir cones and seeds are important indicators for studying their quality and phenotypic variation [4].

Plant phenotypic diversity is an important component of genetic diversity and is the result of the interaction of genes and the environment [5]. Of the tools commonly used for genetic diversity research, such as phenotyping, cytology, biochemistry, and molecular markers, phenotyping is the most feasible and direct method [6]. Assessing the degree of phenotypic variation is research content to reveal the adaptation of organisms to different environments [7]. The phenotypic variation of seed and fruit traits determines the diffusion ability and population distribution pattern of a species, and it is an economically important trait for the breeding of improved forest varieties [8]. Studies of phenotypic variation of the genera *Cunninghamia* R. Br. [9] and *Pinus* Linn [10] have laid a foundation for resource protection and genetic improvement of related tree species [11].

The main goals of seed production in seed orchards are to improve the genetic quality of seeds and to facilitate seed harvesting. The ninth national inventory of forest resources showed that the area of Chinese fir plantations reached 148 million mu, and the stock volume reached 755 million m3, accounting for 1/4 and 1/3 of the total area and stock volume of the country's artificial arbor forests, both ranking first [12]. The cone is the basic unit of seed production of Chinese fir and other coniferous trees [13]. Ayari and Khouja reported that the cone yield of *Pinus* is not uniform and often varies among crown heights [14]. In a study of pine trees, Debain et al. found that the number of cones produced in the upper layer of the crown was higher [15]. Seeds from different layers differ not only in seedling growth potential but also in seed morphology and quality. For example, Shen and Gao studied the seed quality from different crown heights of Chinese fir and found that the proportion of good seeds in the upper layer was high, followed by the middle and lower parts [16]. Therefore, it is very important to study the phenotypic variation of cone number and seed quality of Chinese fir in different layers of crown [17]. Additionally, in a study of seed phenotype, Frank and Robert found that the seed size of forest trees is one of the most stable phenotypic characteristics [18].

At present, a large number of Chinese fir seed orchards have been established in China, and they provide high-quality seeds for large-scale afforestation. However, at present, seed orchards are all non-dwarfed seed orchards. With the gradual increase in the tree body, the seed-bearing layer moves upward, and the operation of seed collection and pollination becomes increasingly difficult. Therefore, the "dwarfing" of seed orchards is the management direction of improved seed bases, and the dwarfing experiments and technical schemes for Chinese fir mother plants are gradually developing. One of the common "dwarfing" schemes of Chinese fir seed orchard refers to the operation of cutting off the upper trunk and pruning the side branches [19]. At home and abroad, tree seed orchards such as Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) [20], Black pine (*Pinus thunbergii* Parl.) [21], Japanese larch (*Larix kaempferi* (Lamb.) Carr.) [22], Masson pine (*Pina massoniana* Lamb.) [23], and other tree species have been cut off the upper trunk and pruned the side branches in order to decline the seed-bearing layer of trees and improve seed yield. It is crucial that we understand the layering characteristics of the Chinese fir cone setting so that seed setting and/or quality are not reduced after the mother plant is dwarfed. Zheng et al. reported that dwarfing *Pina massoniana* clones with different seed setting characteristics increased the amount of female cones of middle- and high-yield clones by more than 20%, but did not significantly improve the low-yield clones. Differences were found for studied cone and seed characters among orchards and crown positions. Variation among grafts within clone was higher than among clones for most characters for any individual characters. The coefficient of variation within clones was often higher than among clones [24]. This result illustrates that dwarfing clones with different seed setting abilities has quite different effects.

To date, little is known about the effect of seed setting characteristics from different crown layers on the seed yield after tree dwarfing and pruning. In this study, we evaluated seed yield and seed quality of Chinese fir from different layers of the crown using modern phenotypic analysis methods. We selected three Chinese fir clones with different seed setting characteristics to study seed quality, germination rate, and phenotypic characteristics at different crown layers. Our results provide a reference for the selection of clones from dwarf Chinese fir seed orchards as well as a theoretical basis and technical reference for the construction and management of a new generation of seed orchards.

2. Materials and Methods

2.1. Study Area

The experimental area is located in Yangkou in a state-owned forest farm near Daoping Village, in Fujian Province (longitude 117.902773°; latitude 26.823542°). It is the central production area of Chinese fir. The farm was founded in 1956 and has a total operating area of 4133 hm^2 and a total forest stock of 590,000 m^3. The seed garden is located in the low mountains and hills of the Wuyi Mountains. The area is dominated by fertile and deep red soil and has a subtropical climate. The annual average temperature is 18.5 °C, the annual average precipitation is 1880 mm, and the frost-free period can reach 305 days. The climate is mild, the rainfall is abundant, and the plant growth period is long, making it a suitable locale for the growth of fir, pine, and other timber species.

2.2. Study Materials

In 2011, 670 clones were collected, and they were grafted onto two-year rootstocks in March 2013. We adopted artificial belt land preparation, with a bandwidth of 1.2 m, excavation specification of 60 cm × 40 cm × 40 cm, and plant row spacing of 3 m × 3 m, to generate the third Chinese fir germplasm gene bank. We selected the following three representative Chinese fir clones from the 670 clones according to their characteristics in the lower crown: (1) more seed bearing in the lower layer (jiang26), (2) medium seed bearing in the lower layer (jiang18), and (3) no seed bearing in the lower layer (jiangA13). We evaluated three clones from each group (Table 1).

Table 1. Basic information about the three Chinese fir clones assessed in the study.

Clone Number	Test Strain Number	Number of Cones on the Upper Sunny Side	Number of Cones on the Upper Shady Side	Number of Cones on the Middle Sunny Side	Number of Cones on the Middle Shade Side	Number of Cones on the Lower Sunny Side	Number of Cones on the Lower Shady Side	Clonal Seed Setting Characteristics
jiang26	jiang26-1	328	194	247	95	354	118	more seed bearing in the lower layer
jiang26	jiang26-2	205	233	286	103	295	84	
jiang26	jiang26-3	170	181	185	76	265	89	
jiang18	jiang18-1	175	183	242	107	138	69	medium seed bearing in the lower layer
jiang18	jiang18-2	151	135	108	70	114	37	
jiang18	jiang18-3	334	110	31	178	174	131	
jiangA13	jiangA13-1	220	118	113	55	0	0	no seed bearing in the lower layer
jiangA13	jiangA13-2	203	121	132	50	0	0	
jiangA13	jiangA13-3	184.	108	122	76	0	0	

2.3. Study Method

In late November 2020, we measured three growth indexes (tree height, diameter at breast height, and crown width) of clones jiang26, jiang18, and jiangA13. We divided the three experimental plants of jiang26 and jiang18 into six layers (upper sunny side, upper shady side, middle sunny side, middle shady side, lower sunny side, and lower shady side) and randomly collected six cones from each layer, for a total of 216 cones. Because the lower crown layer of clone jiangA13 does not bear fruit, we divided it into four layers (upper sunny side, upper shady side, middle sunny side, and middle shady side) and randomly collected six cones from each layer, for a total of 72. In total, we tested 288 cones.

For each experimental plant and layer, we randomly chose three cones, measured their fresh weight (CW, g), and took photos. We then removed the bract scales and seeds from the base of each selected cone [25], took photos, recorded the one seed weight (OSW, g). The quality of a seed is to weigh all the seeds in a cone one by one, and then obtain the average. We then dissected the seeds and recorded the number of empty seeds, seeds filled with tannin-like substance, and good seeds. The phenotypic characteristics of cones, bract scales, and seeds were determined using ImageJ software [26]. These traits included longitudinal diameter of cones (CL, cm), transverse diameter of cones (CD, cm), bract scale area (WPA, cm), bract scale perimeter (WPP, cm), bract scale length (WPL, cm), bract scale width (WPW, cm), bract scale length-width ratio (WPL/WPW), seed area (SA, cm), seed perimeter (SP, cm), seed length (SL, cm), seed width (SW, cm), and seed length-width ratio (SL/SW).

The other three cones from each clone/layer were dried to make the seeds fall off naturally. The seeds were collected from each cone, washed three times with ultrapure water, placed in a Petri dish with filter paper, and placed in an artificial climate room with a temperature of 25 °C and a humidity of 75% for 21 days. We recorded the number of germinated seeds every day to calculate the seed germination rate [27].

2.4. Data Analysis

We analyzed all data using R (4.1.1 is Version of R). We applied single factor analysis of variance (F-test) and multiple comparisons to study the good seed rate, seeds filled with tannin-like substance rate, empty seed rate, total seed number, and seed germination rate. The nested linear model [28] was used to analyze the variance of phenotypic variation among and within the Chinese fir clones. The linear model is as follows:

$$y_{ijk} = \mu + p_j + bi_{(j)} + e_{ijk} \quad (1)$$

where μ is the overall average value; p_j is the family effect; $bi_{(j)}$ is the repetitive effect; and e_{ijk} is a random error. Multiple comparison selection Newman-Keuls.

We used Pearson correlation to analyze the bivariate correlation, and we conducted cluster analysis according to the Euclidean distance of each trait among clones.

The mean value (x), standard deviation (SD), and phenotypic variation coefficient (CV) of each phenotypic trait of cones, bract scales, and seeds of different Chinese fir clones in each crown layer were calculated as follows:

$$\mathrm{CV} = \frac{\mathrm{SD}}{\mathrm{X}} \times 100\% \quad (2)$$

We calculated the phenotypic variation coefficient $v_{(st)}$ as:

$$v_{(ST)} = \frac{\delta^2_{t/s}}{\left(\delta^2_{t/s} + \delta^2_s\right)} \quad (3)$$

where $\delta^2_{t/s}$, δ^2_s are the difference between clones and within clones.

The clonal repeatability (R) of each characteristic was estimated using the following formula [29]:

$$\mathrm{R} = 1 - \frac{1}{\mathrm{F}} \quad (4)$$

where F is the F value in the analysis of variance.

We used the following formula to calculate the seed germination rate (GP%) [30]:

$$GP\% = \frac{N_n}{N} \times 100\% \quad (5)$$

where N_n is the number of germinating seeds and N is the total number of seeds.

3. Results

3.1. Distribution Characteristics of Seed Types in Different Crown Layers

The number of seeds, good seed rate, seeds filled with tannin-like substance rate, and empty seed rate in a single cone differed among the three clones and among different layers of the crown (Table 2). The number of seeds in a single cone of clone jiang26 ranged from 74.33 to 104.67, and the number of seeds differed significantly between the upper sunny side and lower shade side. For clone jiang18, the number of seeds in a single cone ranged from 99.89 to 112.89, and for clone jiangA13 the range was 100.22 to 115. The number of seeds in a single cone of the three clones did not differ among the upper sunny side, upper shady side, central sunny side, and central shady side. However, in the lower sunny side and lower shady side of the crown, the number of seeds in a single cone of clone jiang26 was significantly lower than that of clone jiang18.

The percentage of good seeds in a single cone of clone jiang26 ranged from 12.56% to 44.22%, and the percentage of good seeds in the upper sunny side was significantly higher than that in the central shady side, lower sunny side, and lower shady side. For clone jiang18, the percentage of good seeds in single cone ranged from 11.1% to 22.12%, and the range was 18.24% to 23.96% for clone jiangA13. In the upper sunny side, the good seed rate of jiang26 was extremely significantly higher than that of jiang18 and jiangA13.

The rate of seeds filled with tannin-like substance of a single cone of clone jiang26 ranged from 34.19% to 65.88%. The rate for the upper sunny side was the lowest, and that of the lower shady side was the highest. For clone jiang18, the percentage of seeds filled with tannin-like substance in single cone was 23.97% to 34.88%, and that for clone jiangA13 was 15.67% to 29.90%. The rate for the upper shady side of clone jiang26 was significantly higher than that of clone jiangA13, and the rate for the central shady side of jiang26 was significantly higher than that of clones jiang18 and jiangA13. Additionally, the rates for the lower sunny side and lower shady side of clone jiang26 were significantly higher than those of clone jiang18.

The percentage of empty seeds in a single cone of clone jiang26 was 21.56% to 34.02%, the range for clone jiang18 was 53.11% to 62.49%, and that for clone jiangA13 was 53.70% to 60.38%. The empty seed rate for the upper sunny side, upper shady side, and central sunny side was significantly lower for clone jiang26 than for clones jiang18 and jiangA13, and the empty particle ratio of the central shady side was extremely significantly lower than that of clones jiang18 and jiangA13. The empty seed rates for the lower sunny side and lower shady side of clone jiang26 were significantly lower than those of clone jiang18.

For clone jiang26, the rate of seeds filled with tannin-like substance was significantly higher than the good seed rate and the empty seed rate. For clone jiang18, the good seed rate was significantly lower than that of seeds filled with tannin-like substance and the empty seed rate, and the rate of seeds filled with tannin-like substance was significantly lower than the empty seed rate. The empty seed rate of clone jiangA13 was significantly higher than that of seeds filled with tannin-like substance and the good seed rate.

In general, the two indexes of good seed rate and seeds filled with tannin-like substance rate of the high seed-bearing clones in the lower layer (jiang26) were significantly higher than those of the other two types of clones ($p < 0.001$), and the empty seed rate was significantly lower than those of the other two types of clones ($p < 0.001$). When we compared the high seed-bearing clones (jiang26) and the medium seed-bearing clones (jiang18), there was no significant difference in the number of single cone seeds and the good seed rate in the lower crown layer. The rate of seeds filled with tannin-like substance of the higher seed-bearing clones was significantly higher than that of the clones with medium seed bearing in the lower layer ($p < 0.001$), while the empty seed rate was significantly lower than that of the medium seed bearing clones in the lower layer ($p < 0.001$).

Table 2. Distribution of different quality and types of seeds in different crown layers in the three Chinese fir clones.

Clone Number	Position	Number of Seeds per Cone	Good Seed Rate (%)	Seeds Filled with Tannin-Like Substance Rate (%)	Empty Seed Rate (%)
jiang26	Upper sunny side	104.67 ± 21.20 a	44.22 ± 17.89 a A	34.19 ± 5.29 cd AB	21.59 ± 15.62 c B
jiang26	Upper shady side	95.56 ± 16.12 ab	27.18 ± 16.31 b A	38.80 ± 6.50 bcd A	34.02 ± 17.36 abc A
jiang26	Central sunny side	94.22 ± 20.04 ab	26.48 ± 14.27 b A	41.49 ± 13.74 bc A	32.03 ± 20.22 bc A
jiang26	Central shady side	97.56 ± 9.98 ab	16.72 ± 6.58 b B	55.97 ± 11.02 ab A	27.32 ± 14.81 c B
jiang26	Lower sunny side	92.00 ± 14.32 ab	17.06 ± 9.92 b B	56.62 ± 10.22 ab A	26.32 ± 16.19 c B
jiang26	Lower shady side	74.33 ± 20.63 b	12.56 ± 9.83 b B	65.88 ± 16.83 a A	21.56 ± 21.67 c B
jiang18	Upper sunny side	112.89 ± 18.03 a	22.12 ± 11.42 b B	24.77 ± 11.89 cd B	53.11 ± 13.97 ab A
jiang18	Upper shady side	100.44 ± 16.18 a	15.48 ± 13.18 b B	26.98 ± 21.90 cd B	57.54 ± 22.30 ab A
jiang18	Central sunny side	105.22 ± 23.71 a	12.01 ± 13.44 b B	25.50 ± 14.54 cd B	62.49 ± 24.44 a A
jiang18	Central shady side	101.11 ± 15.46 a	12.19 ± 8.41 b C	29.14 ± 13.46 cd B	58.66 ± 19.40 ab A
jiang18	Lower sunny side	104.11 ± 6.13 a	15.66 ± 10.18 b B	23.97 ± 9.22 cd B	60.36 ± 17.03 ab A
jiang18	Lower shady side	99.89 ± 14.56 a	11.10 ± 9.40 b C	34.88 ± 19.97 cd B	54.02 ± 20.67 ab A
jiangA13	Upper sunny side	115.00 ± 31.92 a	18.31 ± 12.80 b B	22.97 ± 22.44 cd B	58.72 ± 21.11 ab A
jiangA13	Upper shady side	102.56 ± 11.94 a	23.96 ± 16.77 b B	15.67 ± 8.96 d B	60.38 ± 14.06 ab A
jiangA13	Central sunny side	100.22 ± 18.34 a	12.04 ± 9.27 b C	29.90 ± 22.27 cd B	58.06 ± 18.60 ab A
jiangA13	Central shady side	101.67 ± 11.91 a	18.24 ± 13.42 b B	28.05 ± 15.07 cd B	53.70 ± 14.68 ab A

Significant differences among values in the same row are indicated by capital letters: the same letter indicates no significant difference, and different letters indicate significant difference ($p < 0.05$). Significant differences among values in the same column are indicated by lowercase letters: the same letter indicates no significant difference, and different letters indicate significant difference ($p < 0.05$). The same is true in the following tables.

3.2. Variation of Seed Germination Ability in Different Crown Layers

We detected extremely significant differences in seed germination rates in different layers of the canopies of the three Chinese fir clones (Figure 1). The highest germination rate of clone jiang26 occurred on the upper sunny side (51.50%) and the lowest rate on the lower shady side (20.29%). For clone jiangA13, the highest and lowest germination rates were on the upper sunny side (48.33%) and middle sunny side (23.61%), respectively. The average germination rate of clone jiang18 was relatively low. When comparing the same crown layer among clones, we found that the germination rate of clone jiang26 was 1.91 times greater than that of jiangA13 on the central sunny side, and the rates of clones jiang26 and jiangA13 were 1.80 times greater than that of clone jiang18 on the central shady side8. Therefore, among the three clones tested, in the middle layer, the germination rate of the high seed-bearing clone (jiang26) was significantly higher than that of the other two clones ($p < 0.001$). However, the germination rates of the two clones that bore seeds in the lower crown layer (jiang26 and jiang18) did not differ significantly.

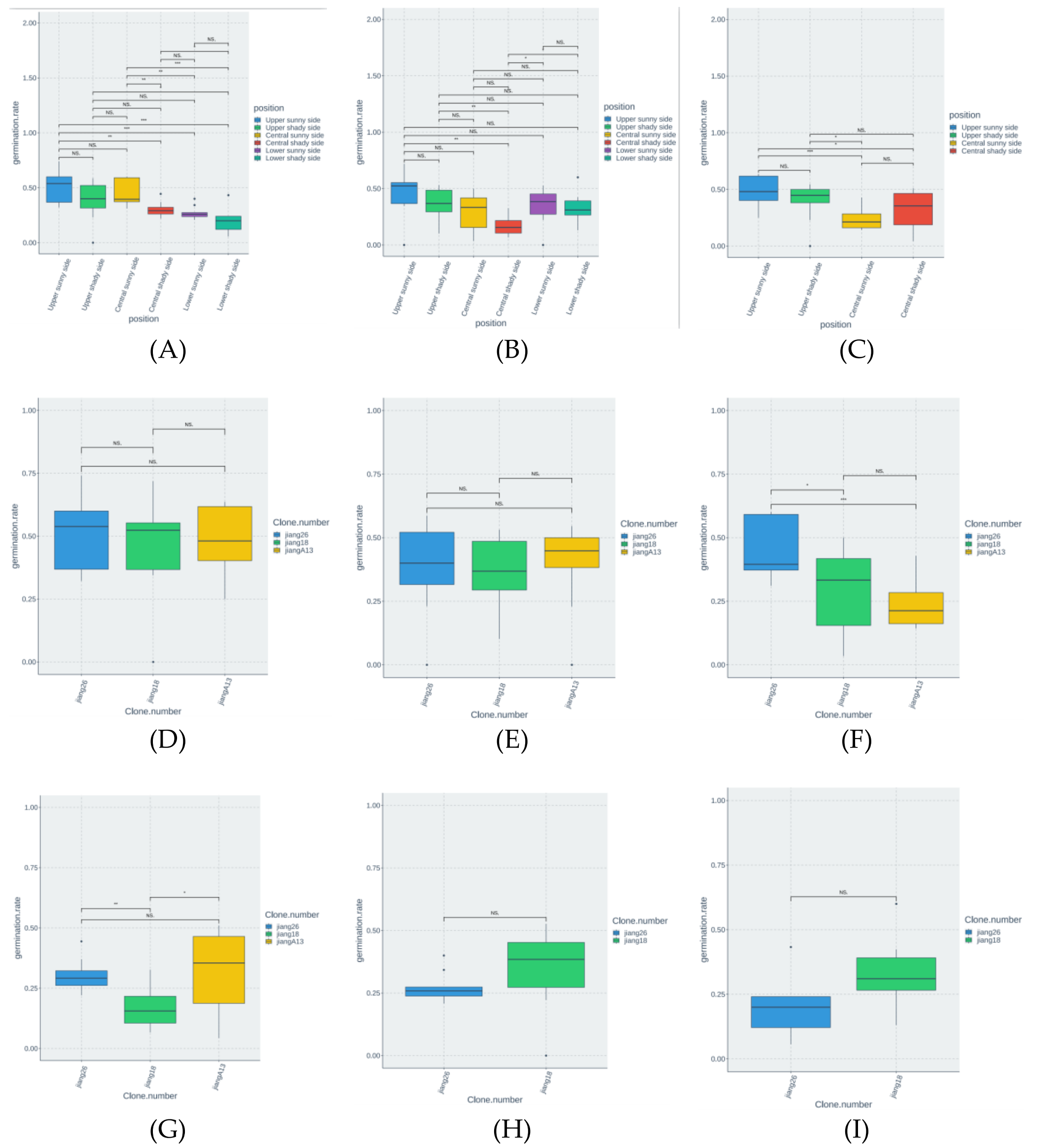

Figure 1. (**A**) Germination rate of Chinese fir clone jiang26 in different crown layers, (**B**) germination rate of clone jiang18 in different crown layers, (**C**) germination rate of clones jiangA13 in different crown layers, (**D**) germination rate of Chinese fir clones from the upper sunny side, (**E**) germination rate of clones from the upper shady side, (**F**) germination rate of clones from the central sunny side, (**G**) germination rate of clones from the central shady side, (**H**) germination rate of clones of different from the lower sunny side, (I) germination rate of clones from the lower shaded side. Significance levels: NS, $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$.

3.3. Phenotypic Variation of Cone and Seed Traits and Differentiation of Phenotypic Traits

Table 3 shows the results of nested variance analysis, variance components, and $v_{(st)}$ of the three clones from the upper sunny side, upper shady side, central sunny side, and central shady side. With the exception of CW, CD, and CL of cones for the within clone comparison, the phenotypic characteristics of cones and seeds were very significantly different within and among the clones. The percentage of variance components among the 14 phenotypic traits varied from 2.42% to 55.46%, with an average of 19.34%. The percentage of variance components in the total variation was 0.00% to 3.20%, with an average of 1.75%. The variance component percentage of random error was 44.54% to 96.46%, with an average of 78.91%. The variance components among clones were greater than those within clones, indicating that the variation among clones was the main explanation of the total phenotypic variation of Chinese fir. At the same time, the variation range of the $v_{(st)}$ of cone and seed traits ranged from 50.00% to 100%, and the average $v_{(st)}$ of the 14 phenotypic traits was 87.97%. This result indicates that the phenotypic variation of the Chinese fir seed orchard population accounted for 87.97% of the variation among clones and 12.03% of that within clones. The diversity among clones also was greater than that within clones. Among the 14 traits, the trait with the largest $v_{(st)}$ was CD (100%), followed by the WPW (99.17%), and SW had the smallest $v_{(st)}$ (50.00%). The $v_{(st)}$ of the 14 phenotypic traits in clones was lower than 50%. These results show that the phenotypic variation in the upper and middle crown layers for the three clones with different seed-bearing characteristics mainly existed among the clones.

Table 4 shows the variance components and $v_{(st)}$ for the two clones that bore seeds in the lower crown layer (jiang26 and jiang18) for the lower sunny side and lower shady side. Thirteen phenotypic traits were significantly different between jiang26 and jiang18, but WPP was not. We found significant differences within clones for 11 phenotypic traits, but not for CL, WPW, or OSW. The percentage of variance components among the 14 phenotypic traits varied from 0 to 65.16%, with an average of 21.03%. The percentage of variance components in the total variation was 0 to 14.95%, with an average of 3.58%, and that of random error was 19.89% to 98.37%, with an average of 75.39%. The variance components among clones were greater than those within clones, indicating that the variation among clones was the main explanation of the total phenotypic variation of Chinese fir. The variation range of $v_{(st)}$ of the 14 cone and seed traits ranged from 0.00% to 100%, with an average of 74.34%. Thus, the phenotypic variation of the Chinese fir seed orchard population accounted for 74.34% of the variation among clones and 25.66% of that within clones. Additionally, the diversity among clones was greater than that within clones. WPW had the largest $v_{(st)}$ (100.00%), followed by OSW (99.46%), but the $v_{(st)}$ was 0.00% for WPL and WPP. The $v_{(st)}$ of all phenotypic traits except WPP, WPL, and WPL/WPW was less than 50% within clones, indicating that the phenotypic variation of the two clones in the lower crown layer mainly existed among the clones.

The repeatability of all phenotypic traits for the three clones in the upper sunny side, upper shady side, central sunny side, and central shady side of the crown ranged from 89.44% to 99.94% (Table 3), and all 14 phenotypic traits had high repeatability ($R > 0.80$). Except for CL ($R = 89.44\%$), the other 13 phenotypic traits were highly heritable ($R \geq 90\%$). The repeatability of all phenotypic traits for the jiang26 and jiang18 clones in the lower sunny side and lower shady side of the crown ranged from 55.63% to 99.85% (Table 4). Except for WPP ($R = 55.63\%$), which had mid and high repeatability, the other 13 phenotypic traits had high repeatability ($R > 0.80$) and strong heritability ($R \geq 90\%$). These results show that the yield and quality of cones and seeds from the three clones were stable, thus these characteristics can be stably inherited in the offspring and are less affected by the environment.

Table 3. Variance component, phenotypic differentiation coefficient, and repeatability of phenotypic characters of cones and seeds of Chinese fir clones in the upper and middle crown layers.

Phenotypic Traits	Variance Component			Variance Component Percentage			V(st) (%)	F		R(%)
	Between Clones	Within Clones	Random Error	Between Clones	Within Clones	Random Error		Between Clones	Within Clones	
CW	4.2880	0.3570	10.2320	28.8200	2.4000	68.7800	92.31	16.4010 ***	1.3140	93.90
CD	12.3010	0.0000	9.8790	55.4600	0.0000	44.5400	100.00	45.2420 ***	0.4130	97.79
CL	5.7630	1.0180	25.5850	17.8000	3.1400	79.0500	84.99	9.4660 ***	1.3580	89.44
WPA	0.0300	0.0030	0.2190	12.0100	1.2400	86.7600	90.91	341.6000 ***	9.5400 ***	99.71
WPP	0.0310	0.0120	1.1800	2.5700	0.9700	96.4600	72.09	70.9680 ***	7.0320 ***	98.59
WPL	0.0100	0.0010	0.1200	7.7700	1.0900	91.1400	90.91	212.6750 ***	8.1920 ***	99.53
WPW	0.0060	0.0001	0.0580	9.1100	0.7300	90.1600	99.17	248.2920 ***	5.8620 ***	99.60
WPL/WPW	0.0390	0.0020	0.2370	13.9300	0.8700	85.2000	95.12	399.2850 ***	7.1350 ***	99.75
OSW	0.0044	0.0002	0.0237	15.6200	0.7700	83.5900	95.65	698.0020 ***	9.5670 ***	99.86
SA	0.0020	0.0002	0.0050	24.0800	3.2000	72.7300	90.91	1260.8600 ***	41.4400 ***	99.92
SP	0.0500	0.0030	0.1130	30.0300	1.9600	68.0100	94.34	1653.4700 ***	27.5400 ***	99.94
SL	0.0060	0.0010	0.0150	27.3700	2.6500	69.9800	85.71	1476.0000 ***	35.8700 ***	99.93
SW	0.0010	0.0010	0.0210	2.4200	2.6700	94.9100	50.00	120.8800 ***	26.8500 ***	99.17
SL/SW	0.7610	0.0900	2.3530	23.7500	2.8000	73.4500	89.42	1227.1100 ***	36.0400 ***	99.92

Significance level: ***, $p < 0.001$. CW is one cone fresh weight; CD is transverse diameter of cones; CL is longitudinal diameter of cones; WPA is bract scale area; WPP is bract scale perimeter; WPL is bract scale length; WPW is bract scale width; WPL/WPW is bract scale length-width ratio; OSW is one seed weight; SA is seed area; SP is seed perimeter; SL is seed length; SW is seed width; SL/SW is seed length-width ratio.

Table 4. Variance component, phenotypic differentiation coefficient, and repeatability of phenotypic characters of cones and seeds of Chinese fir clones in the lower crown layer.

Phenotypic Traits	Variance Component			Variance cOmponent Percentage			V(st)(%)	F		R(%)
	Between Clones	Within Clones	Random Error	Between Clones	Within Clones	Random Error		Between Clones	Within Clones	
CW	3.0630	1.2810	4.9710	32.8800	13.7500	53.3700	70.51	14.4090 ***	3.3190 *	93.06
CD	26.4690	6.0720	8.0790	65.1600	14.9500	19.8900	81.34	66.7370 ***	7.7640 **	98.50
CL	35.2230	5.3520	13.9060	64.6500	9.8200	25.5300	86.81	50.0560 ***	4.464	98.00
WPA	0.0290	0.0030	0.2230	11.2800	1.3300	87.3900	90.63	161.5900 ***	10.0100 ***	99.38
WPP	0.0000	0.0220	1.3530	0.0000	1.6300	98.3700	0.00	2.254	10.8180 ***	55.63
WPL	0.0000	0.0030	0.1420	0.0000	1.9100	98.0900	0.00	12.2100 ***	12.5300 ***	91.81
WPW	0.0020	0.0000	0.0560	4.0700	0.0000	95.9300	100.00	50.1920 ***	0.355	98.01
WPL/WPW	0.0040	0.0050	0.2920	1.4000	1.7500	96.8500	44.44	28.5700 ***	11.7100 ***	96.50
OSW	0.0551	0.0003	0.0232	19.1800	0.1000	80.7200	99.46	394.0820 ***	2.054	99.75
SA	0.0015	0.0000	0.0036	28.7200	0.3800	70.9000	98.72	673.5760 ***	5.4270 **	99.85
SP	0.0300	0.0010	0.0840	26.2800	0.4700	73.2500	96.77	598.1890 ***	6.3760 **	99.83
SL	0.0026	0.0002	0.0114	18.2100	1.1600	80.6300	94.14	385.5400 ***	12.9100 ***	99.74
SW	0.0024	0.0003	0.0140	14.1900	1.8500	83.9600	88.89	298.1800 ***	19.2700 ***	99.66
SL/SW	0.1140	0.0140	1.2390	8.3300	1.0400	90.6300	89.06	162.1600 ***	10.5100 ***	99.38

Significance levels: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3.4. Variation Characteristics of Phenotypic Characters of Chinese Fir Cones and Seeds

Tables 5 and 6 show that the 14 phenotypic traits of Chinese fir varied extensively among clones and crown different layers. The order of the phenotypic CV of the high seed bearing clone in the lower crown layer (jiang26) was as follows: OSW > WPA > WPL/WPW > WPW > CW > WPP > SA > WPL > SW > SL/SW > SP > SL > CD > CL, and that of the medium seed bearing clone in the lower crown layer (jiang18) was OSW > WPA > WPL/WPW > WPW > SA > WPP > WPL > SW > CW > SL > SP > SL/SW > CL > CD. The phenotypic CV of the no seed bearing in the lower crown layer clone (jiangA13) was ordered as follows: OSW > SA > WPL/WPW > WPA > WPW > SW > CW > SL > SP > WPP > SL/SW > WPL > CL > CD. For all three clones, the phenotypic character with the largest CV was OSW. The OSW of the jiang26 clone varied from 0.0075 g to 0.0095 g, that of the jiang18 clone varied from 0.0052 g to 0.0067 g, and that of the jiangA13 clone varied from 0.0038 g to 0.0040 g. The phenotypic traits with the smallest variation range were CL and CD. Our data show that the phenotypic characteristics of the cones and seeds of all three clones provide abundant resources for the breeding of improved Chinese fir varieties.

The variation of the phenotypic characteristics of cones and seeds also differed among the different crown layers. The phenotypic CV in the upper sunny side was in the order of OSW > WPA > WPL/WPW > WPW > SA > CW > WPP > SW > WPL > SL > SP > SL/SW > CL > CD. For the upper shade side, the order was OSW > SA > WPA > WPL/WPW > WPW > SW > CW > SP > SL > WPP > WPL > SL/SW > CL > CD. The order for the central sunny side was OSW > WPA > WPL/WPW > WPW > SA > CW > SW > WPP > SL > SP > WPL > SL/SW > CL > CD. The phenotypic CV for the middle shade side was as follows: OSW > WPA > WPL/WPW > WPW > SA > SW > WPP > CW > WPL > SP > SL > SL/SW > CL > CD. For the lower sunny side, the order was OSW > WPA > WPL/WPW > WPW > SA > WPP > WPL > SW > CW > SL > SP > SL/SW > CD > CL. The order for the lower shade side was OSW > WPA > WPL/WPW > WPW > SA > WPP > CW > WPL > SW > SL > SP > SL/SW > CL > CD.

In the different crown layers, OSW had the largest CV, and CL and CD had the smallest CVs. In the upper sunny side, the phenotypic CV of OSW was 72.73%–83.00% and that of CD was 7.70%–14.24%. In the upper shade side, the phenotypic CV of OSW was 76.28%–129.51%, and that of CD was 7.19%–15.86%. In the middle sunny side, the phenotypic CV of OSW was 51.53%–95.72%, and that of CD was 11.01%–12.48%. In the middle shade side, the phenotypic CV of OSW was 47.19%–80.78%, and that of CD was 5.33%–7.74%. In the lower sunny side, the phenotypic CV of OSW was 59.49%–84.34%, and that of CL was 7.72%–9.23%. In the lower shade side, the phenotypic CV of OSW was 47.51%–74.09%, and that of cone diameter was 9.27%–10.93%. These results show that there were significant differences in cone and seed traits among different layers of Chinese fir. OSW was the most unstable characteristic, and CD and CL were the most stable.

Table 5. Average values of phenotypic characters of bract scales and seeds from different crown layers of the three Chinese fir clones.

Clone Number	Position	CW (g)	CD (cm)	CL (cm)	WPA (cm^2)	WPP (cm)	WPL (cm)	WPW (cm)	WPL/WPW	OSW (g)	SA (cm ^{2})	SP (cm)	SL (cm)	SD (cm)	SL/SW
jiang26	Upper sunny side	11.79 ± 3.92	29.81 ± 4.25	37.18 ± 5.19	1.19 ± 0.55	4.57 ± 1.13	1.66 ± 0.36	0.63 ± 0.25	3.05 ± 1.28	0.0089 ± 0.0064	0.23 ± 0.06	1.82 ± 0.28	0.65 ± 0.10	0.26 ± 0.05	2.60 ± 0.39
jiang26	Upper shady side	14.28 ± 4.08	30.80 ± 2.33	37.81 ± 3.53	1.15 ± 0.58	4.55 ± 1.25	1.65 ± 0.39	0.62 ± 0.27	3.10 ± 1.27	0.0081 ± 0.0062	0.22 ± 0.06	1.81 ± 0.29	0.64 ± 0.09	0.27 ± 0.07	2.46 ± 0.40
jiang26	Central sunny side	10.98 ± 3.58	30.42 ± 3.56	37.24 ± 4.60	1.22 ± 0.63	4.58 ± 1.22	1.63 ± 0.38	0.66 ± 0.27	2.86 ± 1.23	0.0084 ± 0.0043	0.23 ± 0.06	1.81 ± 0.28	0.64 ± 0.10	0.26 ± 0.05	2.49 ± 0.42
jiang26	Central shady side	10.92 ± 2.43	30.47 ± 5.40	41.05 ± 4.36	1.07 ± 0.53	4.42 ± 1.16	1.60 ± 0.36	0.61 ± 0.26	3.11 ± 1.44	0.0075 ± 0.0035	0.21 ± 0.05	1.78 ± 0.25	0.63 ± 0.09	0.25 ± 0.06	2.56 ± 0.43
jiang26	Lower sunny side	12.44 ± 2.62	32.88 ± 4.06	43.17 ± 3.99	1.15 ± 0.59	4.46 ± 1.21	1.61 ± 0.38	0.62 ± 0.26	3.09 ± 1.61	0.0090 ± 0.0053	0.23 ± 0.06	1.84 ± 0.30	0.65 ± 0.10	0.27 ± 0.06	2.46 ± 0.39
jiang26	Lower shady side	9.77 ± 2.87	27.70 ± 3.03	39.39 ± 2.85	1.02 ± 0.56	4.32 ± 1.27	1.56 ± 0.40	0.60 ± 0.27	3.10 ± 1.40	0.0095 ± 0.0045	0.23 ± 0.06	1.86 ± 0.26	0.66 ± 0.10	0.27 ± 0.05	2.54 ± 0.42
jiang18	Upper sunny side	9.54 ± 2.68	23.89 ± 1.84	35.87 ± 4.93	0.87 ± 0.37	4.27 ± 1.08	1.47 ± 0.35	0.66 ± 0.24	2.46 ± 0.90	0.0067 ± 0.0052	0.19 ± 0.06	1.68 ± 0.28	0.61 ± 0.10	0.23 ± 0.05	2.73 ± 0.38
jiang18	Upper shady side	6.62 ± 1.46	21.99 ± 1.58	29.98 ± 4.07	0.78 ± 0.33	4.04 ± 0.95	1.39 ± 0.31	0.62 ± 0.22	2.48 ± 1.01	0.0056 ± 0.0073	0.16 ± 0.06	1.52 ± 0.33	0.55 ± 0.12	0.21 ± 0.06	2.62 ± 0.47
jiang18	Central sunny side	7.71 ± 1.85	23.82 ± 2.62	33.65 ± 2.53	0.78 ± 0.34	4.09 ± 1.08	1.43 ± 0.34	0.61 ± 0.25	2.69 ± 1.20	0.0052 ± 0.0050	0.16 ± 0.07	1.50 ± 0.37	0.55 ± 0.13	0.21 ± 0.06	2.72 ± 0.49
jiang18	Central shady side	8.41 ± 2.01	23.64 ± 1.26	34.40 ± 4.47	0.84 ± 0.38	4.28 ± 1.09	1.50 ± 0.35	0.64 ± 0.25	2.64 ± 1.02	0.0060 ± 0.0048	0.18 ± 0.06	1.60 ± 0.28	0.58 ± 0.10	0.22 ± 0.05	2.70 ± 0.37
jiang18	Lower sunny side	8.51 ± 1.27	23.05 ± 1.58	34.31 ± 2.65	0.84 ± 0.38	4.18 ± 1.06	1.48 ± 0.36	0.60 ± 0.23	2.75 ± 1.01	0.0059 ± 0.0050	0.17 ± 0.06	1.58 ± 0.29	0.57 ± 0.11	0.22 ± 0.05	2.69 ± 0.40
jiang18	Lower shady side	8.05 ± 1.76	22.05 ± 2.04	30.67 ± 4.95	0.84 ± 0.36	4.45 ± 1.12	1.58 ± 0.37	0.65 ± 0.24	2.74 ± 1.03	0.0059 ± 0.0043	0.18 ± 0.06	1.62 ± 0.31	0.59 ± 0.12	0.22 ± 0.05	2.76 ± 0.38
jiangA13	Upper sunny side	11.04 ± 3.34	26.46 ± 2.48	33.40 ± 4.93	1.14 ± 0.52	4.55 ± 1.14	1.66 ± 0.36	0.62 ± 0.25	3.13 ± 1.47	0.0040 ± 0.0033	0.13 ± 0.06	1.34 ± 0.35	0.48 ± 0.13	0.19 ± 0.06	2.68 ± 0.56
jiangA13	Upper shady side	11.19 ± 4.59	26.19 ± 4.15	33.40 ± 7.15	1.02 ± 0.44	4.30 ± 0.99	1.59 ± 0.20	0.56 ± 0.38	3.22 ± 0.42	0.0041 ± 0.0037	0.16 ± 0.15	1.42 ± 0.54	0.52 ± 0.39	0.19 ± 0.40	2.85 ± 0.26
jiangA13	Central sunny side	11.53 ± 3.55	26.29 ± 3.28	34.06 ± 5.86	1.02 ± 0.42	4.32 ± 0.97	1.58 ± 0.31	0.59 ± 0.22	3.10 ± 1.41	0.0038 ± 0.0034	0.12 ± 0.06	1.28 ± 0.35	0.46 ± 0.13	0.18 ± 0.06	2.58 ± 0.50
jiangA13	Central shady side	12.60 ± 3.23	27.52 ± 2.18	36.32 ± 7.10	1.12 ± 0.43	4.56 ± 0.93	1.66 ± 0.31	0.62 ± 0.21	3.02 ± 1.33	0.0040 ± 0.0032	0.13 ± 0.06	1.37 ± 0.34	0.49 ± 0.12	0.19 ± 0.06	2.56 ± 0.40

Table 6. Variation coefficients of phenotypic characters of bract scales and seeds from different crown layers of the three Chinese fir clones.

Clone Number	Position	CW (g) (%)	CD (cm) (%)	CL (cm) (%)	WPA (cm^2) (%)	WPP (cm) (%)	WPL (cm) (%)	WPW (cm) (%)	WPL/WPW (%)	OSW (g) (%)	SA (cm^2) (%)	SP (cm) (%)	SL (cm) (%)	SD (cm) (%)	SL/SW (%)
jiang26	Upper sunny side	33.23	14.24	13.96	46.77	24.70	21.72	39.67	42.07	72.73	25.83	15.12	14.92	21.13	14.97
jiang26	Upper shady side	28.58	7.56	9.34	50.01	27.45	23.72	43.96	40.92	76.28	25.18	15.80	14.50	26.15	16.12
jiang26	Central sunny side	32.60	11.69	12.34	51.45	26.67	23.28	41.28	43.10	51.53	27.41	15.46	15.38	20.10	16.67
jiang26	Central shady side	22.28	17.74	10.62	49.74	26.19	22.54	43.23	46.38	47.19	23.94	13.97	13.58	21.97	16.66
jiang26	Lower sunny side	21.11	12.34	9.24	51.02	27.16	23.83	41.95	52.04	59.49	27.73	16.15	15.80	22.04	15.90
jiang26	Lower shady side	29.43	10.93	7.24	54.74	29.37	25.44	45.26	45.38	47.51	24.39	13.96	14.81	17.02	16.46
jiang18	Upper sunny side	28.08	7.70	13.75	42.76	25.41	23.96	36.53	36.38	78.10	30.25	16.73	17.07	22.03	14.12
jiang18	Upper shady side	22.14	7.19	13.59	42.63	23.58	22.16	35.77	40.49	129.51	37.80	21.60	21.75	27.49	17.92
jiang18	Central sunny side	23.96	11.01	7.51	44.24	26.39	23.86	41.19	44.50	95.72	42.21	24.48	24.61	27.23	17.89
jiang18	Central shady side	23.91	5.33	12.98	44.74	25.43	23.18	38.94	38.59	80.78	32.62	17.79	17.99	21.12	13.84
jiang18	Lower sunny side	14.98	6.86	7.72	44.82	25.43	23.99	38.00	36.92	84.34	33.14	18.11	18.55	21.19	15.06
jiang18	Lower shady side	21.90	9.27	16.16	42.53	25.27	23.45	37.35	37.66	74.09	34.57	19.23	19.71	21.05	13.75
jiangA13	Upper sunny side	30.28	9.36	14.77	45.52	25.08	21.58	40.64	46.81	83.00	46.50	25.86	25.95	31.33	21.04
jiangA13	Upper shady side	41.04	15.86	21.40	43.58	22.98	20.43	37.80	42.18	90.27	92.47	38.14	38.85	39.77	25.55
jiangA13	Central sunny side	30.81	12.48	17.20	41.44	22.53	19.91	37.08	45.34	90.07	49.57	27.56	27.88	31.45	19.21
jiangA13	Central shady side	25.60	7.91	19.56	38.38	20.40	18.51	33.76	44.06	79.58	43.92	24.68	24.50	29.04	15.71

3.5. Correlation between Phenotypic Characters of Chinese Fir Cones and Seeds

Figure 2 shows that there were different degrees of correlation between the phenotypic characters of Chinese fir cones and seeds. OSW was positively correlated with SL ($p < 0.01$, r = 0.92), SA ($p < 0.01$, r = 0.95); and SP ($p < 0.01$, r = 0.92). SL was positively correlated with SA ($p < 0.01$, r = 0.97) and SP ($p < 0.01$, r = 0.99) and negatively correlated with SL/SW ($p < 0.01$, r = –0.95). SA was positively correlated with SP ($p < 0.01$, r = 0.98) and negatively correlated with SL/SW ($p < 0.01$, r = -0.89). SP was negatively correlated with SL/SW ($p < 0.01$, r = –0.93). WPL was positively correlated with WPA ($p < 0.01$, r = 0.80) and WPP ($p < 0.01$, r = 0.92) and negatively correlated with WPL/WPW ($p < 0.01$, r = - 0.96). WPA was positively correlated with WPP ($p < 0.01$, r = 0.94). The correlations between WPA and SP ($p < 0.01$, r = 0.71), SA ($p < 0.01$, r = 0.66), and SL ($p < 0.01$, r = 0.69) were low, as was the correlation between the WPP and SA ($p < 0.01$, r = 0.61). The correlations between CD and CW ($p < 0.01$, r = 0.68) and between CW and CL ($p < 0.05$, r = 0.60) were also low. Overall, the correlation of cone phenotype, bract scale phenotype, and seed phenotype was low. WPP was positively correlated WPA and WPW, and SW was positively correlated with SA, SP, and SW but not with SL.

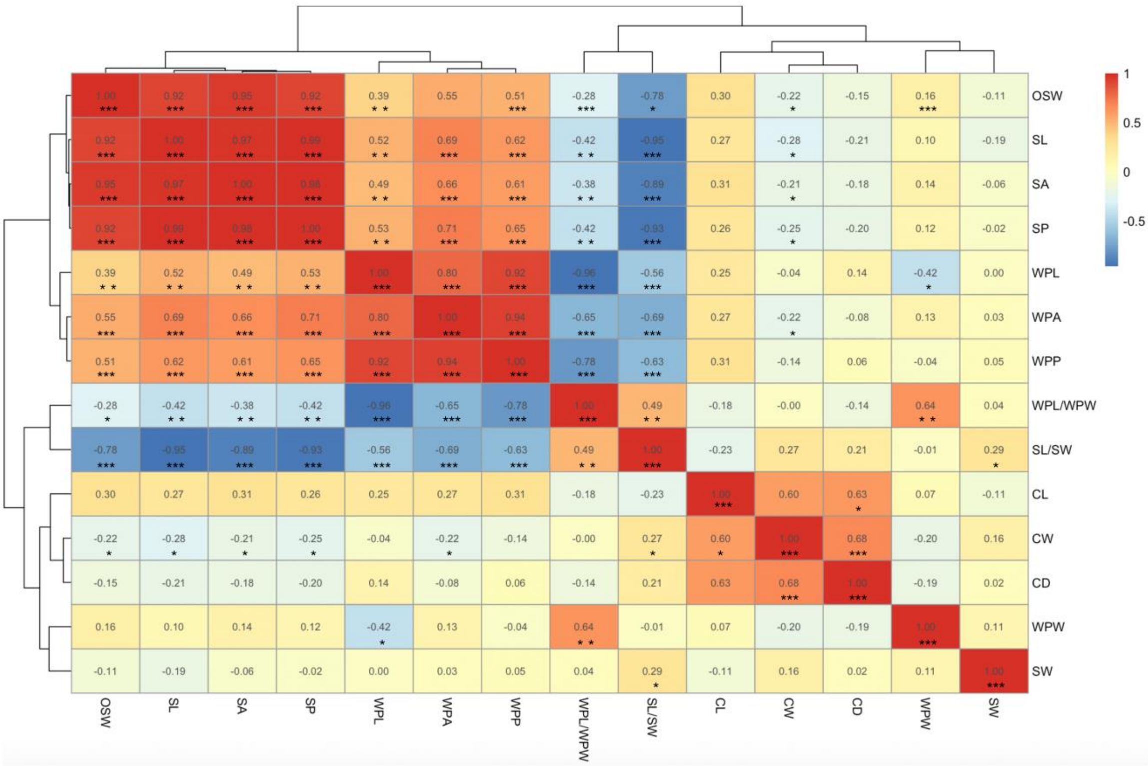

Figure 2. Results of correlation analysis and significant difference tests between Chinese fir cone and seed phenotypic characteristics.

4. Discussion

4.1. Effect of Different Clones and Layers on Seed Types

We found that the parent tree of Chinese fir with different fruit types in the Chinese fir seed orchard affected the total number of seeds produced by cones, the rate of good seeds, and the rate of seeds filled with tannin-like substance, but it did not affect the formation of empty seeds. In the jiang26 clone, the total number of seeds produced by cones decreased significantly with decreasing height of the crown layer; that is, the total number of seeds produced by cones in the upper layer of the crown was significantly higher than that in the lower layer of the crown. However, no significant difference in the total number of seeds produced by cones among the different crown layers was detected in the mother plants of the jiang18 and jiangA13 clones. This result indicates that for Chinese fir clones, the effect of seed setting (i.e., crown layer) is mainly due to the amount of fruit set by the mother plant, which depends on the nutrient supply. Yu et al. reported similar results for Chinese fir [31]. Additionally, Peter et al. reported that when the number of cone seeds of Spreading pine (*Pinus patula* Schltdl. et Cham.) in the upper part of the crown was large, resulting in nutrient deficiency, the number of seeds in the lower cones was small [32]. The percentage of seeds filled with tannin-like substance was significantly high for clone jiang26, and the percentage of empty seeds was significantly high level for clones jiang18 and jiangA13. The genetic difference among clones is an important factor that affects these two values. This scenario was also observed in Japanese larch (*Larix kaempferi* Lamb. Carr) [33]. In another study of three clones with different seed setting ability in the lower crown layer, Weng et al. showed that a higher number of clones resulted in a higher number of seeds filled with tannin-like substance [34]. During the development of Chinese fir seeds, the competition for nutrients among various seeds in the cones is extremely fierce. Gao et al. found that the proportion of seeds filled with tannin-like substance among total seeds collected from fruiting branches with five cones was >70%, whereas the value was only 40% for fruiting branches with only one cone [35].

The effect of crown layer on rate of seeds filled with tannin-like substance and rate of empty seed is different. In our Chinese fir seed orchard, we detected a high rate of good seeds and a low rate of seeds filled with tannin-like substance on the sunny side of the upper crown and a low rate of good seeds and a high rate of seeds filled with tannin-like substance on the shady side of the lower crown. In clone jiang26, the good seed rates on the upper sunny side and lower shady side were 44.22% and 12.56%, respectively, and the rates of seeds filled with tannin-like substance in these settings were 34.19% and 65.88%, respectively. In clone jiang18, the good seed rates on the upper sunny side and lower shady side were 22.12% and 11.10%, and the rates of seeds filled with tannin-like substance were 24.77% and 34.88%, respectively. Yu et al. [36] and Guan et al. [37] reported similar findings. The sunny side of the upper layer of the Chinese fir crown has good light environment conditions, strong photosynthesis, and rich nutrition, which are conducive to the normal development of Chinese fir embryos. In contrast, the shady side of the lower layer of the crown has weak light and poor nutrition conditions, resulting in abnormal development of embryos and endosperm. Aborted seeds are filled with tannins, resulting in the formation of a large number of seeds filled with tannin-like substance [38]. Our results show that the formation of empty seeds has little relationship with the crown layer of the mother tree of Chinese fir, which is consistent with results reported by Ye et al. [39]. This phenomenon is related to the widespread use of artificial pollination in Yangkou Forest. Artificial pollination can effectively eliminate the difference of pollination in different crown layers and different orientations. Determining how to eliminate the self-incompatibility caused by the affinity between artificial pollination and pollinated mother plants requires further research.

4.2. Effect of Different Clones and Different Layers on Seed Quality

The seed germination rate reflects the quality of seed and is also related to the early growth of seedlings [40]. Some researchers believe that excellent seed sources can be selected using the germination ability of seeds as a reference [41]. In our study, the germination rate of Chinese fir seeds was 32.28%. The germination rate of seeds from different parts of different clones also differed significantly, the high seed bearing clone in the lower crown layer have higher germination rate in the upper sun side, and the germination rate of seeds from the sunny side was higher than that of the shady side. The amount of sunlight on the sunny side is greater than that on the shady side, and therefore photosynthesis is stronger, and the endosperm has more nutrients, both of which are conducive to seed germination. We found that the seed germination rate of different Chinese fir clones was significantly related to seed size, with heavy seeds having a higher germination rate. This finding was similar to that reported by Bu et al. [42] but inconsistent with the research results of Chen et al. [43]. Therefore, to evaluate the quality of Chinese fir seeds in the future, we should not only pay attention to the seed germination rate, but also the seed size and weight. These biological characteristics will affect the quality of seedlings and the composition of seed orchard population [44,45].

4.3. Phenotypic Variation of Cones and Seeds

The diversity of phenotypic is generated by the combined action of genetic information and internal and external factors. It is an important representation of the genetic information of a population of organisms [46], and it is also the product of long-term adaptation and evolution [47]. In this study, we evaluated the cones and seeds of three Chinese fir clones that differed in the seed-bearing characteristics of cones in the lower part of the crown. To understand the level of genetic diversity of the population, we used nested variance to analyze the variation of phenotypic traits [48], including the variation of phenotypic traits between and within clones, the phenotypic differentiation coefficient, and repeatability. Most of the 14 phenotypic traits of the cones and seeds studied differed significantly among and within clones, indicating abundant genetic variation of Chinese fir cones. This result laid a foundation for the selection of excellent clones for use in seed orchards. The variation between clones was greater than that within clones, which indicates that Chinese fir has wide adaptability to the environment [49]. Similar results were reported for Phoebe bournei (*Phoebe bournei* (Hemsl.) Yang) [50], Korean pine(*Pinus koraiensis* Sieb. et Zucc.) [51], and Pinus yunnanensis (*Pinus yunnanensis* Franch) [52]. Therefore, inter-clonal variation is not only affected by genetic diversity, but also affected by long-term restricted gene exchange and changes in geographical environmental factors. Its phenotypic differentiation is adaptive evolution in long-term selection [53].

In our study, the average $v_{(st)}$ of the three Chinese fir clones was 81.15%, which is higher than that of Picea crassifolia(*Picea crassifolia* Kom.) by 74.20% [25], and it is higher than that of Sawtooth oak(*Quercus acutissima* Carruth.) by 78.84% [54], as well as lower than that of Sinocalycanthus chinensis(*Calycanthus chinensis* Cheng et S. Y. Chang) by 89.30% [55]. Our results show that the phenotypic differentiation of cones and seeds of the three Chinese fir clones was in the middle of the range and that the differentiation among clones was higher than that within clones. We also found that the repeatability of 14 phenotypic traits was >89.00%, which is similar to that of Korean pine(*Pinus koraiensis* Sieb. Et Zucc.) [56]. They are also significantly higher than 77.67% of the phenotypic traits of Picea crassifolia [57] and 61.00% of Pinus radiata(*Pinus radiata* D. Don) [58]. Therefore, our results indicate that the yield and quality of Chinese fir cones and seeds are stable and that these traits can be stably inherited by their offspring and are less affected by the environment [59].

Phenotypic variation is the most direct manifestation of biological genetic variation, and therefore it is a major focus of phenotypic diversity research [60]. Among the 14 phenotypic traits of Chinese fir that we studied, the CV of OSW was the largest (77.51%), indicating that the number of seed types of different clones differs significantly. The CVs of CL and CD were the smallest, indicating that the cone phenotype was more stable. The CV of SL was small, which is basically consistent with reports of Conjugated oak(*Quercus franchetii* (Skan)) and Quercus mongolica(*Quercus mongolica* Fisch. ex Ledeb) [61,62]. Therefore, the plasticity of different indicators of phenotypic traits among the three Chinese fir clones and different layers of each clone in this study has great differences, and the plasticity of a class of traits (such as cone size, etc.) with strong correlation with fitness is generally low because it is less affected by the environment and relatively stable. The plasticity of a type of character (such as the weight of a single seed) with weak correlation with fitness is generally higher because it is greatly affected by the environment and changes greatly [63]. Our correlation analysis showed that the WPP was highly significantly positively correlated with WPA and WPWE, and OSW was highly significantly positively correlated with SA, SP, and SW, but not with SL. Similar findings were reported for Chinese fir [64–66], European red pine (*Pinus sylvestris* L.) [67], and European spruce (*Picea abies* (L.) Karst.) [68]. Therefore, SW can be used as an index to predict other seed traits in production.

5. Conclusions

In summary, we found that the clones with different seed-bearing characteristics in the lower crown layer had a great impact on the yield and quality of the Chinese fir seed orchard. When establishing a dwarfing Chinese fir seed orchard, the cone situation of different crown layers of different clones must be considered. Clones with more cones in the lower layer should be selected, and indicators such as seed yield, number of good seeds, number of seeds filled with tannin-like substance, and empty seeds as well as high seed weight and seed width should be used in the selection process. When building seed orchards, a good ecological environment with suitable sunshine and fertile soil is needed. Managers should pay attention to the development of each clone, regulate its vegetative and reproductive growth, and conduct artificial pollination to reduce the formation of empty seeds. If necessary, tree thinning, fruit thinning, fertilizer application, hormone application, and other measures should be taken to ensure that cones and seeds have sufficient nutritional conditions, which will improve the yield and quality of seeds.

Author Contributions: J.Z. conceived the project. Y.D. and D.Y. conducted on-site investigation and data collection. Y.D. and T.Y. conducted the statistical analysis. Y.D. wrote the first draft. J.Z.

contributed to improving the quality of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no competing interests.

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