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Fertility variability among clones and genetic diversity of seed crops at seed orchards of *Chamaecyparis obtusa* in South Korea*

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Abstract *Chamaecyparis obtusa* (Siebold & Zucc.) Endl., commonly known as Japanese cypress or hinoki cypress, is widely cultivated for its high-quality timber and ornamental use. Despite its economic and ecological importance, the species faces challenges in adapting to various climates, necessitating the distribution of high-quality seeds and seedlings. This study aims to enhance the genetic improvement and stability of seed production by analyzing fertility characteristics and genetic traits from two clonal seed orchards of *C. obtusa* in two locations (Gochang and Seogwipo), South Korea. We examined patterns of strobilus and cone production from 2020 to 2022, assessed effective parent numbers, genetic diversity, and heritability estimates, and analyzed parental contribution for the reproductive traits. Results indicated significant annual variation in strobilus and cone production among clones, with higher male fertility variance than female parents. The effective parent number and genetic diversity were generally maintained sustainably, though Seogwipo showed slightly lower diversity. The study underscores the need for management practices to balance clonal contributions, and sustain long-term productivity and genetic diversity in the seed orchards of *C. obtusa*. Future research should focus on understanding environmental influences on clonal contributions and refining selection criteria for superior clones in the advanced generation breeding program.

Keywords: Japanese cypress, gene diversity, fertility variation, genetic parameter, effective parent number, cone production.

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Introduction

Forestry, unlike typical crops, faces challenges due to its long growth period, large volume, and difficulty in indoor management (Isik & McKeand 2019, Orji et al. 2022). It is highly sensitive to local environmental changes, characterized by significant genetic heterogeneity and vulnerability to local disturbances (Bottero et al. 2024, Zhang et al. 2024). Therefore, forestry breeding requires the gradual selection of superior trees and a long-term approach to ensure genetic diversity by using high-quality seeds for improvement. One method for stepwise improvement through selection is the composition technique of seed orchards (Namkoong et al. 1988, Funda et al. 2009, Grattapaglia et al. 2018).

Seed orchards aim to provide genetically improved seeds through genetic interventions, silvicultural adjustments, and effective population size evaluation (Fries 1994; Kang et al. 2023). To maximize improvement effects and maintain stable genetic diversity, conditions such as prevention of external pollen inflow, uniform flowering across all clones, synchronized flowering times, complete random mating, and minimal inbreeding need to be met. However, achieving these conditions is challenging in reality (Ei-Kassaby & Ritland 1992, Nikkanen & Ruotsalainen 2000, Lenz et al. 2020, Wang et al. 2023).

Gene flow in tree populations involves both pollen and seed movement. Changes in gene frequency within a population are influenced by factors such as population size, individual fertility differences, gene migration, mutation, and mating systems (Burczyk & Chybicki 2004, Burczyk et al. 2004a, b). Flowering characteristics in seed orchards contribute to variations in effective population size, influencing opportunities for uniform-quality seeds and random mating (Kang 2001). Often, the uneven contribution of clones in seed production leads to reduced genetic diversity, especially in small-scale groups, increasing the risk of inbreeding and self-pollination, which can reduce seed soundness and germination rates (Nakashizuka 2009, Wang et al. 2023). Effective population size is typically assessed at levels between 20 to 30 (Kang et al. 2023).

Maintaining genetic diversity through random mating is a crucial indicator for the quality of seeds within seed orchards and ensuring adaptability in afforestation regions. However, limited gene flow can shape spatial genetic structures within a population, leading to increased inbreeding and reduced genetic diversity due to decreased allelic variation (Yang et al. 2017, Chaloupková & Lstibůrek 2022). Estimating effective population size in relation to the variation in flowering and seed production, determining pollen movement, and mating patterns within seed orchards is crucial. This information helps preserve genetic diversity and assess the outcomes of long-term forestry breeding (Namkoong et al. 1988, Grattapaglia et al. 2018).

Chamaecyparis obtusa (Siebold & Zucc.) Endl., also known as Japanese cypress or hinoki cypress, is extensively cultivated for its high-quality timber and ornamental use in parks and gardens globally (Maruyama et al. 2005, Havens 2020, Górski et al. 2024). The species is valued for its diverse functional substances, making it an attractive choice for afforestation (Kang et al. 2021, Eltayeb et al. 2023, Jang et al. 2023). However, the need to adapt to various climates emphasizes the urgency of distributing high-quality seeds and seedlings. The creation of breeding populations aims to enhance patterns of gene flow and ensure stable seed production for widespread application (Lenz et al. 2020, Gamba & Muchhala 2020, Cortés et al. 2020).

C. obtusa has been adopted as a major afforestation species in South Korea since the 1960s. The seed orchard of *C. obtusa* was established for mass seed production in 1969 (Hong 1969, Lee et al. 2006, Park et al. 2020). The forestry breeding goals in South Korea have shifted from recovering deforested areas in the 1990s to focusing on enhancing traits related to current growth and development (Kim & Zsuffa 1994, Shin et al. 2007). Recently, there has been an increased interest in forest resources considering their economic value in timber production as well as diverse ecological services like forest recreation and healing (Jo et al. 2020, Park et al. 2021, Park 2022). Afforestation species are chosen regardless of the region to establish a variety of functional forests. However, these species face challenges in adapting to diverse climates, necessitating urgent distribution of high-quality seeds and seedlings. Therefore, research on flowering determination and the genetic improvement effects of seeds derived from seed orchards is crucial for ensuring a stable supply of superior seeds.

To meet afforestation needs and address rising timber demands, systematically assessing genetic diversity in existing breeding populations is imperative for establishing the next generation of seed orchards. In South Korea, research on the growth and adaptability of *C. obtusa* in progeny tests has been conducted (Lee et al. 1986, Choi et al. 2012). While some studies address fertility at the seed orchard of *C. obtusa* in Korea (Kang & Mullin 2007), there is a notable gap in research for newly established seed orchards of *C. obtusa* after the year 2010. A comprehensive evaluation of genetic diversity and parameters remains essential for enhancing seed production adaptability and establishing a robust research base for future generations in seed orchards (El-Kassaby 1995, Funda & El-Kassaby 2012, Li et al. 2021, Pakull et al. 2021).

This study aims to estimate the contribution proportion of clones by analyzing the variations in strobilus and cone production, and to predict genetic control of the traits at the two clonal seed orchards of *C. obtusa*. The specific objectives include analyzing variation among clones in (female and male) strobili and cone production, estimating the effective parent numbers, determining the genetic variance and heritability of the traits, and evaluating genetic diversity of seed crops resulted from the seed orchards.

Materials and Methods

Seed orchard and strobilus assessment

The seed orchard of *C. obtusa* in Gochang is located in the south coast (N35°19’22”, E126°33’28”) of South Korea. The seed orchard was established by cuttings with 61 clones (a total of 1,095 ramets) in 2015. All ramets were planted at 7 ha with a 7 m × 7 m spacing randomly to enhance unrelated mating and genetic diversity.

The seed orchard of *C. obtusa* in Seogwipo is located in the Jeju Island of South Korea (N33°18’37”, E126°33’38”). The seed orchard was established by grafts with 26 clones in 1969, a total of 480 trees were planted with 4 ha. The planting followed a random arrangement method with a 5 m × 5 m spacing. The two seed orchards are geographically isolated from other stands of *C. obtusa* therefore, the likelihood of pollen contamination from external sources is considered negligible.

At the time of data collection, the total number of ramets in Gochang was 945, ranging from 1 to 68 per clone, and the average number of ramets per clone was 15.5. In the case of ramets in Seogwipo was ranged from 1 to 71 per clone, and the average number of ramets was 9.1.

Estimation of fertility and cone production variation

The productions of female and male strobili, and mature cones were surveyed at the seed orchards of *C. obtusa* in Gochang and Seogwipo, South Korea during the years 2020 to 2022. The data for each clone were log-transformed [LN(x+1)], and both overall mean and standard deviation were calculated. Furthermore, the coefficient of variation (CV) was determined to illustrate the relative variability in cone production among clones. The number of strobili were estimated by multiplying the number of strobili on a branch representing the overall strobilus density in a single tree by the total number of branches bearing female and male strobili in early May. In September, the mature cones on a single branch were counted. This count was then multiplied by the total number of branches bearing cones to estimate the total number of cones on the entire tree. The total number of cones per tree was then determined by multiplying the count by the number of branches with cones (Burczyk & Chalupka 1997, Sharma & Khanduri 2012).

Fertility is defined as the relative ability (probability) to produce seeds in the seed orchard, rather than genetically confirmed gametic contribution. We acknowledge this as a phenotypic proxy for fecundity (e.g., number of strobili and cones), not a direct measure of fertility. Fertility variation (Ψ, called a sibling coefficient) among clones in the seed orchard was calculated as

$$\Psi = CV^2 + 1 \tag{1}$$

where CV is the coefficient of variation in female and male strobilus, or cone production among clones. Specially, the CV of cone production represents clonal fertility (Kang 2001).

One-way analysis of variance (ANOVA) was performed using SAS JMP Pro Software (ver. 16.2) with a significance level (p) at 0.05. For the relationships between fertility variation and cone production, Pearson's and Spearman's correlation coefficients among annual female and male strobilus production were performed in the surveyed three years. Additionally, Tukey-Honestly Significant Difference post-hoc tests were implemented to investigate significant differences identified through the analyses.

Effective parent number and gene diversity

Effective parent number (N_p) is defined as the number of genotypes divided by the fertility variation (Ψ)), and relative effective parent number (N_r) is the proportion of the effective number against census number (N) (Lindgren & Mullin 1998, Kang & El-Kassaby 2002) as below,

$$N_p = N/\Psi \text{ and } N_r = 1/\Psi \tag{2}$$

where N is the number of clones in the seed orchard (Roberds et al., 1991). The effective parent number can be divided into female and male gametic parents.

Gene diversity (GD) of seed crops was estimated for parental (clone) level. Although genetic diversity (GD) is a function of the effective parent number (Np), we included GD as it expresses the proportional loss of diversity from the parental to offspring generation in a more interpretable form.

$$GD = 1 - 0.5/N_p \tag{3}$$

Parental balance curve

Parental balance was assessed through a curve depicting the cumulative contribution of gamete production from each individual. The contribution of each clone was evaluated using the parental balance curve within the seed orchard (El-Kassaby & Cook 1994). The number of female and male strobili were ordered by clones from high to low production, and then the percentages of cumulative contribution were plotted against the proportion of the clones (Reynolds & El-Kassaby 1990, El-Kassaby & Askew 1991, Chaisurisri & El-Kassaby 1993, El-Kassaby & Cook 1994).

Heritability

The meaning of heritability adjusts the bias of clones. Variance components were calculated by one-way analysis of variance (ANOVA) to estimate the variance of female and male strobilus production. Heritability (h^2) was then estimated based on these variance components to determine the genetic contribution to trait variation (Kang et al. 2023).

$$h^2 = \frac{\sigma_c^2}{\sigma_P^2} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2/k} \tag{4}$$

where σ^2_P, σ^2_c, and σ^2_e are the variances of total (phenotypic), clone (additive), and error (environmental), and k is the coefficient of variance component, respectively.

Results

Clonal variation of strobilus and cone production

The log-transformed mean production values of female strobili at the seed orchard of Gochang were 3.14, 5.37, and 5.31 in the years 2020, 2021, and 2022, respectively. The male strobili during the same period showed values of 3.87, 5.88, and 6.87. The mature cone production among clones increased steadily from 2.47 in 2020 to 5.28 in 2022, indicating a continuous upward trend in strobilus and cone production in Gochang, Korea (Figure 1A). The coefficient of variation (CV) for male strobilus production among clones was higher than that for female strobilus production. Notably, there was a decreasing trend in the variability of strobilus and cone production across the years (Figure 1A).

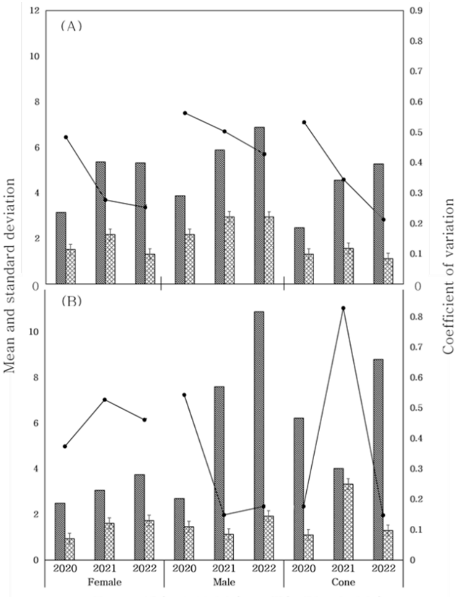

Figure 1 Production of female and male strobilus, and mature cone at the seed orchard of *C. obtusa* in Gochang (A) and Seogwipo (B), Korea during 2020~2022.

The production of female strobili at the seed orchard of Seogwipo was 2.49, 3.05, and 3.74 in the years 2020, 2021, and 2022, respectively. The average mature cone production was 6.22, 4.00, and 8.79 in the years 2020, 2021, and 2022, respectively. The male strobili during the same period showed values of 2.69, 7.59, and 10.88 (Figure 1B). The coefficient of variation of male strobilus production among clones exhibited an opposite trend to that of female strobilus production. Specifically, the coefficient of variation of male strobili increased while the coefficient of variation of female strobili decreased over the same period (Figure 1B).

Effective parent number and gene diversity of seed crops

The sibling coefficient (Ψ), the effective parent number (*Np*), the relative effective parent number (*Nr*), and the gene diversity (*GD*) for female and male strobili, and cone production were calculated at the seed orchard of *C. obtusa* in Gochang and Seogwipo, Korea from 2020 to 2022 (Tables 1 & 2). The sibling coefficient is 1 when all clones have the same fertility. However, the sibling coefficient varied and exceeded 1 across the years and between male and female strobili, and cones.

In general, the sibling coefficients (Ψ) were higher for male strobili compared to female strobili and cones, indicating greater fertility variance among males in Gochang, Korea (Table 1). The effective parent number ranged from 23.64 (2021) to 37.38 (2022) and from 15.52 (2021) to 19.84 (2022) at gamete level, respectively. The effective parent number at the zygote (which is considered as cone production) level ranged from 20.15 (2022) to 35.94 (2021). Similarly, the sibling coefficient was higher for male strobili, indicating a greater fertility skew at the seed orchard of *C. obtusa* in Seogwipo, Korea (Table 2). Among the 23 clones at the seed orchard of Seogwipo, the effective parent numbers of female and male strobilus ranged from 6.4 (2021) to 15.45 (2022) and from 4.32 (2021) to 9.58 (2022) at gamete level, respectively. The effective parent numbers at zygote (clone) level ranged from 7.3 (2020) to 10.71 (2021). The effective parent number was generally lower than those in Gochang.

However, the relative parent number (N_r) for both seed orchards in Gochang and Seogwipo are generally comparable (Tables 1 & 2). In 2022, the relative parent number for female, male strobili, and cone production in Gochang were 61.28%, 32.52%, and 33.04%, respectively. Similarly, in Seogwipo, the relative parent number for female, male strobili, and cone production were 67.16%, 41.66%, and 45.94%, respectively. This indicates that the relative effective parent numbers in both seed orchards are within a similar range.

The genetic diversity was consistently above 0.97 over the three years, with a loss of gene diversity estimated at approximately 3%. Genetic diversity remained relatively high across all years and fertility traits, indicating maintained genetic diversity at the seed orchard of Gochang, Korea (Table 1). In Seogwipo, genetic diversity was slightly lower than that in Gochang, and a loss of gene diversity was estimated at approximately 11.6% in genetic diversity at the male fertility level compared to the entire population (Table 2).

Parental balance curves

The parental balance curves for female strobili, male strobili, and mature cone production among clones in Gochang seed orchard are present for consecutive three years in Figure 2. If the contributions from all clones were equal, the data points would form a straight line. However, the actual curves deviate from the straight line. Among the 61 clones at the seed orchard of Gochang, 12 to 15 clones (20 to 25% of the total) and 6 to 9 clones (10 to 15% of the total) produced 50% female and male strobili, respectively. These results indicated that some clones contribute more significantly than others. Comparatively, the accumulative contribution line for strobilus production in 2022 was closer to equal contribution than those in poor years (2020 & 2021).

Figure 3 shows similar data for the seed orchard in Seogwipo over the same period. The accumulated contribution curves for female strobili, male strobili, and mature cone production again demonstrate that certain clones have a more pronounced contribution. Among the 23 clones at the seed orchard of Seogwipo, the best five parents (5 to 21% of the total) and the best three parents (5 to 15% of the

Table 1 Sibling coefficient (Ψ) effective parent number (N_p), relative effective parent number (N_r), and genetic diversity (*GD*) for female and male strobili, and cone production at the clonal seed orchard of *C. obtusa* in Gochang, South Korea.

Parameter	2020 (*N** = 61)			2021 (*N* = 61)			2022 (*N* = 61)		
	female	male	cone	female	male	cone	female	male	cone
Ψ	1.935	3.398	2.299	2.580	3.931	1.697	1.632	3.075	3.027
N_p	31.52	17.95	26.54	23.64	15.52	35.94	37.38	19.84	20.15
N_r (%)	51.68	29.43	43.50	38.76	25.44	58.92	61.28	32.52	33.04
GD	0.984	0.972	0.981	0.979	0.968	0.986	0.987	0.975	0.975

Note: **N*: census number

Table 2 Sibling coefficient (Ψ) effective parent number (N_p), relative effective parent number (N_r), and genetic diversity (*GD*) for female and male strobili, and cone production at the seed orchard of *C. obtusa* in Seogwipo, South Korea.

Parameter	2020 (*N** = 23)			2021 (*N* = 23)			2022 (*N* =23)		
	female	male	cone	female	male	cone	female	male	cone
Ψ	2.303	3.748	3.152	3.591	5.325	2.148	1.489	2.401	2.177
N_p	9.989	6.136	7.297	6.404	4.319	10.71	15.45	9.581	10.57
N_r (%)	43.43	26.68	31.72	27.85	18.78	46.54	67.16	41.66	45.94
GD	0.950	0.919	0.931	0.922	0.884	0.953	0.968	0.948	0.953

Note: **N*: census number

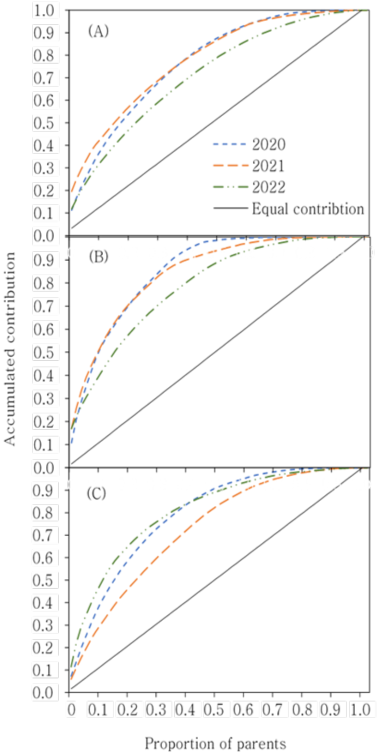

Figure 2 Parental balance curves for accumulated contribution of female (A) and male strobilus (B), and mature cone production (C) to the proportion of parental clones at the seed orchard of *C. obtusa* in Gochang, Korea during 2020~2022. Straight line represents equal contribution among the clones.

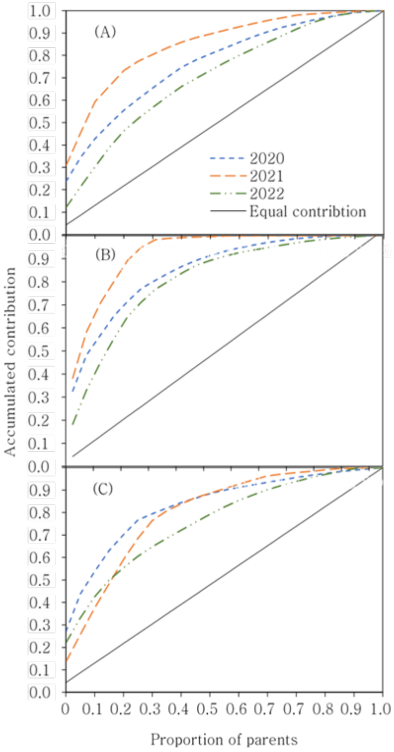

Figure 3 Parental balance curves for accumulated contribution of female (A) and male strobilus (B), and mature cone production (C) to the proportion of parental clones at the seed orchard of *C. obtusa* in Seogwipo, Korea during 2020~2022. Straight line represents equal contribution among the clones.

total parents) produced 50% of female and male strobili, respectively, among three years.

Both seed orchards exhibit a pronounced imbalance in clonal contributions, with certain clones contributing significantly more to cone production. This suggests that the genetic pool within the seed orchard is dominated by a few clones.

Estimation of heritability and correlation

The estimation of variance components and narrow-sense heritability (h^2) for female and male strobilus production, and cone production were estimated at the seed orchards in Gochang and Seogwipo, respectively (Table 3). The ANOVA showed all significant ($p < 0.001$) among clones in female and male strobili production over three years.

Table 3 Analysis of variation and heritability (h^2) estimates for the strobilus and cone production at the seed orchard of *C. obtusa* in Gochang, South Korea.

Source	df	Female strobili			Male strobili			Mature cone		
		2020	2021	2022	2020	2021	2022	2020	2021	2022
Among clones	60	22.64	20.89	17.22	55.85	77.92	77.89	19.76	21.00	9.321
Within clones	884	5.823	2.767	4.646	14.18	9.613	13.43	5.909	4.054	7.904
F-value		3.888^{***}	7.549^{***}	3.707^{***}	3.940^{***}	8.105^{***}	5.814^{***}	3.343^{***}	5.179^{***}	1.178^{ns}
$h^2_{individual}$		0.159	0.309	0.150	0.161	0.326	0.239	0.133	0.221	0.013
h^2_{clone}		0.743	0.868	0.730	0.746	0.877	0.828	0.701	0.807	0.152

ns, not significant; ***, $p < 0.001$

The individual heritabilities of female and male strobili, and cone production ranged from 0.150 (2022) to 0.309 (2021), from 0.161 (2020) to 0.326 (2021), from 0.013 (2022) to 0.133 (2020), respectively in Gochang, Korea (Table 3).

At the seed orchard of Seogwipo, the narrow-sense heritabilities of female and male strobili, and cone production were estimated from 0.086 (2022) to 0.297 (2021), from 0.077 (2021) to 0.489 (2020), from 0.156 (2022) to 0.408 (2021), respectively (Table 4). The narrow-sense heritability of female strobili was lower than that of male strobili for all years, implying that environmental factors affect female strobili compared to male strobili production.

Pearson's correlation coefficients between female and male strobilus production were statistically significant in both regions (Table 5). The correlation coefficients between female, male strobili, and cone production were statistically significant for 2020, 2021, and 2022 ($p < 0.001$). Similar significant correlations were observed in Seogwipo. The strobilus and cone production were positively correlated and statistically significant, except for the male strobilus and cone production at the seed orchard of Seogwipo in 2020 (Table 5). These results indicate that the female strobilus production paralleled the male strobilus production. These coefficients show the strength and direction of the relationship between female strobili, male strobili, and cone production across different years.

Spearman's rank correlation coefficients among years for female, male strobili, and cone production were mostly positively correlated and significant at the seed orchards of Gochang and Seogwipo, Korea (Table 6). The correlation coefficients between the years were mostly significant and positive only except between cone production in 2021 and 2022 at the seed orchard of Gochang. The Spearman's rank correlation coefficients

Table 5 Pearson's (above the diagonal) and Spearman's (below the diagonal) correlation coefficients among female and male strobili, and cone production at the seed orchards of *C. obtusa* in Gochang and Seogwipo, South Korea.

Location/ Year	Variable	Female	Male	Cone
Gochang				
2020	Female	-	0.406^{***}	0.763^{***}
	Male	0.412^{***}	-	0.383^{***}
	Cone	0.789^{***}	0.389^{***}	-
2021	Female	-	0.484^{***}	0.732^{***}
	Male	0.484^{***}	-	0.360^{***}
	Cone	0.709^{***}	0.337^{***}	-
2022	Female	-	0.704^{***}	0.103^{**}
	Male	0.638^{***}	-	0.111^{**}
	Cone	0.132^{***}	0.124^{***}	-
Seogwipo				
2020	Female	-	0.273^{***}	0.353^{***}
	Male	0.249^{***}	-	0.158^{***}
	Cone	0.365^{***}	0.090^{ns}	-
2021	Female	-	0.384^{***}	0.632^{***}
	Male	0.336^{***}	-	0.199^{***}
	Cone	0.634^{***}	0.225^{***}	-
2022	Female	-	0.309^{***}	0.451^{***}
	Male	0.253^{***}	-	0.330^{***}
	Cone	0.549^{***}	0.370^{***}	-

ns, not significant; **, $p < 0.01$; ***, $p < 0.001$

Table 4 Analysis of variation and heritability (h^2) estimates for the strobilus and cone production at the seed orchard of *C. obtusa* in Seogwipo, South Korea.

Source		Female strobili			Male strobili			Mature cone		
	df	2020	2021	2022	2020	2021	2022	2020	2021	2022
Among clones	22	2.518	10.20	6.700	9.684	19.79	23.47	11.85	13.03	5.935
Within clones	186	1.080	2.414	3.922	1.169	12.123	6.763	3.987	2.084	2.454
F-value		2.332^{**}	4.225^{***}	1.708^{*}	8.287^{***}	1.633^{**}	3.470^{***}	2.972^{***}	6.252^{***}	2.419^{***}
$h^2_{individual}$		0.149	0.297	0.086	0.489	0.077	0.245	0.206	0.408	0.156
h^2_{clone}		0.743	0.868	0.730	0.746	0.877	0.828	0.701	0.807	0.152

ns, not significant; ***, $p < 0.001$

among years for female, male strobili, and cone production provide insights into the stability and consistency of these traits over time (Table 6). Positive correlation coefficients between years suggest stable fertility output, which is crucial for long-term productivity.

Table 6 Spearman's correlation coefficients among years at the seed orchards of *C. obtusa* in Gochang and Seogwipo, South Korea

Location	Variable	Year	2020	2021
Gochang	Female	2021	0.295^{***}	-
		2022	0.029^{ns}	0.085^{*}
	Male	2021	0.239^{***}	-
		2022	0.103^{***}	0.326^{***}
	Cone	2021	0.171^{***}	-
		2022	0.158^{***}	-0.045^{ns}
Seogwipo	Female	2021	0.400^{**}	-
		2022	0.074^{ns}	0.207^{***}
	Male	2021	0.306^{***}	-
		2022	0.556^{***}	0.354^{***}
	Cone	2021	0.283^{***}	-
		2022	0.113^{ns}	0.452^{***}

ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Discussion

When trees (clones) from different regions are planted together, there can be significant differences in the annual variation in fertility (Funda & El-Kassaby 2012). The coefficient of variation, calculated as the ratio of the standard deviation to the mean, provides a normalized measure of dispersion. It allows for the comparison of variability between clones with differing levels of production. A high coefficient of variation exhibits greater relative variability in production among clones, indicating less consistent output year over year. Conversely, a low coefficient of variation demonstrates more stable production levels among clones, suggesting better reliability for seed production purposes (Kang et al. 2023).

Although statistically significant, several correlation coefficients and genetic diversity indices showed only moderate values. These findings should be interpreted with caution, particularly given the limited three-year observation period and the absence of genetic marker-based validation. As such, extrapolation to long-term patterns of reproductive consistency and genetic stability should be made carefully.

The analysis of production from 2020 to 2022 at the seed orchards of *C. obtusa* in Gochang and Seogwipo, Korea highlights several key findings. In both Gochang and Seogwipo, there is a noticeable difference in the fertility output between female and male strobili. The higher mean values and greater coefficient of variation for male strobili suggest greater fertility variance among clones. This variance can lead to a reduction in effective parent number over time if not managed appropriately. The continuous upward trend in mature cone production at Gochang indicates successful management practices enhancing overall fertility output. However, the seed orchard in Seogwipo exhibited a different pattern in coefficient of variation trends compared to Gochang.

The coefficient of variation in fertility over the three years indicates that variability in Seogwipo is increasing, which may be due to environmental sensitivity or inherent genetic characteristics, and therefore, target management practices should be developed to mitigate this issue (Foster 1992, Namkoong et al. 2012). The findings suggest that while fertility output is increasing, managing fertility variation is crucial to maintaining genetic diversity (Namkoong et al. 2012). Strategies such as controlled pollination and balanced clone representation should be considered to mitigate the effects of high fertility variance in male strobili (Wang et al. 2023). The observed trends in the coefficient of variation highlight the need for ongoing monitoring and adaptive management to ensure both high productivity and genetic health of the seed orchards (Rosvall et al. 2003).

The fertility outcomes within seed orchards are subject to a variety of influencing factors including fertility phenology, pollen dispersal, pollen vitality, self-incompatibility, and overall fertility achievement. Conversely, strobilus production can be used as an indicator for estimating potential gamete contribution and fertility (Eriksson et al. 1973, Apsit et al. 1989, El-Kassaby & Ritland 1992, Matziris 1994). A tree can produce large quantities of pollen but might not manage to fertilize a single egg cell. Likewise, a tree can produce large amounts of seeds, but they may all be empty. These are extreme situations but serve to demonstrate that fecundity may not be a reliable indicator of fertility, especially for the male component. With the widespread use of genetic markers, particularly SNPs in recent years, fertility and its variation across orchard accessions could be more accurately assessed (El-Kassaby & Ritland 1986, Funda & El-Kassaby 2012).

A theoretical state of perfect sexual equilibrium is posited under conditions where clones contribute equivalently to the production of both female and male strobili. However, such perfect balance is rare (Kang et al. 2005). The contribution of clones over three years revealed a skewed distribution toward a few clones at the seed orchards of Gochang and Seogwipo, Korea. Such an imbalance can reduce genetic diversity within the seed orchard, potentially compromising the long-term genetic health and adaptability of the population. To maintain genetic balance, management strategies should aim to adjust the proportion of high-contributing clones or increase the contribution of less dominant clones. For example, planting more of the underrepresented clones or limiting the propagation of dominant clones can be considered. Ensuring genetic diversity is crucial for the seed orchard to adapt to environmental changes and enhance disease resistance. Thus, a diverse set of clones should be utilized to maintain a healthy gene pool. Also, environmental factors significantly influence clonal contributions. Local climate, soil conditions, and management practices likely impact cone production and should be considered when developing site-specific management. Further research is needed to determine which environmental factors enhance or inhibit the contributions of specific clones. Understanding these interactions will help in designing more effective management and breeding programs.

Clonal fertility variation refers to the difference in reproductive output among clones. Seed orchard programs cannot effectively produce genetically superior seeds if clone fertility is insufficient. Equal clonal contribution is a key assumption in such programs, but in practice, this is rarely achieved. Variation in clonal fertility influences both genetic improvement and gene diversity in progeny (Kang 2001). The sibling coefficient indicates the probability that two random gametes are identical by descent and is elevated when clonal fertility is uneven (Kang 2001). Therefore, $\Psi=1$ means the equal contribution of clones to the gamete gene pool in the seed orchard. Thus, if there is fertility variation like half-sib or full-sib mating, the coefficient exceeds 1, leading to a decrease in genetic diversity and effective parent number. The sibling coefficient was consistently above 1 for male and female strobilus over the three years in both regions. The relative parent numbers at Gochang were found to be higher than those in Seogwipo, Korea. This could be due to differences in site management, environmental conditions, or inherent genetic differences in the planted clones (El-Kassaby & Ritland 1986). Skewed male fertility was reflected in elevated sibling coefficients, but genetic diversity remained relatively high, suggesting that current practices are maintaining diversity effectively (Isik & McKeand 2019).

The heritabilities (h^2) of female and male strobili in both Gochang and Seogwipo, Korea were relatively high, indicating that genetic differences among clones significantly affect strobili and cone production. For instance, the broad-sense heritability of male strobili in Gochang in 2021 was notably high at 0.877, emphasizing the importance of clone selection in genetic improvement programs. In all years surveyed, the heritability values for female production were lower than those for male production. The narrow-sense heritability indicates that male strobilus production is under moderate genetic control, whereas female strobilus production is under stronger environmental effects than male strobili. Heritability of cone production was generally higher in Seogwipo compared to Gochang, suggesting that the Seogwipo seed orchard has higher genetic potential for cone production (El-Kassaby & Ritland 1986).

Significant correlations among female, male strobili, and cone production in both regions suggest consistent fertility performance among clones, which is an important factor to consider when selecting clones (El-Kassaby & Cook 1994). The significant correlations between strobili and cone production over the years, such as the correlation coefficient of 0.295 between female strobili production in 2020 and 2021, also 0.085 between 2021 and 2022 in Gochang, indicate year-to-year consistency, which is a positive sign for long-term productivity.

Considering the variability and heritability, both seed orchards require strategies to prevent the over-representation of certain clones and to maintain genetic diversity. Controlled pollination and balanced clone representation can help maintain genetic health. Continuous monitoring and adaptive management are essential to ensure high productivity and genetic diversity in the seed orchards.

Conclusions

This study provides insight into the genetic composition of seed lots from two *C. obtusa* seed orchards in South Korea, under the assumption that phenotypic fecundity reflects true fertility. Although genetic marker-based validation was not performed, the results offer foundational data for predicting genetic improvement in seed orchard progeny. By addressing imbalances in clonal contributions and applying effective management strategies, long-term genetic diversity and productivity can be sustained. Future studies should aim to identify the causes of production variability and improve selection criteria to enhance resilience under changing environmental conditions.

Conflict of interest

The authors declare no financial or personal interests could influence the work presented in this paper.

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Data availability

Data supporting the findings of this study are included in the manuscript, and may be provided upon request.

Author contribution statement

JM designed the study, conducted the fieldwork, and prepared the draft manuscript for publication. Y-J, KJ and S-J have helped compile and analyze the data. K-S supervised the study at all stages and contributed to the manuscript draft. We are pleased to announce that all authors have read and approved of the final manuscript.

References

Apsit VJ., Nakamura RR., Wheeler N.C., 1989. Differential male reproductive success in Douglas fir. *Theor Appl Genet* 77: 681–684.

Bottero A., Moos C., Stritih A., Teich M., 2024. Editorial: Impacts of global change on protective forests in mountain areas. *Front For Glob Change* 7. https://doi.org/10.3389/ffgc.2024.1375285

Burczyk J., Chalupka W., 1997. Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard. Annales des Sciences Forestières. EDP Sciences.

Burczyk J., Chybicki I.J., 2004. Cautions on direct gene flow estimation in plant populations. *Evolution* 58: 956–963. https://doi.org/10.1111/j.0014-3820.2004.tb00430.x

Burczyk J., DiFazio S., Adams W., 2004a. Gene flow in forest trees: how far do genes really travel? *For Genet* 11: 179.

Burczyk J., Lewandowski A., Chalupka W., 2004b. Local pollen dispersal and distant gene flow in Norway spruce (*Picea abies* [L.] Karst.). *For Ecol Manag* 197: 39–48. https://doi.org/10.1016/j.foreco.2004.05.003

Chaisurisri K., El-Kassaby Y.A., 1993. Estimation of clonal contribution to cone and seed crops in a Sitka spruce seed orchard. Annales des sciences forestières. EDP Sciences.

Chaloupková K., Lstibůrek M., 2022. Spatial optimization of genetic thinning in seed orchards. *Ann For Sci* 79: 37. https://doi.org/10.1186/s13595-022-01158-4

Choi H-S., Kang Y-J., Kim I-S., et al., 2012. Growth and heritability of Hinoki Cypress (*Chamaecyparis obtusa*) families in Jeju. Korean J Agric For Meteorol 14: 236–245.

Cortés A.J., Restrepo-Montoya M., Bedoya-Canas L.E., 2020. Modern Strategies to Assess and Breed Forest Tree Adaptation to Changing Climate. Front Plant Sci 11: 583323. https://doi.org/10.3389/fpls.2020.583323

El-Kassaby Y.A., 1995. Evaluation of the tree-improvement delivery system: factors affecting genetic potential. Tree Physiol 15: 545–550.

El-Kassaby Y.A., Askew G.R., 1991. The relation between reproductive phenology and reproductive output in determining the gametic pool profile in a Douglas-fir seed orchard. For Sci 37: 827–835.

El-Kassaby Y.A., Cook C., 1994. Female reproductive energy and reproductive success in a Douglas-fir seed orchard and its impact on genetic diversity. Silvae Genet 43: 243–245.

El-Kassaby Y.A., Ritland K., 1986. Low levels of pollen contamination in a Douglas-fir seed orchard as detected by allozyme markers. Silvae Genet 35: 224–229.

El-Kassaby Y.A., Ritland K., 1992. Frequency-dependent male reproductive success in a polycross of Douglas fir. Theor Appl Genet 83: 752–758. https://doi.org/10.1007/BF00226694

Eltayeb L.M., Yagi S., Mohamed H.M., et al., 2023. Essential oils composition and biological activity of *Chamaecyparis obtusa*, *Chrysopogon nigritanus* and *Lavandula coronopifolia* grown wild in Sudan. Molecules 28: 1005. https://doi.org/10.3390/molecules28031005

Eriksson G., Jonsson A., Lindgren D., 1973. Flowering in a clone trial of *Picea abies* Karst. Studia Forestalia Suecica. Nr.110, 45pp.

Eriksson G., Schelander B., Åkebrand V., 1973. Inbreeding depression in an old experimental plantation of *Picea abies*. Hereditas 73: 185–193.

Foster G.S., 1992. Estimating yield: beyond breeding values. Handbook of quantitative forest genetics. Springer.

Fries A., 1994. Development of flowering and the effect of pruning in a clonal seed orchard of lodgepole pine. Can J For Res 24(1): 71-76. https://doi.org/10.1139/x94-011

Funda T., El-Kassaby Y.A., 2012. Seed orchard genetics. CABI Rev 1–23.

Funda T., Lstibůrek M., Lachout P., et al., 2009. Optimization of combined genetic gain and diversity for collection and deployment of seed orchard crops. Tree Genet Genomes 5: 583–593. https://doi.org/10.1007/s11295-009-0211-3

Gamba D., Muchhala N., 2020. Global patterns of population genetic differentiation in seed plants. Mol Ecol 29: 3413–3428. https://doi.org/10.1111/mec.15575

Górski K.M., Kowalczyk T., Picot L., et al., 2024. The Precious potential of the sacred tree *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. as a source of secondary metabolites with broad biological applications. Int J Mol Sci 25: 2723. https://doi.org/10.3390/ijms25052723

Grattapaglia D., Silva-Junior O.B., Resende R.T., et al., 2018. Quantitative genetics and genomics converge to accelerate forest tree breeding. Front Plant Sci 9: 1693. https://doi.org/10.3389/fpls.2018.01693

Havens T.R., 2020. Land of plants in motion: Japanese botany and the world. University of Hawaii Press.

Hong S.K., 1969. Forestry in Korea.

Isik F., McKeand S.E., 2019. Fourth cycle breeding and testing strategy for *Pinus taeda* in the NC State University Cooperative Tree Improvement Program. Tree Genet Genomes 15: 70. https://doi.org/10.1007/s11295-019-1377-y

Jang Y-A., Kim S-G., Kim H-K., Lee J-T., 2023. Biological Activity and Component Analyses of *Chamaecyparis obtusa* Leaf Extract: Evaluation of Antiwrinkle and Cell Protection Effects in UVA-Irradiated Cells. Medicina (Mex) 59: 755. https://doi.org/10.3390/medicina59040755

Jang-Hwan J., So-Hee P., JaChoon K., et al., 2020. Preferences for ecosystem services provided by urban forests in South Korea. For Sci Technol 16: 86–103. https://doi.org/10.1080/21580103.2020.1762761

Kang K-S., 2001. Genetic gain and gene diversity of seed orchard crops. Silvestria 187, 75pp.

Kang K-S., Bilir N., Jeon K., Kim Y-J., 2023. Fertility Variation and Gene Diversity in Forest Populations. Forests 14: 2172. https://doi.org/10.3390/f14112172

Kang K-S., El-Kassaby Y., 2002. Considerations of correlated fertility between genders on genetic diversity: the Pinus densiflora seed orchard as a model. Theor Appl Genet 105: 1183–1189. https://doi.org/10.1007/s00122-002-1064-4

Kang K-S., Mullin T.J., 2007. Variation in clone fertility and its effect on the gene diversity of seeds from a seed orchard of *Chamaecyparis obtusa* in Korea. Silvae Genet 56: 134–137.

Kang D-B., Sung J.W., Lee D-H., 2021. Effects of shading and fertilizer treatments on the growth characteristics of *Chamaecyparis obtusa* (S. et Z.) Endlicher seedlings. For Sci Technol 17: 125–134. https://doi.org/10.1080/21580103.2021.1957722

Kim K., Zsuffa L., 1994. Reforestation of South Korea: The history and analysis of a unique case in forest tree improvement and forestry. For Chron 70: 58–64.

Lee K.Y., Han Y.C., Ryu K.O., et al., 1986. Growth of open-pollinated progeny of *Chamaecyparis obtusa* plus trees introduced from Japan. Res Rep Inst For Genet

Korea Repub 111–115.

Lee I-K., Kang Y-J., Kim C-S., Kim Y-K., 2006. Effects of Thinning on Soil Properties and Seed Productivity in Seed Orchards of *Cryptomeria japonica* and *Chamaecyparis obtusa*. *J* Ecol Environ 29: 495–501. https://doi.org/10.5141/JEFB.2006.29.6.495

Lenz P.R., Nadeau S., Azaiez A., et al., 2020. Genomic prediction for hastening and improving efficiency of forward selection in conifer polycross mating designs: an example from white spruce. Heredity 124: 562–578. https://doi.org/10.1038/s41437-019-0290-3

Li Y., Li X., Zhao M-H., et al., 2021. An overview of the practices and management methods for enhancing seed production in conifer plantations for commercial use. Horticulturae 7: 252. https://doi.org/10.3390/horticulturae7080252

Lindgren D., Mullin T.J., 1998. Relatedness and status number in seed orchard crops. Can J For Res 28(2): 276-283. https://doi.org/10.1139/x97-217

Matziris D.I., 1994. Genetic Variation in the Phenology of Flowering in Black Pine. Silvae Genetica 43(5): 321-328.

Maruyama E., Ishii K., Hosoi Y., 2005. Efficient plant regeneration of Hinoki cypress (*Chamaecyparis obtusa*) via somatic embryogenesis. J For Res 10: 73–77. https://doi.org/10.1007/s10310-004-0105-z

Nakashizuka T., 2009. Sustainability and Diversity of Forest Ecosystems. Springer.

Namkoong G., Kang H., Brouard J., 1988. Tree Breeding: Principles and Strategies. Springer-Verlag. N Y.

Namkoong G., Kang H-C., Brouard J.S., 2012. Tree breeding: principles and strategies: principles and strategies. Springer Science & Business Media.

Na S.J., Lee H.S., Han S.U., et al., 2015. Estimation of genetic gain and diversity under various genetic thinning scenarios in a breeding seed orchard of *Quercus acutissima*. Scand J For Res 30: 377-381. https://doi.org/10.1080/02827581.2015.1018936

Nikkanen T., Ruotsalainen S., 2000. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica 34(3): 205–222. https://doi.org/10.14214/sf.626

Orji K., Mbah E., Akpan A., 2022. Agroforestry: A review of its importance, problems and prospects in crop production. Ratarstvo I povrtarstvo 59(3): 64-75. DOI: 10.5937/ratpov59-39353

Pakull B., Eusemann P., Wojacki J., et al., 2021. Genetic diversity of seeds from four German Douglas fir (*Pseudotsuga menziesii*) seed orchards. Eur J For Res 140: 1543–1557. https://doi.org/10.1007/s10342-021-01419-3

Park K-H., 2022. Analysis of urban forest healing program expected values, needs, and preferred components in urban forest visitors with diseases: A pilot survey. Int J Environ Res Public Health 19: 513. DOI: 10.3390/ijerph19010513

Park J-M., Kang H-I., Yeom D-B., et al., 2020. Gender, reproductive output covariation and their role on gene diversity of *Pinus koraiensis* seed orchard crops. BMC Plant Biol 20: 418. https://doi.org/10.1186/s12870-020-02632-9

Park S., Kim S., Kim G., et al., 2021. Evidence-based status of forest healing program in South Korea. Int J Environ Res Public Health 18: 10368. https://doi.org/10.3390/ijerph181910368

Reynolds S., El-Kassaby Y.A., 1990. Parental balance in Douglas-fir seed orchards-cone crop vs. seed crop. Silvae Genet 39: 40–42.

Rosvall O., Mullin T.J., Lindgren D., 2003. Controlling parent contributions during positive assortative mating and selection increases gain in long-term forest tree breeding. For Genet 10: 35-53.

Sharma C.M., Khanduri V.P., 2012. Pollen cone characteristics, pollen yield and pollen-mediated gene flow in *Cedrus deodara*. Curr Sci 102: 394–397. https://www.jstor.org/stable/24083884

Shin J.H., Park P.S., Lee D.K., 2007. Forest restoration in Korea. Keep Asia Green 2: 55–79.

Wang F., Zhang S., Zhu P., et al., 2023. The effects of fertility and synchronization variation on seed production in two Chinese fir clonal seed orchards. Sci Rep 13: 627. https://doi.org/10.1038/s41598-022-27151-5

Yang J., Mezmouk S., Baumgarten A., et al., 2017. Incomplete dominance of deleterious alleles contributes substantially to trait variation and heterosis in maize. PLoS Genet 13: e1007019. https://doi.org/10.1371/journal.pgen.1007019

Zhang X., Yu M., Su J., et al., 2024. Leaf nutrient traits of planted forests demonstrate a heightened sensitivity to environmental changes compared to natural forests. Front Plant Sci 15: 1372530. https://doi.org/10.3389/fpls.2024.1372530