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ARTICLE

Impact of Space Mutagenesis on Growth and Cone Variation in *Cunninghamia lanceolata*

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ABSTRACT: Space-induced mutagenesis, as a tool for generating genetic variation, has been widely exploited in crop improvement. However, its mutagenic impact on long-lived forest trees—especially the long-term effects on traits expressed at reproductive maturity—remains systematically unassessed. In this study, we used Chinese fir (*Cunninghamia lanceolata*), a key timber species in China, to evaluate the influence of the space environment on mature-phase traits. We compared growth and cone characters between a 14-year-old space-mutated plantation derived from seeds flown aboard the Shijian-8 recoverable satellite and a contemporaneously planted ground control stand. Mean diameter at breast height and individual stem volume were 18.0% and 44.4% higher, respectively, and needle length was 15.7% higher in the space-mutated stand compared with the control, while mean tree height was slightly reduced. Both positive and negative mutations were detected: some individuals outperformed the control, while others performed worse. This bidirectional effect suggests that space mutagenesis may have contributed to expanding the genetic diversity of Chinese fir, rather than guaranteeing universal improvement. The expanded variation provides valuable breeding materials, but rigorous selection is required to identify and fix beneficial alleles. These findings indicate that space mutagenesis should be viewed as a variation-generating tool for Chinese fir breeding, not as a direct yield-enhancing treatment.

KEYWORDS: Space mutagenesis; *Cunninghamia lanceolata*; inter-individual variation; inter-population variation; phenotypic variation

1 Introduction

Mutation breeding, also known as artificial mutation breeding [1–3], is a plant breeding technology that uses artificial means to induce changes in the genetic material of plant material [4–6]. The resulting mutants can be screened for valuable traits. In the case of forest trees, artificial mutation breeding can partially overcome some of the challenges that traditional breeding cannot address [7–9]. However, it is essential to recognize that conventional mutation breeding, including space mutagenesis, is largely untargeted. The vast majority of induced mutations are neutral or deleterious, and only a small minority are beneficial. Therefore, the primary value of mutagenesis lies not in guaranteeing immediate trait improvement but in expanding the genetic variation pool, from which superior alleles can subsequently be extracted through rigorous phenotypic selection and progeny testing.

Chemical mutation breeding has been successful in white birch (*Betula platyphylla*) [10], Sapium sebiferum (*Sapium sebiferum*) [11], and seashore hibiscus hamabo (*Hibiscus hamabo*) [12], among other forest tree species. According to a study, radiation breeding has a significant effect on the germination and growth of forest tree seeds such as *Camellia oleifera* [13] and *Acer palmatum* [14]. Space mutation breeding is a novel approach to breeding [15–17]. Space mutagenesis exposes biological materials to space-specific factors to induce mutations, then uses propagation, cultivation and evaluation to obtain stable genetic material with desirable traits (e.g., high yield, quality, stress resistance). Space research has attracted widespread attention and has made significant progress in the field of deep space exploration. Beyond the ozone layer, the absence of gravity [18], magnetic fields [19], air [20], and atmospheric pressure in space [21], coupled with high levels of radiation, induces extensive genomic mutations [22,23]. These conditions lead to the emergence of new characteristics and traits in organisms. Currently, space mutagenesis breeding has been applied to crops such as rice [24], peanut [25], wheat [26], Huang Qin [27], rosemary [28], and tomato [29], with broader research ongoing worldwide. However, almost all of these successful cases are annual crops. For forest trees—especially long-generation conifers such as Chinese fir—the application of space mutagenesis remains extremely limited. A few exploratory studies have reported space-induced variants in conifers. For example, *Pinus massoniana* seeds flown on the Shijian-8 satellite have shown notable gains in height (50.7%), diameter at breast height (87.5%), and individual volume (318.9%) [30]. Similarly, *Pinus elliottii* seeds from the Shenzhou-8 mission showed potential volume increases of 110.5–139.4% [31]. Furthermore, seeds of *Picea crassifolia* and *Juniperus przewalskii* from the Shijian-19 satellite have recently entered on-ground cultivation trials, though no growth data have yet been reported [32]. However, the existing reports on conifers have focused primarily on early growth stages without assessing mature-phase traits. Consequently, no systematic study has evaluated the long-term effects of spaceflight on commercially relevant, mature-phase traits in gymnosperms. Existing research has mainly focused on seed germination and early seedling growth, leaving a significant gap in our understanding of whether and how space-induced mutations persist to reproductive maturity.

Cunninghamia lanceolata (Lamb.) Hook., commonly known as fir, is a fast-growing timber species that is naturally distributed in latitudes 19°30′~34°03′ N and longitudes 101°30′~121°53′ E in southern China [33–35]. Its timber production accounts for 1/4 of the national commercial timber production [36–38]. In China, fir has been an important research subject for the genetic improvement of forest trees [39–41]. Currently, the 3rd generation of fir seed orchard has been established. However, advanced-generation breeding programs now face a critical challenge: a narrowing genetic base that limits further genetic gains in growth, wood quality, and stress tolerance [41]. Currently, the only known location of ‘space fir’ trees is in the Yangkou State Forest in Fujian Province. In 2006, the seeds of these trees were sent into space aboard the ‘Practice 8’ satellite. The resulting seedlings were then grown in a nursery in Yangkou Forest in March 2007 and planted in February 2008, and currently, 126 plants are alive [42]. This forest plantation is now considered to be the most significant in China. Fir has a long growth cycle [43–46]. The effects of space mutation are more noticeable in the seedling stage than in the ground control [47–49]. There are fewer studies on the differences in effects after establishment. The growth cycle is long, and differences in standing wood traits can only be observed and measured after seedling planting [50,51]. Temperature, rainfall, geologic hazards, and human activities during the growth cycle can affect the structure and growth of the forest stand, which may subsequently impact the observation of traits [52–54]. Space mutation breeding can alter the growth traits of fir to some extent, making it significant to select good trait plants and cultivate them in the establishment of large-diameter fir timber forests. Therefore, direct measurement of mature individuals is essential, rather than extrapolation from seedling data. The 14-year-old space-mutated

Chinese fir stand in Yangkou State Forest provides a rare opportunity to assess the long-term effects of space mutagenesis on growth and reproductive traits at a commercially relevant stage.

This study used a 14-year-old space-mutagenized Chinese fir (*Cunninghamia lanceolata*) stand and an adjacent, contemporaneously established ground-control stand to evaluate the long-term effects of space mutagenesis at both the population and individual levels. Specifically, the objectives were: (i) to assess the long-term phenotypic effects of space mutagenesis at the stand/population level by comparing growth traits, trait ranges, and coefficients of variation between the space-mutagenized and control stands; and (ii) to screen and characterize potentially superior mutant individuals based on individual stem volume and related growth traits, thereby identifying candidate germplasm for future Chinese fir breeding. In addition, cone and seed traits of cone-bearing space-mutated trees were described to provide preliminary information on reproductive performance. By distinguishing population-level phenotypic responses from individual-level selection, this study aimed to clarify whether space mutagenesis not only alters mature-stage phenotypic variation but also generates selectable mutant materials for subsequent clonal propagation, progeny testing, and breeding utilization.

2 Materials and Methods

2.1 Experimental Site Overview

The study area is located in the southern part of the Wuyi Mountain Range in Yangkou State Forest, Shunchang County, Nanping City, Fujian Province. The area is characterized by low mountainous and hilly terrain, and the space mutagenic forest stand is located in Yangkou State Forest (117°53′32″). The experimental stand (26°49′13.27″ N, 117°53′32.14″ E) and the control stand (26°49′37.61″ N, 117°52′19.20″ E) are both located in the Huangkengtou Work Area of Yangkou State Forest. They are situated in an area with a standing index of 18 and experience similar climatic conditions. The study area has a middle subtropical monsoon humid climate with sufficient light and abundant rainfall. The sunshine duration is between 1668–1972 h. It has an average annual temperature of 18.6°C, with an extreme maximum temperature of 41.4°C and an extreme minimum temperature of 5.8°C. The annual precipitation ranges from 1600–1900 mm and the average annual evapotranspiration ranges from 1308–1587 mm. The area's elevation ranges from 185 to 292 m. The soil is a dark reddish loam, more than 90 cm deep, and relatively fertile.

2.2 Experimental Design and Investigation

This study was conducted to evaluate the effects of spaceflight-induced mutagenesis on the growth and phenotypic traits of *Cunninghamia lanceolata* at the stand level. The space-mutagenized and ground control stands were established from seeds of a common genetic source. Detailed information on space treatment, seed propagation, and experimental conditions is provided below (Section 2.3).

A control group of twenty standing trees was selected within the control stand, and a per-tree survey was conducted on 126 trees in the space-mutagenized stand and the control group in 2021. Growth indicators, including diameter at breast height (DBH), tree height, crown spread, height under branches, leaves, and bark, were determined for the selected individual plants. Leaf and bark color were measured using a colorimeter for colorimetry. Bract scales, leaf length, leaf width, and bark thickness were measured using vernier calipers. Intact bark was selected from standing trees at 1.3 m above the ground, and 1.5 square centimeters of bark were taken from each plant for measurement.

2.3 Space Treatment and Seedling Propagation

A total of approximately 1000 seeds of Chinese fir (*Cunninghamia lanceolata*), sourced from the second-generation seed orchard of Yangkou State Forest, were selected for space mutagenesis. These seeds, along with other biological materials, were packed according to material type and placed in the return capsule of the Shijian-8 recoverable scientific satellite. The satellite was launched from the Jiuquan Satellite Launch Center on 09 September 2006, and operated in a near-Earth orbit (perigee approx. 180 km, apogee approx. 460 km, inclination 63°) for a total of 355 h before being successfully recovered on 24 September 2006 at Suining, Sichuan Province. During the mission, the seeds were exposed to the combined space environment, including cosmic radiation, microgravity, high vacuum and variable magnetic fields. Onboard measurements indicated an average ion flux of 4.44 ions/cm^2·d for space heavy ions and an average dose of 4.79 mGy from low linear energy transfer (LET) radiation to the plant seeds.

Upon recovery, the space-exposed seeds were returned to Yangkou State Forest, Fujian Province, where they were germinated in a nursery under standard conditions in 2007. The resulting seedlings were outplanted in the field in 2008, together with seedlings from the contemporaneous ground control, at a spacing of 2 m × 2 m in the Huangkengtou Work Area. Both the space-mutagenized and control stands share comparable site index (18), edaphic conditions and slope orientation, and have been managed under identical silvicultural practices. A total of 126 space-mutated trees survive at present.

The ground control seeds originated from the same seed lot as the space-exposed batch, were stored under ambient temperature and humidity conditions at Yangkou State Forest during the flight mission, and were subjected to the same nursery and field establishment procedures as the space-utagenized group. Since the spacecraft returned to Earth and the seeds were processed, the two groups have been maintained in parallel without further differential treatment.

2.4 Trait Calculation and Data Analysis

Single-plant volume (V) was calculated based on tree height and diameter at breast height, and single-plant volume was calculated using the binary stumpage model for cedar published in DB35/T 1823-2019 Binary Stumpage Tables for Major Tree Species:

$$V = 0.000\,070\,609\,4\,D^{1.801\,671}\,H^{0.997\,998} \tag{1}$$

where: V is lumber volume; D is diameter at breast height; H is tree height.

The phenotypic coefficient of variation formula [55] is:

$$Y = \frac{\sigma_p^2}{\overline{X}} \tag{2}$$

where: Y is the phenotypic coefficient of variation; $\frac{\sigma_p^2}{\overline{X}}$ is the phenotypic variance; $\overline{X}$ is the average value of a certain trait.

The superior-tree selection criterion was defined as individual stem volume greater than the population mean plus one standard deviation, whereas the inferior-tree selection criterion was individual stem volume less than the population mean minus one standard deviation.

The data were analyzed by SPSS (Version 25.0, IBM Corp.) statistical analysis software, and one-way ANOVA was performed to analyze the differences between the growth traits of the space-mutagenized forest and the control forest.

3 Results and Analysis

3.1 Analysis of Genetic Variation of Fir Traits in Space Mutant and Control Forests

Growth-trait measurements of the 14-year-old space-mutated Chinese-fir stand (HT) and the control stand (CK) revealed that the space-mutated stand showed significantly higher mean values for several traits and a broader range of variation (Table 1).

With respect to mean values, DBH and individual stem volume of the mutated stand significantly exceeded those of the control. Mean DBH in the mutated stand was 15.7 cm, 18.0% greater than the 13.3 cm recorded for the control, while mean individual volume reached 0.13 m^3, 44.4% higher than the 0.09 m^3 observed in the control. In contrast, mean tree height in the mutated stand (11.3 m) was slightly lower than that of the control (12.4 m), and height to crown base (5.8 m) was also below the control value (7.9 m). Crown width averaged 3.4 m in the mutated stand, surpassing the 1.9 m measured in the control.

Analysis of trait ranges demonstrated that the mutated stand exhibited broader variation for every investigated trait. The DBH range in the mutated stand was 22.6 cm, far exceeding the 11.6 cm observed in the control; similarly, the individual volume range (0.37 m^3) was markedly wider than the 0.20 m^3 recorded for the control. Coefficients of variation (CV) were generally higher in the mutated stand; for example, CV of height to crown base was 0.36 versus 0.16 in the control, and CV of individual volume was 0.59 compared with 0.53 in the control. These patterns suggest that the space-mutated population exhibits substantially expanded phenotypic variation, implying that space mutagenesis may have contributed to this expansion. Importantly, this expanded variation was bidirectional. Although mean DBH and volume were higher in the mutated stand, the minimum values in the space-mutated group were substantially lower than those in the control: minimum DBH was 4.4 cm versus 8.1 cm; minimum individual volume was 0.01 m^3 versus 0.02 m^3. Similarly, the maximum values were higher (DBH 27.0 cm vs. 19.7 cm; volume 0.38 m^3 vs. 0.22 m^3). These patterns indicate that the space-mutated population exhibits a wider phenotypic spectrum, implying that space mutagenesis may have contributed to this expansion.

3.2 Comparison of Superior and Weaker Plant Traits in the Space Mutant Forest with the Control Forest

Based on the selection criteria, superior and inferior individuals were identified in the two stands to evaluate the extent of phenotypic variation. Six superior individuals were selected from the space-mutagenized stand, whereas two were selected from the control stand, using individual stem volume as the selection criterion. The selected superior individuals in the space-mutagenized stand had heights of 13.5–14.9 m, DBH values of 22.8–27.0 cm, crown widths of 3.8–6.5 m, crown-base heights of 3.6–7.8 m, and individual stem volumes of 0.280 0–0.388 8 m^3.

Six inferior individuals were selected from the space-mutagenized stand, and two from the control stand, using individual stem volume as the selection criterion. The selected inferior trees in the space-mutagenized stand had heights of 5.0–7.9 m, DBH values of 4.4–8.2 cm, crown widths of 1.9–2.8 m, crown-base heights of 1.3–7.5 m, and individual stem volumes of 0.005 1–0.024 6 m^3.

Table 2 shows that the selected healthy single plants had a higher diameter at breast height (DBH), crown diameter, and lumber volume than the control group. In contrast, the selected weaker single plants had a lower DBH, tree height, and single tree volume per plant than the control group. The coexistence of extreme superior and extreme inferior individuals in the same space-mutated stand underscores the bidirectional nature of untargeted mutagenesis and highlights why selection is indispensable in mutation breeding programs.

Table 1: Variation of growth traits of 14-year-old Chinese fir in two stands.

Type	Tree Height/m		Diameter at Breast Height/cm		Crown Base Height/m		Crown Diameter/m		Single Tree Volume/m^3		Bark Thickness/cm		Leaf Length/cm		Leaf Width/cm	
	HT	CK	HT	CK	HT	CK	HT	CK	HT	CK	HT	CK	HT	CK	HT	CK
Mean value	11.3	12.4	15.7	13.3	5.8	7.9	3.4	1.9	0.13	0.09	8.20	8.90	5.16	4.26	0.30	0.32
Minimum value	5.0	7.9	4.4	8.1	1.3	4.8	1.9	1.3	0.01	0.02	2.77	5.51	2.83	3.81	0.06	0.28
Maximum Value	14.6	15.8	27.0	19.7	10.6	9.4	6.5	2.9	0.38	0.22	13.56	13.83	8.08	5.43	0.50	0.36
extreme deviation	9.6	7.9	22.6	11.6	9.3	7.6	4.6	1.6	0.37	0.2	10.79	8.32	5.25	1.62	0.44	0.08
Standard deviation	2.05	2.43	4.39	3.33	2.14	1.27	0.82	1.9	0.08	0.49	3.02	2.48	1.03	0.53	0.07	0.02
Coefficient of variation	0.18	0.19	0.27	0.25	0.36	0.16	0.23	0.49	0.59	0.53	0.36	0.28	0.19	0.12	0.24	0.08

Note: HT represents the space mutation forest, while CK stands for the control forest.

Table 2: Comparison of traits of superior and inferior individual plants with control group.

Dominant Wood							Weak Wood						
Number	DBH/cm	Tree Height/m	Crown Base Height/m	Crown Width/m	Single Tree Volume/m^3	Height-Diameter Ratio	Number	DBH/cm	Tree Height/m	Crown Base Height/m	Crown Width/m	Single Tree Volume/m^3	Height-Diameter Ratio
90	27.0	14.6	4.0	5.7	0.388 8	0.54	102	4.4	5.0	1.3	2.6	0.005 1	1.13
52	24.8	14.9	7.0	3.8	0.340 4	0.60	64	5.9	7.9	7.4	1.9	0.013 6	1.33
12	26.2	12.6	4.0	6.5	0.317 9	0.48	53	7.7	6.5	6.0	2.1	0.018 1	0.84
87	22.8	14.9	7.8	3.4	0.292 6	0.65	38	7.1	7.6	6.7	2.3	0.018 3	1.07
120	22.8	14.8	3.6	4.3	0.290 6	0.65	20	7.4	8.3	7.5	2.0	0.021 5	1.12
131	23.5	13.5	4.5	3.9	0.280 0	0.57	78	8.2	7.9	6.3	2.8	0.024 6	0.96
CK 18	19.7	15.0	8.5	2.7	0.226 4	0.76	CK 4	8.1	7.9	4.8	1.3	0.024 2	0.97
CK 8	18.7	15.5	8.5	2.3	0.212 9	0.82	CK 12	9.5	11.2	8.6	1.6	0.045 4	1.17

3.3 Correlation Analysis of the Growth Traits of Standing Trees in Space-Mutagenized Forests

Table 3 presents the correlation coefficients among growth traits of *Cunninghamia lanceolata* in the space-mutagenized forest. The three core growth traits—diameter at breast height (DBH), tree height, and single tree volume—exhibited extremely significant positive correlations with each other. DBH showed the strongest correlation with single tree volume (r = 0.958, $p < 0.01$), followed by tree height with single tree volume (r = 0.800, $p < 0.01$) and DBH with tree height (r = 0.731, $p < 0.01$).

Crown width was extremely significantly positively correlated with DBH (r = 0.642, $p < 0.01$), tree height (r = 0.240, $p < 0.01$), and single tree volume (r = 0.545, $p < 0.01$), but extremely significantly negatively correlated with crown base height (r = −0.426, $p < 0.01$). Crown base height was only extremely significantly positively correlated with tree height (r = 0.279, $p < 0.01$), with no significant correlation with DBH, single tree volume, or bark thickness.

Bark thickness was significantly positively correlated with DBH (r = 0.194, $p < 0.05$) and tree height (r = 0.193, $p < 0.05$), and extremely significantly positively correlated with single tree volume (r = 0.271, $p < 0.01$). However, bark thickness showed no significant correlation with either crown base height or crown width.

Table 3: The correlation coefficient between the growth traits of Chinese fir in mutant forest.

Character	DBH	Tree Height	Single Tree Volume	Crown Base Height	Crown Width	Bark Thickness
DBH	1					
Tree height	0.731**	1				
Single tree volume	0.958**	0.800**	1			
Crown base height	−0.059	0.279**	0.019	1		
Crown width	0.642**	0.240**	0.545**	−0.426**	1	
Bark thickness	0.194*	0.193*	0.271**	0.082	0.074	1

Note: **indicates a significant difference at the 0.01 level between the space mutagenesis treatment and the ground control, and *indicates a significant difference at 0.05 level between the space mutagenesis treatment and the ground control.

3.4 Bark and Leaf Chromaticity Analysis and Evaluation of Space Mutagenized and Control Forests

The relationship between the growth status of fir and the chromaticity of its bark and leaves is close. Table 4 shows a comparison of bark chromaticity. The brightness value (L*) of the bark in the space-mutagenized forest was smaller than that of the control forest. The average value of bark brightness was 21.52 in the space-mutagenized forest, while that of the control forest was 34.43. Meanwhile, the red-green index (a*) and yellow-blue index (b*) of bark in the space-mutagenized forest were both higher than those in the control forest, and the total color difference (ΔE) was also significantly higher (46.90 vs. 42.77).

Table 4: Comparison of bark color between space mutation forest and control group.

Standing forest	ΔL	Δa	Δb	ΔE
Space mutation forest	21.52 ± 15.92	23.95 ± 28.55	2.05 ± 15.50	46.90 ± 25.04
Control stand	34.43 ± 6.15	16.16 ± 1.23	19.27 ± 2.35	42.77 ± 5.67

The leaf lightness value (LL*) of the space-mutagenized forest (24.18) was slightly higher than that of the control forest (23.06) (Table 5). The leaf yellow-blue index (Lb*) (63.43) was significantly higher than that of the control (16.10), indicating that leaves from the space-mutagenized forest were more yellowish. The leaf red-green index (La*) was higher in the space-mutagenized group (−19.80 vs. −86.26), suggesting

a significantly lower greenness compared with the control. In addition, the total color difference (ΔE) of leaves in the space-mutagenized forest was slightly lower than that in the control forest (75.21 vs. 94.53).

Table 5: Comparison of leaf colorimetry between space-mutated and control stands.

Standing Forest	ΔL	Δa	Δb	ΔE
Space mutation forest	24.18 ± 6.19	−19.80 ± 15.51	63.43 ± 46.50	75.21 ± 42.24
Control stand	23.06 ± 5.39	−86.26 ± 76.32	16.10 ± 4.71	94.53 ± 71.92

3.5 Analyzing and Evaluating the Type of Cones and Bract Scale Traits in Space-Induced Forests

Fir cones and seed quality are important economic and quality indicators of *Cunninghamia lanceolata*. In the space-mutated stand, tree No. 77 produced eight cones and tree No. 122 produced three cones. Most cones of tree No. 77 belonged to the broad-scaled wrapping type (KF), with only one cone classified as the broad-scaled loose type (KS). Among the cones collected from tree No. 122, two were classified as the broad-scaled tight-wrapping type (KJ) and one as the broad-scaled loose type (KS). All cones showed semicircular bract scales. Cone length ranged from 13 to 22 mm and cone width from 11 to 16 mm. Tree No. 77 had a low proportion of healthy seeds, with most seeds being empty or abortive. Tree No. 122 had a higher proportion of healthy seeds than tree No. 77, although empty and abortive seeds still accounted for a considerable proportion of the total.

Cone morphology and seed quality are important economic and quality indicators of Chinese fir. In the space-mutated stand, tree No. 77 produced eight cones and tree No. 122 produced three cones. The cone types were classified according to bract scale morphology as follows: Broad-scaled warping type (KF): bract scales are broadly shaped and distinctly curved or warped outward. Broad-scaled loose tension type (KS): bract scales are broadly shaped but loosely arranged, with visible gaps between scales. Broad-scaled tight wrapping type (KJ): bract scales are broadly shaped and tightly wrapped around the cone axis, with minimal gaps.

Most cones of tree No. 77 were of the broad-scaled warping type (KF), with only one being broad-scaled loose tension type (KS). Two cones of tree No. 122 were broad-scaled tight wrapping type (KJ) and one was broad-scaled loose tension type (KS). All cones had a semicircular bract scale morphology. Cone length ranged from 13 to 22 mm and width from 11 to 16 mm.

Tree No. 77 had a low proportion of healthy seeds, with most seeds being empty or abortive. Tree No. 122 had a higher proportion of healthy seeds than tree No. 77, but empty and abortive seeds still constituted a significant portion of the total (Table 6).

Table 6: Growth of fir cones and seeds in space mutagenized forests.

Standing Tree Number	Cone				Seed				
	Number	Type	Long/mm	Wide/mm	Length/mm	Width/mm	Empty Seed/%	Abortive Seed/%	Healthy Seed/%
77	1	KF	22.67	15.72	7.96	7.83	64.3	21.4	14.3
	2	KS	17.89	12.52	6.97	6.63	70.8	20.8	8.3
	3	KF	18.80	13.68	7.78	7.60	50.0	30.0	20.0
	4	KF	16.15	13.34	7.20	7.25	75.0	16.7	8.3
	5	KF	15.59	15.09	7.56	7.48	80.0	15.0	5.0
	6	KF	13.63	11.13	7.41	6.63	63.6	9.1	27.3
	7	KF	13.63	12.92	6.85	7.16	91.7	0	8.3
	8	KF	16.76	12.53	6.68	6.92	51.9	22.2	25.9

Table 6: *Cont.*

Standing Tree Number	Number	Cone Type	Cone Long/mm	Cone Wide/mm	Seed Length/mm	Seed Width/mm	Seed Empty Seed/%	Seed Abortive Seed/%	Seed Healthy Seed/%
122	1	KS	15.45	14.79	7.43	7.86	26.3	50.9	22.8
	2	KJ	17.11	11.75	8.26	7.89	23.0	41.0	36.1
	3	KJ	13.54	12.72	8.28	8.07	75.6	10.0	14.4

Note: KF indicates the broad-scaled wrapping type, KS the broad-scaled loose type, and KJ the broad-scaled tight-wrapping type; the data in the table pertains to the percentage of seeds in that state compared to all the seeds in the standing tree.

4 Discussion and Conclusion

As a technique that can efficiently induce genomic variation and create novel germplasm, space mutagenesis has been successfully applied in various crops [15–17,56]. However, in timber tree species with extremely long growth cycles such as Chinese fir (*Cunninghamia lanceolata*), the long-term effects of space mutagenesis on growth, phenotypic and reproductive traits at mature stages remain systematically unclear. Using a 14-year-old space-mutagenized Chinese fir plantation, this study provides the first systematic evaluation of mature-stage growth and reproductive traits in a long-rotation forest tree species after space mutagenesis, documenting sustained differences in key economic traits and expanded phenotypic variation relative to the control. The results show that, compared with the control, the space-mutagenized population exhibits a wider range of phenotypic variation, producing both superior and inferior individuals, thereby providing valuable phenotypic variation and preliminary breeding materials for advanced-generation Chinese fir breeding.

To date, reports on space-induced mutations in forest trees are sparse and largely restricted to early growth stages. Several exploratory studies in conifers, such as those on *Pinus massoniana* [33] and *Pinus elliottii* [34], have reported substantial gains in juvenile height, diameter, and volume. However, these studies did not assess mature-phase traits, lacked heritability estimates, and were based on single, unreplicated spaceflights. More recently, seeds of *Picea crassifolia* and *Juniperus przewalskii* have been flown and entered ground trials [35], but no growth data have yet emerged. For broadleaf trees, similar limitations exist. Thus, the present study is the first to systematically evaluate mature-phase traits (including stem volume, crown architecture, cone and seed characteristics) in a long-generation conifer after space mutagenesis, thereby providing a benchmark for future research.

Ground-based physical and chemical mutagens have been applied to several tree species. For example, gamma irradiation of *Camellia oleifera* seeds [13] and chemical mutagenesis in white birch [10] have produced phenotypic variants, but typically at the cost of high deleterious mutation loads and reduced survival. In contrast, the space environment involves a synergistic combination of cosmic radiation, microgravity, high vacuum and weak magnetic fields, which may be associated with different genomic or regulatory responses [25,26]. The broader phenotypic variation observed in our space-mutated stand (e.g., CV of individual volume 0.59 vs. 0.53 in the control) suggests that space mutagenesis might produce a wider spectrum of variation than single-agent ground mutagenesis, although direct comparisons are difficult due to differences in starting genetic materials and experimental designs. Importantly, both approaches share the same fundamental limitation: most induced mutations are neutral or deleterious, and rigorous selection is essential.

One intriguing finding is that mean tree height in the space-mutated stand was slightly reduced (11.3 m vs. 12.4 m in the control), whereas DBH and individual volume increased by 18.0% and 44.4%, respectively, and needle length also increased (15.7%). This pattern suggests a possible shift in resource

allocation: space-induced mutations may favour radial growth and leaf elongation over height extension. A similar phenomenon has been observed in some gamma-irradiated tree populations, where stem diameter growth was less affected than height growth [13,14]. The underlying mechanisms could involve altered gibberellin or auxin signalling, changes in cell wall composition, or differential expression of genes regulating secondary xylem formation. However, without molecular data, these explanations remain speculative. The observed reduction in height to crown base (5.8 m vs. 7.9 m) further supports a change in crown architecture, which may affect light interception and biomass partitioning. Beyond these individual trait responses, such full-trait and large-scale variation expansion is consistent with the hypothesis that composite space mutagenesis may induce efficient genomic perturbation, and it aligns with the core logic of mutation breeding: "expand variation first, then select elites" [57]. Under the background that advanced-generation breeding of Chinese fir is facing a narrow genetic basis and slowed improvement progress [44,45], the rich variation library created by space mutagenesis can provide sufficient materials for simultaneous multi-trait selection and help broaden the selection base for advanced-generation Chinese fir breeding.

Several limitations must be acknowledged. First, the spaceflight treatment lacked biological replication, and the ground control was not subjected to launch/re-entry conditions, so the observed differences cannot be unequivocally attributed solely to space radiation and microgravity. Second, differential mortality (12.6% survival) may have biased the surviving population, meaning the mean superiority might partly reflect selective survival. Third, heritability of space-induced traits is unknown, and the study is limited to a single site and age (14 years); longer-term monitoring is needed. It is important to note that direct molecular evidence for specific genomic changes (e.g., DNA base substitutions, structural variations, or epigenetic modifications) is lacking in the present study. Confirmation of such mechanisms will require future work using whole-genome resequencing, transcriptomics, and methylation analysis. Despite these limitations, the identified superior individuals have biological and operational significance as preliminary candidate plus trees. They should not be interpreted as proven elite cultivars at this stage, because their heritability and phenotypic stability across sites have not yet been validated. However, they are not merely statistical extremes within a broadened distribution. First, they satisfied the predefined superior-tree selection criterion based on individual stem volume exceeding the population mean plus one standard deviation. Second, their growth performance exceeded not only the mean level of the space-mutagenized population, but also the selected superior individuals observed in the ground control under the same site and management conditions. For example, the individual stem volumes of trees No. 90, 52, 12, 87, 120, and 131 ranged from 0.2800 to 0.3888 m^3, whereas the two selected superior trees in the control stand had individual stem volumes of 0.2264 and 0.2129 m^3. These results suggest that these space-mutated individuals showed relatively favorable phenotypic performance within the scope of the present experiment.

Nevertheless, because this study did not include a direct side-by-side comparison with currently deployed advanced-generation Chinese fir clones or breeding populations, we cannot conclude that these individuals already exceed existing operational breeding materials. Instead, their breeding value should be regarded as potential rather than confirmed. Their practical importance lies in providing novel candidate germplasm that may broaden the selection base of advanced-generation Chinese fir breeding, particularly under the current background of a narrowing genetic base. Therefore, these individuals should be prioritized for vegetative propagation, multi-site clonal trials, controlled crossing with elite breeding materials, progeny testing, and molecular characterization. Only after such validation can their true genetic gain, phenotypic stability, and operational breeding value be determined.

In conclusion, this study demonstrates that space mutagenesis altered the mature-stage growth pattern of Chinese fir in a trait-specific and selection-relevant manner. Compared with the ground control, the

14-year-old space-mutagenized stand exhibited higher DBH and individual stem volume, whereas mean tree height was slightly lower. These results indicate that space mutagenesis did not uniformly enhance all growth traits, but instead produced differentiated growth responses, with improved radial growth and stem-volume accumulation accompanied by reduced height performance.

The expanded phenotypic variation observed in the space-mutagenized population was practically meaningful for breeding because it generated both inferior individuals and selectable superior trees. In particular, trees No. 90, 52, and 12 showed relatively superior growth performance, with tree No. 90 exhibiting the highest DBH and individual stem volume among the selected individuals. Trees No. 87, 120, and 131 also showed comparatively high stem-volume performance. These individuals may therefore be regarded as promising candidate mutant germplasm for future Chinese fir improvement, rather than simply as evidence of increased phenotypic variation. They should be prioritized for clonal propagation, multi-site validation, controlled crossing, progeny testing, and molecular characterization. However, because the heritability and molecular basis of these phenotypes remain unconfirmed, their operational breeding value must be further validated before large-scale deployment. Overall, space mutagenesis should be regarded as a variation-generating approach capable of producing selectable elite materials for advanced-generation Chinese fir breeding, rather than as a direct and universal yield-enhancing treatment.

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