STUDY ON SEED QUALITY OF *CUPRESSUS GIGANTEA* UNDER DIFFERENT SEED SETTING PATTERNS

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Abstract. This study took the unique species of *Cupressus gigantea* in Xizang as the research object, the *Cupressus gigantea* under the seed setting pattern of cone size and position, crown orientation and position, site type and slope direction. 14 indexes of the *Cupressus gigantea* cone and seed were measured and analyzed, to improve the seed quality, guide the field return, and establish the seed quality evaluation system of *Cupressus gigantea*. The results showed that: (1) When collecting the seeds of *Cupressus gigantea*, the mother tree growing on the piedmont slope and sunny slope should be selected first, followed by the terrace, and the larger cones in the south and middle of the crown should be collected first. (2) When returning *Cupressus gigantea* in the field, it is suggested that the returning site should be the sunny slope of the piedmont slope and the terrace. (3) Germination rate, 1000 grain weight, seed viability, superoxide dismutase (SOD) activity and catalase (CAT) activity could be used as representative indices to establish the seed quality evaluation system of *Cupressus gigantea*.
Keywords: *Xizang, Cupressus gigantea, seed quality, field regression*

Introduction

Cupressus gigantea is an arbor of *Cupressus* in Cupressaceae, which is endemic to Xizang, and it was identified as a national first-class protected species (Wang and Xie, 2004). It is mainly distributed in Bayi, Milin and Lang County of Nyingchi along the Yarlung Zangbo River and its tributaries, or forms a sparse forest on the sunny slope with an altitude of 3000~3400 m.

At present, the research of *Cupressus gigantea* mainly focuses on seedling cultivation, community ecology and seed breeding (Yin et al., 2017; Li et al., 2017; Zhang et al., 2006; Zheng et al., 2007; Wang et al., 2005; Chang and Zhao, 2018; Chang et al., 2019), lacking systematic research on seed quality evaluation of *Cupressus gigantea*. Through long-term investigation, the endangered status of *Cupressus gigantea* may be closely related to poor seed quality (Zhang et al., 2006; Zheng et al., 2007; Wang et al., 2005). This paper studied the seeds of *Cupressus gigantea* under different seed setting patterns, and discussed the effective indices for evaluating the seed quality of *Cupressus gigantea* based on the analysis results of 14 indexes. The purpose is to effectively guide researchers and local people to collect and screen out high-quality seeds quickly and comprehensively, thus improving the quality of seedlings, and preparing for the later field regression. By improving seed quality, the endangered *Cupressus gigantea* can be effectively alleviated, and laying a foundation for species protection and artificial propagation.

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Materials and methods

Test materials

Cupressus gigantea seed collection stand is located in Zhaxitang, Dongga, Lang County, Nyingchi, Xizang, 28°59'49"N, 93°18'0"E, 3060 m. The study area is a high mountain canyon area with dry and hot valley climate characteristics. The annual precipitation is about 400 mm. The precipitation is mainly concentrated in April to September, and the highest in June and July. It is a transition area from semi humid to semi-arid in temperate zone. The seeds were collected under different seed setting patterns in May 2017, dried the seeds naturally and stored them at 5℃.

Collected 3 *Cupressus gigantea* mother trees growing on the terrace, and divided the cones at the lower part of the crown in the west into four grades according to the size: < 16 mm, 16~19 mm, 19~22 mm and > 22 mm, 50 cones per grade; The layer of seed scales close to the branches and trunks was defined as the lower cone, the other two layers were the middle cone and upper cone respectively. Three mother trees of *Cupressus gigantea* growing on the terrace were selected, and 50 cones were collected respectively from the lower part of the crown in the south, and the seeds were classified.

The crown of *Cupressus gigantea* was divided into four directions and three layers: East, West, South, North, and upper, middle, lower. Three mother trees of *Cupressus gigantea* growing on the terrace were selected to collect 50 cones from four directions of the crown and 50 cones from different parts of the crown in the south respectively (*Fig. 1*).

Figure 1. *The map of the distribution area and the sampling stand*

The site types of *Cupressus gigantea* are diverse, but most of the seeds came from *Cupressus gigantea* growing on the piedmont slope, terrace, terrace slope and waterline. Three mother trees of *Cupressus gigantea* were selected from different sites, and 50 cones were collected respectively for research.

Piedmont slope can be divided into sunny slope and shady slope according to different slope directions. Three mother trees of *Cupressus gigantea* were selected, and 50 cones growing on sunny and shady slopes were collected respectively for research.

This study collected a total of 21 independent samples of giant cypress, including three independent samples: cone size, cone location, crown orientation, and crown source. Three independent samples were collected for different site types and slope orientations (*Table 1*).

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Table 1. *The seed-setting patterns*

The seed-setting patterns		Number of sampled trees	Number of test seeds	Seed traits and quality
Cone size	< 16 mm	There are 3 independent samples in total, and each independent sample is divided into a small sample on the left	50 × 3	Germination potential (%), Germination rate (%), Cone diameter (mm), Seed length (mm), 1000 grain weight (g), Seed viability (%), Emergence rate (%), Seedling survival rate (%), Soluble protein content (μg), Soluble sugar content (μg), SOD activity (U/g), CAT activity (μg/g), POD activity (U/g), MDA content (μmol/g)
	16~19 mm		50 × 3	
	19~22 mm		50 × 3	
	> 22 mm		50 × 3	
Cone location	Upper		50 × 3	
	Middle		50 × 3	
	Lower		50 × 3	
Crown orientation	East		50 × 3	
	West		50 × 3	
	South		50 × 3	
	North		50 × 3	
Crown source	Upper		50 × 3	
	Middle		50 × 3	
	Lower		50 × 3	
Site types	Piedmont slope	3 independent samples for each sample	50 × 3	
	Terrace		50 × 3	
	Terrace slope		50 × 3	
	Waterline		50 × 3	
Slope orientations	Sunny	3 independent samples for each sample	50 × 3	
	Shady		50 × 3	

Test method

Index measurement

The collected *Cupressus gigantea* samples were mixed according to the seed setting pattern.

The average transverse diameter of 50 cones was used to characterize the size of cones; The average longitudinal length of 100 seeds was used to characterize the seed size; The weight of seeds was characterized by the weight of 1000 seeds and repeated for 3 times.

Seedling test was carried out with the soil of the test site as the substrate, 50 seeds per replicate, 3 replicates in total. The number of seedlings under each seed setting pattern was observed every 3 d from the 15 d, and the survival number of seedlings under each seed setting pattern was observed every half month from the 30 d to 6 months, and the emergence rate and seedling survival rate were calculated.

The seed germination test adopted sand culture method (Chang et al., 2019). The observation was started on the 4th day until the 15th day. The germination quantity was counted, and the germination potential and germination rate were calculated.

The seed viability was measured by TTC staining (Wang et al., 2005). In three repetitions, 50 seeds in each repetition were soaked in 50℃ water for 36.5 h (Chang and Zhao, 2018), and the seed life vitality was determined after full imbibition.

Anthrone-H_2SO_4 colorimetric method, Coomassie brilliant blue G-250 staining method and thiobarbituric acid method (TBA) were used to determine the soluble sugar content, soluble protein content and malonaldehyde (MDA) content of seeds respectively. The activities of superoxide dismutase (SOD), catalase (CAT) and

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peroxidase (POD) were determined by NBT method, ultraviolet spectrophotometry (Tris-HCl) and guaiacol method (Gao, 2006).

$$\text{Germination potential (\%)} = \text{When the number of germinated seeds reaches its peak (7 d)} / \text{Total number of tested seeds} \times 100\%$$

$$\text{Germination rate (\%)} = \text{Number of germinated seeds} / \text{Total number of tested seeds} \times 100\%$$

Data statistical analysis

The main physiological and biochemical indicators during germination, artificial aging, and storage of giant cypress seeds, as well as data on 14 quality evaluation indicators of giant cypress seeds under different fruiting patterns, were organized using Excel 2003 software. The SPSS 25.0 software was used to conduct significant difference testing and correlation analysis on the main physiological and biochemical indicators during the germination, artificial aging, and storage processes of giant cypress seeds. The quality evaluation indicators of giant cypress seeds under different fruiting patterns were subjected to significant difference testing, correlation analysis, principal component analysis, and cluster analysis, and the representative indicators of 14 evaluation indicators were jointly judged, and a quality evaluation system for giant cypress seeds was established using Bayes discriminant formula. We used Photoshop software to organize the images of seed germination morphology and used Origin9.0 software to draw the images.

Results and analysis

Analysis on differences of seed characters and quality of Xizang Cupressus gigantea under different seed setting patterns

It could be seen from *Table 2* that under different seed setting patterns, the seed germination potential was 8~51.33%, the germination rate was 21.33~66.00%, the cone diameter was 17.53~21.86 mm, the seed length was 3.77~5.08 mm, the 1000 grain weight was 2.29~5.34 g, the seed viability was 32.00~76.00%, the seedling survival rate was 5.00~42.50%, the soluble protein content was 123.90~271.86 μg, the soluble sugar content was 61.81~164.97 μg, SOD activity was 903.23~7410.53 U/g, and POD activity was 4.60~107.93 μg/g. Among them, the seed germination potential, germination rate, cone diameter, seed length, seed viability and seedling survival rate of *Cupressus gigantea* growing on the piedmont slope were significantly higher, the activities of SOD and POD in seeds were significantly lower; The seed germination potential, seed length, 1000 grain weight, seed viability, soluble protein and soluble sugar content of cones < 16 mm were significantly lower.

The emergence rate was 6.67~56.67%, the emergence rate of *Cupressus gigantea* growing on the waterline was significantly lower, and the emergence rate of seeds in the middle of the crown was significantly higher; The CAT activity was 0.79~68.94 U/g, it was significantly lower in the cones of 16~19 mm, and it was significantly higher in the seeds growing in the west of the crown; The MDA content was 2.38~15.96 μmol/g, it in the seeds of *Cupressus gigantea* growing in the middle crown was significantly lower, and it in the seeds of the sunny slope was significantly higher.

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Table 2. *Analysis on differences of seed characters and quality of Xizang Cupressus gigantea under different seed setting patterns (ANOVA, P ≤ 0.05)*

Seed setting pattern		Germination potential (%)	Germination rate (%)	Cone diameter (mm)	Seed length (mm)	1000 grain weight (g)	Seed viability (%)	Emergence rate (%)
Cone size	< 16 mm	8.00 a	22.00 a		3.77 a	2.29 a	32.00 a	21.67 abcd
	16~19 mm	11.33 a	29.33 ab		4.25 abcd	3.49 c	50.00 c	26.67 abcd
	19~22 mm	12.67 a	32.00 abc		4.50 bcdef	4.24 fgh	52.00 c	33.33 cde
	> 22 mm	22.00 ab	54.67 ef		4.95 ef	5.34 k	76.00 h	40.00 ef
Cone position	Upper	8.67 a	28.00 ab		4.63 cdef	3.22 b	50.00 c	20.00 abcd
	Middle	9.33 a	32.00 abc		4.89 ef	3.51 cd	54.00 cd	35.00 def
	Lower	18.67 ab	38.00 abcde		4.52 cdef	3.63 cd	62.00 ef	21.67 abcd
Crown orientation	East	16.00 a	42.00 bcde	18.33 ab	4.79 def	3.89 e	76.00 h	35.00 def
	West	18.00 a	37.33 abcde	17.53 a	4.61 cdef	3.88 e	56.00 de	18.33 abcd
	South	24.67 abc	48.67 cdef	19.48 abc	5.08 f	4.39 h	78.00 h	36.67 def
	North	14.00 a	34.00 abcd	20.31 abc	4.66 cdef	3.68 cde	54.00 cd	10.00 ab
Crown position	Upper	17.33 a	40.67 bcde	18.56 ab	3.90 ab	3.61 cd	66.00 fg	30.00 bcde
	Middle	26.67 abc	43.33 bcde	19.33 abc	4.45 bcde	4.05 ef	70.00 g	56.67 f

Table 2. *Continuation*

Seed setting pattern		Seedling survival rate (%)	Soluble protein content (μg)	Soluble sugar content (μg)	SOD activity (U/g)	CAT activity (μg/g)	POD activity (U/g)	MDA content (μmol/g)
Crown position	Lower	10.67 a	37.33 abcde	17.85 ab	4.37 abcde	3.71 de	56.00 de	16.67 abcd
Site type	Piedmont slope	51.33 d	66.00 f	21.86 c	5.06 f	4.71 i	76.00 i	45.00 ef
	Terrace	24.67 abc	53.33 ef	20.72 bc	4.61 cdef	4.27 gh	61.00 ef	33.33 cde
	Terrace slope	38.00 b	51.33 def	19.98 abc	4.25 abcd	4.16 fg	53.00 cd	11.67 abc
	Waterline	21.33 ab	37.33 abcde	17.97 ab	4.05 abc	3.24 b	43.00 b	6.67 a
Slope direction	Sunny	43.33 c	47.33 cde	20.22 abc	4.53 cdef	4.95 j	49.00 c	26.67 abcd
	Shady	21.33 ab	21.33 a	20.81 bc	4.18 abc	4.22 fgh	34.00 a	18.33 abcd
Cone size	< 16 mm	17.50 abc	123.90 a	61.81 a	2507.01 abcd	1.60 a	25.29 a	5.49 e
	16~19 mm	21.67 abcd	144.33 abcd	85.38 cde	3962.79 bcde	0.79 a	56.90 bc	6.73 g
	19~22 mm	25.00 cd	127.71 ab	117.96 g	4776.00 bcde	8.76 ab	23.68 a	9.06 i
	> 22 mm	25.00 cd	132.81 abc	116.57 g	6225.00 e	9.94 ab	56.90 bc	10.53 j
Cone position	Upper	7.50 ab	155.14 cd	95.15 ef	2452.63 abcd	3.38 a	83.91 de	12.43 k
	Middle	20.00 abc	168.71 d	112.22 g	1810.40 abc	7.62 ab	76.44 cd	6.05 f
	Lower	22.50 bcd	156.86 cd	117.33 g	2571.43 abcd	2.70 a	75.86 bcd	9.01 i
Crown orientation	East	37.50 de	151.14 bcd	91.43 ef	2353.40 abcd	4.10 a	58.05 bc	4.90 d
	West	7.50 ab	136.95 abc	81.35 bcde	7410.53 e	2.81 a	107.93 f	6.76 g
	South	26.67 cde	154.86 bcd	93.19 ef	2079.31 abcd	11.70 ab	58.05 bc	11.70 k
	North	7.50 ab	144.67 abcd	81.35 bcde	5092.68 de	21.06 b	89.66 def	6.63 g
Crown position	Upper	25.00 cd	134.05 abc	66.29 ab	4300.99 bcde	52.25 de	15.52 a	3.22 b
	Middle	25.00 cd	232.86 e	104.28 fg	3242.11 abcd	68.94 f	99.43 def	2.38 a
	Lower	15.00 abc	143.29 abcd	70.70 abc	4867.92 cde	1.43 a	75.86 bcd	7.55 h
Site type	Piedmont slope	42.50 e	269.00 f	83.43 cde	903.23 a	4.62 a	4.60 a	7.06 gh
	Terrace	23.33 bcd	271.86 f	85.70 cde	2505.11 abcd	8.37 ab	53.45 bc	4.64 cd
	Terrace slope	5.00 a	253.10 f	89.16 def	2033.68 abcd	39.46 cd	107.47 ef	4.41 cd
	Waterline	5.00 a	268.71 f	75.30 abcd	2529.73 abcd	2.44 a	94.71 def	4.18 c
Slope direction	Sunny	17.50 abc	271.52 f	164.97 h	1315.07 ab	3.17 a	52.30 b	15.96 l
	Shady	5.00 a	267.43 f	87.21 de	2625 abcd	62.63 ef	87.93 def	3.69 bc

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Correlation analysis of seed quality evaluation indexes of Cupressus gigantea

According to *Table 3*, it can be seen that in the evaluation indicators of the quality of giant cypress seeds, germination vigor is significantly positively correlated with seed vitality and soluble sugar content, and extremely significantly positively correlated with germination rate, thousand grain weight, and soluble protein content; The germination rate is significantly positively correlated with seed length, seedling survival rate, soluble protein content, and extremely significantly positively correlated with thousand grain weight and seed viability; There is a significant positive correlation between cone diameter and seedling emergence rate, a highly significant positive correlation with thousand grain weight and soluble protein content, and a highly significant negative correlation with SOD activity; The seed length is significantly positively correlated with seedling emergence rate and soluble sugar content, and extremely significantly positively correlated with thousand grain weight, seed viability, seedling survival rate, and MDA content; The thousand grain weight is significantly positively correlated with seedling emergence rate, soluble protein content, and extremely significantly positively correlated with seed vitality, soluble sugar content, and MDA content; The seed vitality is significantly positively correlated with the emergence rate and seedling survival rate; The emergence rate is significantly negatively correlated with POD activity, and highly significantly positively correlated with seedling survival rate; The survival rate of seedlings is significantly negatively correlated with POD activity; The content of soluble protein is significantly positively correlated with CAT activity, and extremely significantly negatively correlated with CAT activity; There is a highly significant positive correlation between soluble sugar content and MDA content; There is a highly significant negative correlation between CAT activity and MDA content.

Principal component analysis of seed quality evaluation indexes of Xizang Cupressus gigantea

The principal component analysis was carried out on the 14 indexes of seed quality evaluation of *Cupressus gigantea*, and the eigenvalues and variance contribution rate of each evaluation index were obtained.

It could be seen from the table that the eigenvalues of the first four factors were greater than 1, and the cumulative variance contribution rate was 72.562% (>70%). That is, the 4 principal components could basically describe 72.562% of the information of 14 seed quality evaluation indexes.

It could be seen from *Table 4* that in the first principal component, the load of 1000 grain weight (0.839), germination rate (0.698), germination potential (0.658), SOD activity (-0.619) and seedling survival rate (0.619) were the largest; In the second principal component, the load of seed viability (-0.721) and soluble protein content (0.638) were the largest; The load of CAT activity (0.768) and MDA content (-0.713) were the largest in the third principal component; And the load of germination potential (-0.617) was the largest in the fourth principal component (4 components were extracted).

The results of principal component analysis showed that 14 indexes can be used to evaluate the seed quality of *Cupressus gigantea*, but the main evaluation factors were germination potential, germination rate, 1000 grain weight, seed viability, seedling survival rate, soluble protein content, SOD activity, CAT activity and MDA content.

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Table 3. *Correlation analysis of seed quality evaluation indexes of Cupressus gigantea*

Evaluating indicator	Germination potential (%)	Germination rate (%)	Cone diameter (mm)	Seed length (mm)	1000 grain weight (g)	Seed viability (%)	Emergence rate (%)	Seedling survival rate (%)	Soluble protein content (μg)	Soluble sugar content (μg)	SOD activity (U/g)	CAT activity (μg/g)	POD activity (U/g)	MDA content (μmol/g)
Germination potential (%)	1													
Germination rate (%)	0.704**	1												
Cone diameter (mm)	0.216	0.147	1											
Seed length (mm)	0.100	0.314*	0.146	1										
1000 grain weight (g)	0.503**	0.566**	0.494**	0.451**	1									
Seed viability (%)	0.300*	0.604**	0.078	0.524**	0.535**	1								
Emergence rate (%)	0.113	0.213	0.261	0.266*	0.289*	0.421**	1							
Seedling survival rate (%)	0.141	0.300*	0.166	0.348**	0.238	0.569**	0.643**	1						
Soluble protein content (μg)	0.524**	0.308*	0.424**	-0.007	0.328*	-0.042	0.004	-0.065	1					
Soluble sugar content (μg)	0.285*	0.183	0.209	0.289*	0.547**	0.075	0.169	0.119	0.171	1				
SOD activity (U/g)	-0.149	0.059	-0.509**	-0.109	0.038	0.036	-0.102	-0.171	-0.405**	-0.122	1			
CAT activity (μg/g)	0.027	-0.027	0.103	-0.235	0.132	-0.034	0.188	-0.058	0.261*	-0.103	-0.038	1		
POD activity (U/g)	-0.173	-0.188	-0.187	0.048	-0.093	-0.230	-0.307*	-0.464**	0.159	-0.001	0.081	0.231	1	
MDA content (μmol/g)	0.109	0.109	0.082	0.346**	0.357**	0.089	-0.094	0.010	-0.153	0.623**	-0.013	-0.500**	-0.153	1

* indicates significant correlation at the 0.05 level, ** indicates extremely significant correlation at the 0.01 level

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Table 4. *Principal component matrix*

Evaluation index	Principal component			
	1	2	3	4
Germination potential（%）	0.658	0.252	-0.029	-0.617
Germination rate (%)	0.698	-0.194	-0.078	-0.569
Cone diameter (mm)	0.492	0.282	0.390	0.265
Seed length (mm)	0.507	-0.299	-0.323	0.361
1000 grain weight (g)	0.839	0.335	-0.034	0.181
Seed viability (%)	0.549	-0.721	-0.017	0.012
Emergence rate (%)	0.511	-0.398	0.504	0.188
Seedling survival rate (%)	0.619	-0.581	0.211	0.119
Soluble protein content (μg)	0.391	0.638	0.375	-0.202
Soluble sugar content (μg)	0.542	0.565	-0.222	0.176
SOD activity (U/g)	-0.619	-0.346	-0.321	-0.188
CAT activity (U/g)	-0.216	0.101	0.768	0.096
POD activity (μg/g)	-0.595	0.384	0.036	0.127
MDA content (μmol/g)	0.481	0.292	-0.713	0.261
Eigenvalue	4.529	2.486	1.955	1.188
Variance contribution rate (%)	32.350	17.761	13.967	8.484
Cumulative variance contribution rate (%)	32.350	50.110	64.078	72.562

Extraction method: principal component analysis, the first 4 components

Cluster analysis of seed quality evaluation indexes of Xizang Cupressus gigantea

A representative factor can be selected among the indicators with strong correlation. It could be seen from *Figure 2* that the 7 evaluation indexes of germination potential, germination rate, 1000 grain weight, soluble sugar content, MDA content, soluble protein content and cone diameter were similar. There was a very significant positive correlation between 1000 grain weight and germination potential, germination rate, cone diameter, seed viability, soluble sugar content and MDA content, that is, the bigger the seeds, the heavier the seeds, the higher the germination rate and the better the seeds. Among them, the correlation between germination potential and germination rate, and the correlation between 1000 grain weight, soluble sugar content and MDA content were the strongest, the germination rate could more comprehensively reflect the seed germination ability. 1000 grain weight had a significant positive correlation with soluble sugar content, MDA content, soluble protein content and cone diameter, which could more comprehensively and intuitively reflect the seed fineness, so germination rate and 1000 grain weight could be used as representative factors.

Seed viability, seedling survival rate, emergence rate and seed length were similar levels. There was a very significant positive correlation between seed viability and emergence rate and seedling survival rate and seed length, which could more intuitively and quickly reflect seed vitality and could be used as a representative factor. SOD activity and POD activity were similar levels, in which SOD activity could more comprehensively reflect the stress resistance of seeds and could be used as a representative factor.

Therefore, combining the results of cluster analysis and principal component analysis, the original 14 evaluation indexes could be simplified to 5 representative indexes, namely germination rate, 1000 grain weight, seed viability, SOD activity and CAT activity.

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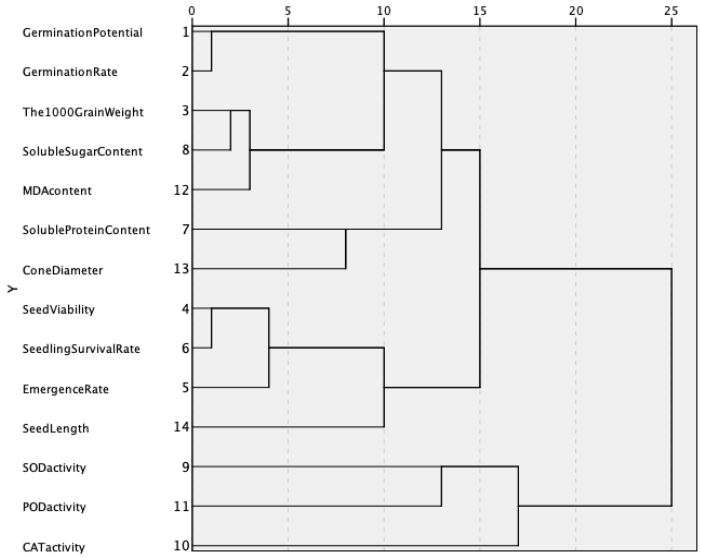

Figure 2. *Cluster dendrogram of seed quality evaluation indexes of Xizang Cupressus gigantea*

Discriminant analysis of seed quality evaluation indexes of Xizang Cupressus gigantea

Through the analysis of germination rate, viability, emergence rate and other indicators of *Cupressus gigantea* seeds under different seed setting patterns, judging the seed quality. The seed quality was divided into three grades: the level 1 is the best, the level 2 is the second, and the level 3 is the worst.

Discriminant analysis was carried out on the 5 evaluation indexes (germination rate, 1000 grain weight, seed viability, SOD activity and CAT activity) of the seed quality of *Cupressus gigantea* obtained through principal component analysis and cluster analysis. The results were shown in *Table 5*.

Table 5. *Classification function coefficient*

Evaluation index	Seed grades		
	Level 1 (Y_1)	Level 2 (Y_2)	Level 3 (Y_3)
Germination rate (X_1)	0.449	0.185	0.150
1000 grain weight (X_2)	15.241	13.434	11.916
Seed viability (X_3)	1.320	1.058	0.807
SOD activity (X_4)	0.000	8.840E-6	0.000
CAT activity (X_5)	0.152	0.087	0.085
(Constants)	-63.466	-43.207	-30.629

From the classification function coefficient, 3 grades discriminant functions for seed quality evaluation of *Cupressus gigantea* were obtained:

Level 1: $Y_1 = -63.466 + 0.449 \times X_1 + 15.241 \times X_2 + 1.320 \times X_3 + 0.152 \times X_5$

Level 2: $Y_2 = -43.207 + 0.185 \times X_1 + 13.434 \times X_2 + 1.058 \times X_3 + 8.840\ E - 6 \times X_4 + 0.087 \times X_5$

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Level 3: $Y_3 = -30.629 + 0.150 \times X_1 + 11.916 \times X_2 + 0.807 \times X_3 + 0.085 \times X_5$

Among them, X_1 is the germination rate index, X_2 is the 1000 grain weight index, X_3 is the seed viability index, X_4 is the SOD activity index, X_5 is the CAT activity index; Y_1 is the seed score of level 1, Y_2 is the seed score of level 2 and Y_3 is the seed score of level 3.

Calculate the value of each evaluation index measured by the sample with the discriminant to obtain the Y value. The category with the largest Y value is the grade of the sample, and then judge the quality grade of the *Cupressus gigantea* seed sample.

Discussion

The seed quality was affected by cone size and position, crown orientation and position, site type and slope direction. The bigger the cone, the better the seed quality; The seed quality of the lower part of the cone was better than that of the middle and upper part of the cone; The seed quality of *Cupressus gigantea* collected from different crown positions was the highest in the south, and the regularity was South > East, West > North; The seed quality in the middle of the crown was better than that in the upper and lower part of the crown; Under different site types, the law of seed quality of *Cupressus gigantea* was piedmont slope > terrace > terrace slope > waterline; The seed quality of the mother tree of *Cupressus gigantea* growing on the sunny slope was better than that on the shady slope.

The development of cones and seeds were affected by light conditions, which may be the main reason for the poor quality of seeds in the shady slope and the north of the crown; The bigger the cone was and the closer it was to the branches, the better the nutrition supply was. Moreover, the upper branches of the crown were younger and the lower branches were older, which were not conducive to the nutrition supply and affected the healthy growth of the seeds; The worse the growth environment was, the more restricted the nutrition supply of the mother tree to its seed development was, and the seed quality was relatively poor.

There were a large number of non-germinated seeds in the seeds of *Cupressus gigantea*. Through the analysis of abortive seeds in the seeds of Cunninghamia lanceolata, it is found that the formation of abortive seeds is closely related to site conditions, climatic conditions, pollination methods, embryo competition, embryo development, genetics and other factors (Yu and He, 1989; Shi, 1998). Therefore, the reason of low seed quality of *Cupressus gigantea* was speculated and further analyzed.

The 5 indexes of germination rate, 1000 grain weight, seed viability, SOD activity and CAT activity were taken as the evaluation indexes of seed quality of *Cupressus gigantea*. Among them, the germination rate, 1000 grain weight and seed viability were in line with the evaluation indexes of forest seed quality (Hampton et al., 1995), and as the quality evaluation indexes of plant seeds for many times and establish the classification standard (Xu et al., 2009; Niu, 2014).

The germination potential and germination rate are both indicators reflecting the germination ability of seeds, and show a highly significant positive correlation. The thousand grain weight, cone diameter, and soluble protein content are all significantly positively correlated. The three indicators and soluble sugar content can to some extent express the degree of material accumulation of cones and seeds, thereby reflecting the goodness of seeds. The MDA content can reflect the degree of stress damage to plants.

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Research has found that the soluble sugar content in giant cypress seeds is significantly positively correlated with the MDA content, indicating that the measurement of MDA content in plant tissues is mainly affected by the interference of soluble sugars (Zhou et al., 2013). The seed length, seed vitality, emergence rate, and seedling survival rate of giant cypress are significantly positively correlated, which can reflect the germination ability and seed vitality of seeds to a certain extent, and thus reflect seed quality. SOD, POD, and CAT can synergistically reflect the anti-aging ability of plants.

Conclusion

(1) When collecting the seeds of *Cupressus gigantea*, the mother tree growing on the piedmont slope and sunny slope should be selected first, followed by the terrace, and the larger cones in the south and middle of the crown should be collected first.

(2) When returning *Cupressus gigantea* in the field, it is suggested that the returning site should be the sunny slope of the piedmont slope and the terrace.

(3) The seed quality evaluation system of *Cupressus gigantea* was established by the germination rate, 1000 grain weight, seed viability, SOD activity and CAT activity, and the seed quality was divided into three grades.

As a unique national first-class protected species in Xizang, *Cupressus gigantea* is of great significance for environmental protection and paleocatate research in Xizang because of its long growth period. This paper preliminarily discusses the relationship between *Cupressus gigantea* seed quality and its endangered. In the future, the relationship between seeds and endangered of *Cupressus gigantea* will be discussed from the cellular and genetic level, the seedlings will be the research object to understand the causes of endangered, and the field regression of *Cupressus gigantea* will be practiced to explore the best conditions.

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