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Polyploid induction and characteristic analysis of *Pteroceltis tatarinowii Maxim*

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Abstract: *Pteroceltis Tatarinowii Maxim is a deciduous tree of eulaceae in China. It is a unique fibrous tree species and a national first-grade key protected plant, mainly distributed in 19 provinces such as Anhui, Fujian, Zhejiang, Hubei, etc. It is usually born in the thin forest of limestone mountains by valleys and streams. Pteroceltis Tatarinowii Maxim is a pioneer in afforestation of limestone, sandstone, mountain and riverbank. In addition, Pteroceltis Tatarinowii Maxim sets papermaking, wood, feed, medicinal value in one, especially the unique properties of Pteroceltis Tatarinowii Maxim bast fiber, is an irreplaceable raw material for making rice paper, with important market value. Referring to relevant papers and literatures, it is found that there are few researches on Pteroceltis Tatarinowii Maxim in foreign countries, while there are relatively more researches on Pteroceltis Tatarinowii Maxim in China at present, which are limited to seed breeding, development and utilization, afforestation experiments and other experimental research directions. Many research institutions or enterprises are studying the key technologies and commercial development of forest germplasm innovation, but there are few researches on the germplasm innovation of Pteroceltis Tatarinowii Maxim. There is only one book of Pteroceltis Tatarinowii Maxim China written by Nanjing Forestry University on the market, but there is no research on the breeding of Pteroceltis Tatarinowii Maxim. In this study, Pteroceltis Tatarinowii Maxim seedlings were selected as the research object, and a new variety of Pteroceltis Tatarinowii Maxim was screened by coldnarcissus solution soaking growing point treatment with different concentration and time, and the bark fiber characteristics of new Pteroceltis Tatarinowii Maxim germplasm were analyzed, aiming at filling the blank of Pteroceltis Tatarinowii Maxim breeding in China and cultivating a new variety of Pteroceltis Tatarinowii Maxim with rapid growth, high quality and resistance.*

Keywords: *Pteroceltis Tatarinowii Maxim; polyploid induction; Pteroceltis Tatarinowii Maxim; characteristic analysis*

1. Introduction

Pteroceltis tatarinowii Maxim. is a deciduous tree of the genus Dalbergia of Ulmaceae, also known as Pteroceltis Tatarinowii Maxim bark, peeling elm and magnolia. It is an endemic fiber tree species and national rare and endangered tree species in China, mainly distributed in Anhui, Zhejiang, Fujian and other 19 provinces. Pteroceltis Tatarinowii Maxim has strong adaptability, likes calcium, likes to be born in limestone mountains, and can also grow in granite and sandstone areas. The stem bark and branch bark fiber of Pteroceltis Tatarinowii Maxim are the high quality raw materials for making well-known calligraphy and painting Xuan paper at home and abroad; Pteroceltis Tatarinowii Maxim is solid, compact, tough and wear-resistant for furniture, agricultural tools, drawing boards and cabinetwork; seeds can extract oil. In addition, Pteroceltis Tatarinowii Maxim is a unique single-genus plant in China, which has important academic value for the study of the phylogeny of Ulmaceae.

Chromosome is the carrier of genetic material DNA. It is generally believed that the plant after chromosome doubling is characterized by huge shape, thickened leaves, deepened leaf color, strong resistance and so on, which is an effective means for selecting timber and resistant tree species[1]. In the process of polyploid induction, chromosome mutations, such as chromosome inversion, translocation, etc., often occur, which produces many types of variation in the process of polyploid induction. It shows great genetic plasticity. At present, the polyploid induction of plants with colchicine has been successful in a variety of plants. As an important wood species and resistant tree species for rice paper, the current research is mainly focused on population distribution, resistance test, cultivation and reproduction, development and application, genetic structure analysis and so on[2]. whether polyploid

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Pteroceltis Tatarinowii Maxim has advantages in phloem fiber synthesis and resistance, and the genetic plasticity of polyploid Pteroceltis Tatarinowii Maxim is mostly a blank in Pteroceltis Tatarinowii Maxim breeding at present. The research in this aspect not only fills the blank of ploidy breeding of Pteroceltis Tatarinowii Maxim in China, but also is an effective way to broaden the cultivation of new varieties of fast-growing, high-quality and stress-resistant Pteroceltis Tatarinowii Maxim. In the genetic study of polyploid plants, most scholars have studied the variation rate and heritability of naturally formed polyploids[3]. however, there are few studies on artificially induced polyploid plants, and it has not been reported in Pteroceltis Tatarinowii Maxim. In this study, autotetraploid Pteroceltis Tatarinowii Maxim was induced by colchicine, and the quality difference of different ploidy Pteroceltis Tatarinowii Maxim bark was analyzed, which provided a theoretical basis for further polyploid breeding and giving full play to the advantage of pterodendron polyploid.

2. Materials and methods

2.1 Experiment time and place

This experiment was conducted in Taishan Forestry Research Institute from October 2020 to June 2021.

2.2 The experimental method

2.2.1 Polyploid induction in Pteroceltis Tatarinowii Maxim

At the end of October to early November 2020 to collect the same maternal of preroceltis tatarinowii maxim, sowing in hole after sand hidden budding disc, the following early April seedlings, unearthed from cotyledon flattened stay true leaf stirring, will dip in with concentration of 0.4%, 0.6%, 0.8% and 1.2% colchicine solution of absorbent cotton ball on the stem tip of seedlings growing point, respectively processing 24 h, 48 h, 72 h. Drop 1 to 3 drops each time in the morning and evening, and then cover with plastic film and sun screen. Each treatment is 30 plants and 4 times, a total of 120 plants.Calculate the treatment time from the first drop, remove the absorbent cotton ball after the predetermined time, and rinse with clean water to remove the effect of the liquid medicine. To identify the ploidy of plants with existing variation (thick and coarse leaves, short plants).

2.2.2 Polyploid identification of Pteroceltis Tatarinowii Maxim

Chromosome identification: The stem tips of *Pteroceltis Tatarinowii* Maxim to be measured were extracted, and the number of chromosomes in the stem tips of diploid and tetraploid *Pteroceltis Tatarinowii* Maxim seedlings was identified by conventional stem tip tablet method. ZEISS optical microscope was used for observation and imaging.

Flow cytometry identification: About 0.2g of seedling top leaves were taken for ploidy identification by BD FACS Calibur flow cytometry at Shandong Agricultural University.

2.2.3 Morphology of Pteroceltis Tatarinowii Maxim fiber

The 2-year twigs of diploid and tetraploid Pteroceltis Tatarinowii Maxim were taken, and the fibers were extracted by the method of Fang Shengzuo(2001), and the fibers were sliced and digitally imaged with DT2000 biological microscope. The length and width of fibers were measured systematically, and 30 fibers were measured per treatment.

2.2.4 Pteroceltis Tatarinowii Maxim characteristic analysis

10 strains of 2-year old diploid and tetraploid Pteroceltis Tatarinowii Maxim were randomly selected to determine and calculate the basic density, water content, ash content, benzene-alcohol extract, synthetic cellulose, acid insoluble lignin, acid soluble lignin and other components according to the national standard method.

2.2.5 Comprehensive evaluation of Pteroceltis Tatarinowii Maxim properties

The measured values of each indicator are converted quantitatively by the membership function calculation formula, which is as follows:

Xij=(xij-ximin)/(ximaxin), Xij represents the ith measurement index of the j sample, $U(Xi) \in [0,1]$. The membership function values of all indexes of each variety were accumulated to obtain the average membership function values of each variety.

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2.3 The data analysis

SPSS19.0 was used to conduct anOVA and DUNCAN multiple comparison on the obtained data, and the significance of difference between different ploidy was tested at the level of 0.05. Use Excel2010 for data collation and chart drawing.

3. Results and analysis

3.1 Induction effect of Colchicine on Rosewood seedlings

The induction results of different colchicine concentrations on seedlings were shown in Table 1 and Figure 1. The results showed that the morphological variation rate of colchicine was 33.3% and 34.2%, the mortality rate was 12.5% and 13.3%, and the induction effect was the best.The tetraploid Pteroceltis Tatarinowii Maxim showed broad and sturdy leaves. Leaves dark green; The leaf surface has the growth characteristic of wrinkle and rapid growth.Since most of the plants with external morphological variation will have chromosomal ploidy changes, colchicine induced morphological variation of Pteroceltis Tatarinowii Maxim can be used as a preliminary identification index for polyploid plants.Treatment with too high or too low colchicine concentration has a great influence on inducing polyploid effect. Therefore, 72h treatment with 0.6% -- 0.8% colchicine concentration is appropriate for inducing polyploid of Pteroceltis Tatarinowii Maxim.

Table 1 Induction effects of colchicines concentrations and treatment timeson seeds of Pteroceltis tatarinowii Maxim.

Concentration /%	Treatmen t time/h	Seedling number	Deaths number	Mortality/%	Variation number	Mutation rate/%
0	0	120	0	0e	0	0h
	24	120	0	0e	2	1.7 ±0.0096gh
0.2	48	120	0	0e	5	4.2 ±0.0083fgh
	72	120	0	0e	7	5.8 ±0.0083fg
	24	120	10	8.3 ±0.0215d	16	13.3 ±0.0136bcde
0.4	48	120	12	10 ±0.0236d	18	15 ±0.0215bc
	72	120	16	13.3 ±0.0272cd	39	32.5 ±0.0160a
	24	120	11	9.2 ±0.0285d	10	8.3 ±0.0096def
0.6	48	120	12	10 ±0.0136d	17	14.2 ±0.0160bcd
	72	120	15	12.5 ±0.0285cd	40	33.3 ±0.0304a
	24	120	11	9.2 ±0.0083d	11	9.2 ±0.0210cdef
0.8	48	120	15	12.5 ±0.0250cd	20	16.7 ±0.0136b
	72	120	16	13.3 ±0.0136cd	41	34.2 ±0.0438a
	24	120	20	16.7 ±0.0236bc	15	12.5 ±0.0083bcde
1.2	48	120	25	20.8 ±0.0160ab	10	8.3 ±0.0167def
	72	120	29	24.2 ±0.0160a	9	7.5 ±0.0160ef

Note:Different small letters in the same arrange data indicate significant difference(P<0.05).

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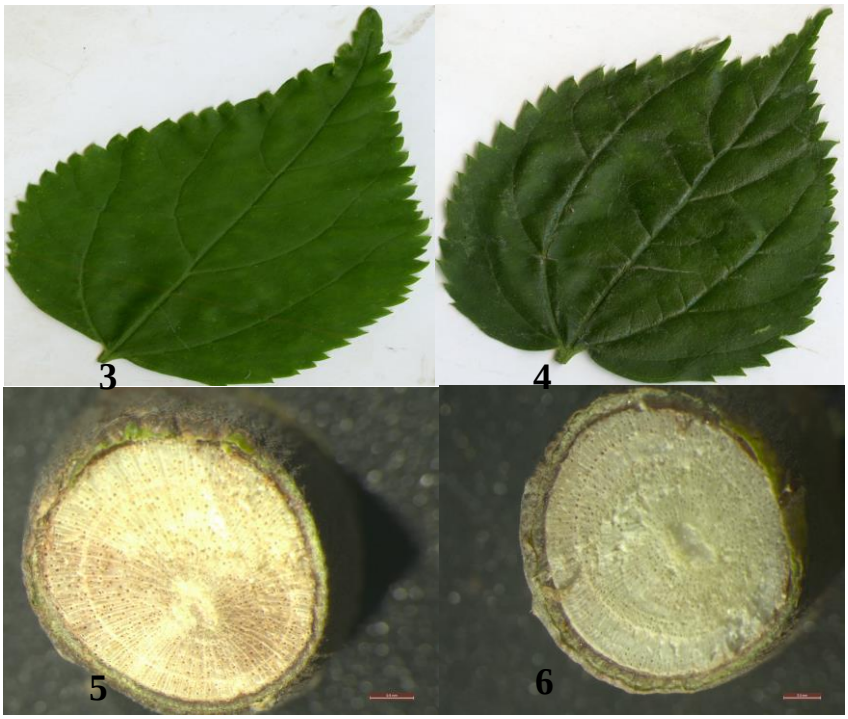

Fig.1 Induction effects of colchicines on seeds variation of Pteroceltis tatarinowii Maxim.

(note:Number of 1, 3, 5 is the diploid;Number of 2, 4, 6 is the tetraploid)

3.2 Polyploid identification of Pteroceltis Tatarinowii Maxim

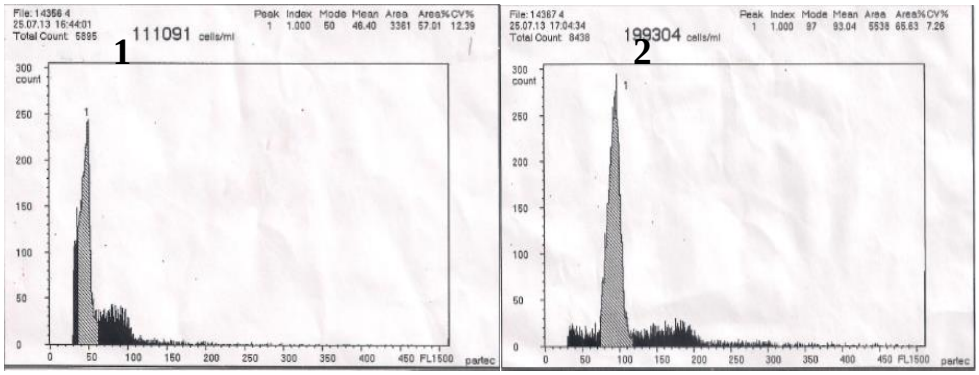

Fig.2 Flow cytometric appraisal results of Pteroceltis tatarinowii Maxim.

(note:Number of 1 is the diploid;Number of 2is the tetraploid)

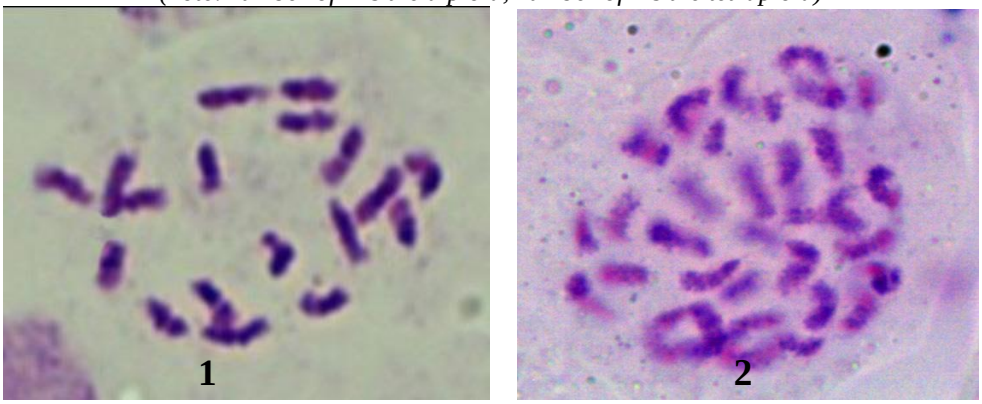

Fig.3 Chromosome observation of apices for Pteroceltis tatarinowii Maxim.

(note: Number of 1 is the diploid;Number of 2is the tetraploid)

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Flow cytometry was used to detect the cell DNA content of both diploid and suspected tetraploid plants (FIG. 2).The results showed that there was only one unimodal peak at the position of relative fluorescence intensity of about 50, and only one unimodal peak at the position of relative fluorescence intensity of about 100, indicating that the DNA content in the cells was doubled compared with the control, which was tetraploid.

Of tetraploid plant cell chromosome counting identification of preroceltis tatarinowii maxim, the conventional tabletting method for natural green wingceltis of crops grown in the diploid and tetraploid induced plant stem tip chromosomal examination, found the diploid chromosome number of preroceltis tatarinowii maxim 2 n = 2 x = 18, after colchicine solution treatment of plants, although there is a certain percentage of the high proportion of diploid and Mosaic, but also won the more homozygous tetraploid, through chromosome number is 2 n = 4 x = 36 (figure 3).

3.3 Morphological analysis of Pteroceltis Tatarinowii Maxim fiber

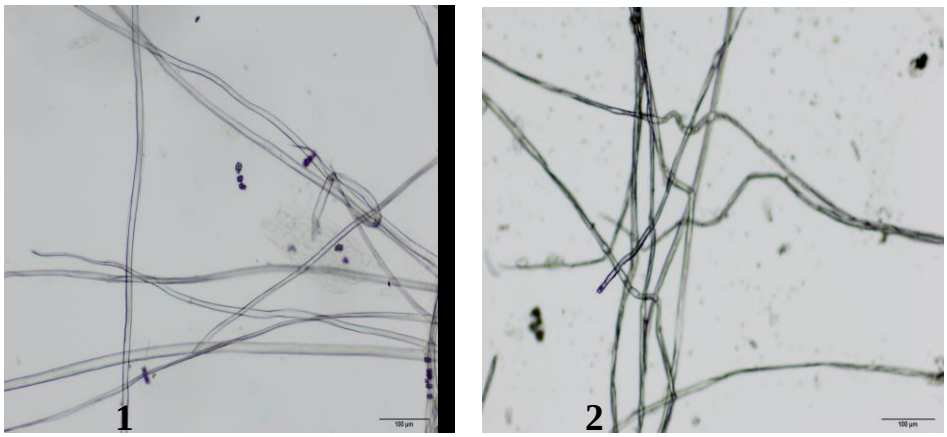

Fig.4 fiber observation of apices for Pteroceltis tatarinowii Maxim.(note:Number of 1 is the diploid;Number of 2is the tetraploid)

Table 2 Fiber observation of different apices of Pteroceltis tatarinowii Maxim.

Sample	fiber length/um	fiber width/um	fiber length / width
Diploid	2450.884±89.2096a	10.52850±0.5367a	207.5894±15.5410a
Tetraploid	3053.7016208.6007b	11.91300±0.5941b	270.9973±15.2714b

Note: Different small letters in the same arrange data indicate significant difference(P<0.05).

As shown in Figure 4 and Table 2, the average fiber length, width and length-width ratio of tetraploid Pteroceltis Tatarinowii Maxim was significantly higher than that of diploid Pteroceltis Tatarinowii Maxim. The average fiber length, width and length-width ratio of diploid Pteroceltis Tatarinowii Maxim were 2450.88um, 10.53um and 207.59 um, respectively. The average fiber length, width and length-width ratio of tetraploid Pteroceltis Tatarinowii Maxim were 3053.70um, 11.91um and 270.99 respectively. The average fiber length, width and length to width ratio of tetraploid Pteroceltis Tatarinowii Maxim were 24.59%, 13.11% and 30.54% higher than diploid, respectively.Generally considered ordinary tan leather average fiber length value in 2126 ~ 2788 um, average width in 9.84 ~ 11.99 um, average aspect ratio in 186.40 ~ 268.98, is a very good paper making raw materials, this study found that tetraploid wingceltis of preroceltis tatarinowii maxim leather fibre length, length-width ratio were significantly higher than that of control, therefore, tetraploid green wingceltis may be a good raw material to make paper.

3.4 Pteroceltis Tatarinowii Maxim characteristic analysis

The 7 fiber components of Pteroceltis Tatarinowii Maxim in 20 samples are shown in Table 3. Average is a parameter of the set of reaction variables and an important characteristic number of variables.As can be seen from Table 2, the basic density, moisture content, ash content, benzene-alcohol extract, total cellulose content, acid soluble lignin content and acid insoluble lignin content in diploid Pteroceltis Tatarinowii Maxim bark are 0.2512, 40.03%, 6.31%, 8.15%, 65.86%, 0.71% and 17.02%, respectively. The basic density, moisture content, ash content, benzoyl alcohol

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extract, synthetic cellulose content, acid lysolvable lignin content and acid insoluble lignin content were 0.2928, 31.03%, 6.25%, 7.70%, 71.22%, 0.59%, 18.8%, respectively.Compared with diploid, tetraploid Pteroceltis Tatarinowii Maxim bark's basic density, total cellulose content and acid insoluble lignin content were 14.21%, 7.52% and 9.89% higher than diploid, respectively; water content, ash content, benzene-alcohol extract and acid insoluble lignin content were 22.48%, 0.95%, 5.52% and 16.90% lower than diploid.Standard deviation, standard error and coefficient of variation can represent the discrete characteristics of variables. The larger the statistical value is, the greater the dispersion degree of variables is.Based on the three indexes, it can be seen that among the components of Pteroceltis Tatarinowii Maxim leather, in diploid Pteroceltis Tatarinowii Maxim and tetraploid Pteroceltis Tatarinowii Maxim, the dispersion of benzene-alcohol extract is the largest, while that of brown cellulose is the smallest.The dispersion of basic density, water content, ash content and acid insoluble lignin content in tetraploid Pteroceltis Tatarinowii Maxim fiber was higher than that in diploid Pteroceltis Tatarinowii Maxim fiber, and that of benzene-alcohol extract, synthetic cellulose and acid insoluble lignin content was lower than that in diploid Pteroceltis Tatarinowii Maxim fiber.

Table 3 Description statistics for bark compositition of P.tatarinow

Variation characteristics		Basic density	moisture /%	ash/%	benzene-alcohol extract/%	holocellulose /%	acid-soluble lignin/%	acid-insoluble lignin/%
Average number	Diploid	0.2512	40.03	6.31	8.15	65.86	0.71	17.02
	Tetraploid	0.2928	31.03	6.25	7.70	71.22	0.59	18.89
Standard error	Diploid	0.0083	0.4145	0.0920	0.0308	1.1536	0.6622	0.8998
	Tetraploid	0.0080	0.0168	0.1086	0.1491	2.4694	0.5388	0.6660
Standard deviation	Diploid	0.0143	0.7054	0.1593	0.0533	2.1139	0.0217	1.6444
	Tetraploid	0.0140	0.0284	0.1794	0.2579	4.2561	0.0149	1.1536
Minimum value	Diploid	0.2376	40.02	6.305	8.10	62.8	0.67	14.56
	Tetraploid	0.2691	40.06	6.311	8.21	66.4	0.74	17.35
Maximum value	Diploid	0.2627	31.01	5.944	7.34	67.5	0.56	17.22
	Tetraploid	0.2961	31.06	6.284	7.84	75	0.61	19.32
Coefficient of variation	Diploid	0.246	0.285	0.180	0.831	0.138	0.512	0.308
	Tetraploid	0.248	0.291	0.184	0.826	0.132	0.493	0.312

Table 4 Correlativity between each two bark compositions of Pteroceltis tatarinowii Maxim.

(The lower left is diploid, and the right is tetraploid)

Fiber characteristics	1	2	3	4	5	6	7
1	1.000	0.428	-0.011	0.486	0.285	0.154	0.735
2	0.771	1.000	-0.629	0.116	0.362	-0.469	-0.033
3	0.017	0.696	1.000	-0.504	-0.931	0.579	-0.136
4	0.475	0.817	0.876	1.000	0.180	0.385	0.962
5	0.316	0.264	0.819	0.626	1.000	-0.772	-0.133
6	0.754	0.095	-0.739	-0.329	-0.856	1.000	0.450
7	0.365	0.902	0.913	0.838	0.480	-0.363	1.000

Note:1.Basicdensity;2.Moisture;3.Ash;4.Benezene-alcoholextraction;5.Holocallulos;6.Acid-solublelignin;7.Acid-insoluble lignin

Bivariate correlation analysis of the percentage content of 7 fiber components in Pterostilbene (Table 4) showed that there was a great correlation between different fiber components.The correlation between ash and acid insoluble lignin was 0.913. The correlation between basic density and water content was the lowest, 0.017. In pairwise comparison, ash was negatively correlated with acid-lysol, benzene-alcohol extracts with acid-lysol, syncellulose and acid-lysol, while other components were positively correlated with each other.In tetraploid Pteroceltis Tatarinowii Maxim, the correlation between benzene-alcohol extract and acid-insoluble lignin was the highest, 0.962. The correlation between basic density and ash content is the lowest, which is -0.011. In pairwise comparison, the basic density was negatively correlated with ash, water and acid insoluble lignin, water and acid insoluble lignin, ash and acid insoluble lignin, syncellulose and acid insoluble lignin, syncellulose and acid insoluble lignin, and other components were positively correlated pairwise. Visible, different polyploidy between Pteroceltis Tatarinowii Maxim fiber composition difference, easy to be affected by other factors.

3.5 Comprehensive evaluation of Pteroceltis Tatarinowii Maxim properties

As can be seen from the results of the comprehensive evaluation of the characteristics of Pteroceltis Tatarinowii Maxim with different ploidy in table 5, the comprehensive score of Pteroceltis Tatarinowii

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Maxim diploid was 0.26, and that of Pteroceltis Tatarinowii Maxim tetraploid was 0.31. The ranking of the comprehensive evaluation values was: Pteroceltis Tatarinowii Maxim tetraploid >. Therefore, according to the experimental data, it was concluded that the characteristics of the two year tetraploid Pteroceltis Tatarinowii Maxim were better than that of the diploid Pteroceltis Tatarinowii Maxim.

Table 5 Evaluation of the quality between each two bark compositions of Pteroceltis tatarinowii Maxim

Variety	Fiber length	Fiber width	fiber length / width	Basic density	moisture	ash	benzene -alcohol extract	holocellulose	acid-soluble lignin	acid-insoluble lignin	Synthesis
Diploid	0.04	0.11	0.26	0.13	0.35	0.12	0.36	0.32	0.27	0.39	0.26
Tetraploid	0.27	0.43	0.36	0.17	0.13	0.29	0.29	0.45	0.29	0.47	0.31
Weight	0.05	0.05	0.10	0.10	0.05	0.10	0.15	0.15	0.10	0.15	1

4. Discussion

Colchicine concentration and treatment time were the key factors affecting the induced polyploid, and the different varieties and types of induced materials also greatly affected the induced polyploid rate[4-6]. Zhang Zhisheng et al. showed that after anthurium was cultured in liquid medium containing 0.2g Ł$^{-1}$ colchicine for 14 days, polyploid induction effect was the best (45.5%). Zhang haifeng et al. found that 0.1% colchicine treatment for 12 h induced the tetraploid eucommia ulmoides was the best.In this study, it was found that the best combination was treated with 0.6-0.8% colchicine solution for 72 h, and the highest tetraploid induction rate was 34.2%.

In this paper, the characteristics of different ploidy Pteroceltis Tatarinowii Maxim were studied for the first time. The preliminary conclusions are as follows: the average fiber length, width and lengthen width ratio of tetraploid Pteroceltis Tatarinowii Maxim were 24.59%, 13.11% and 30.54% higher than diploid. The basic density, total cellulose content and acid insoluble lignin content of tetraploid Pteroceltis Tatarinowii Maxim were 14.21%, 7.52% and 9.89% higher than diploid Pteroceltis Tatarinowii Maxim, respectively.

That can be used to make paper irreplaceable raw materials, fiber characteristics of preroceltis tatarinowii maxim and the rice paper product, there are many process between fiber characteristics of preroceltis tatarinowii maxim ink and paper properties such as embellish, deformation, etc should be further research[7].The relationship between the yield and quality is our next research direction, the relation between the clear tetraploid wingceltis of preroceltis tatarinowii maxim leather production, quality and rice paper on the basis of performance, subject to further analysis phloem fiber synthase gene expression and regulation of preroceltis tatarinowii maxim, paper pulp forest of preroceltis tatarinowii maxim directive breeding in the future, tan leather quality evaluation and improve the quality of our paper provide a scientific basis.

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