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Comparative Study on Pollen Viability of *Camellia oleifera* at Four Ploidy Levels

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Abstract: Oil tea (*Camellia oleifera* Abel.) is one of the most important woody edible oil tree species in China, with intraspecific polyploid. In order to study the variation in pollen size and vigor of *C. oleifera* at ploidy level, four ploidy covers a total of 32 types of Camellia pollens as the material for the experiment. The results showed that the pollen sizes of diploid, tetraploid, hexaploidy, and octaploid were positively correlated with the ploidy level. Pollen viability of *C. oleifera* was determined by fluorescein diacetate (FDA) dye solution staining and medium containing 10% sucrose, 0.01% boric acid, and 1% agar germination in vitro, which indicated that the pollen viability and germination rate of the hexaploid were relatively high among the four ploidy levels, at 79.69% and 71.78% respectively. The pollen vigor of diploid NR-3, tetraploid DP43, hexaploid CJ-12, and octoploid YNYC-1 was higher than that of other materials with the same ploidy level. Knowledge of different ploidy pollen sizes and pollen viability provides basic information for formulating pollen breeding plans and pollination methods of *C. oleifera*.

Keywords: oil tea; ploidy; pollen size; pollen viability; pollen germination rate

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1. Introduction

Oil tea (*Camellia oleifera* Abel.) belongs to the genus Camellia in the family Theaceae, which, together with oil palm (*Elaeis guineensis* Jacq.), olive tree (*Olea europaea* L.), and coconut palm (*Cocos nucifera* L.), is considered to be one of the four major woody oil crops in the world [1,2]. Tea oil, extracted from camellia seeds, is known as "oriental olive oil" because of its high nutritional value and health benefits [3]. It is rich in unsaturated fatty acids (more than 80% of the total oil content), mainly containing monounsaturated fatty acids (oleic acid, accounting for more than 68% of the total oil content) and part of polyunsaturated fatty acids (linoleic acid and linolenic acid) [3,4]. Studies have shown that camellia oil intake has health benefits, such as helping to lower blood fats and prevent cardiovascular disease [5]. In order to meet the rapidly growing demand for healthy vegetable oil, the Chinese government planned to increase the planting area of *C. oleifera* to more than 4 million hectares and the annual output of *C. oleifera* to 2.5 million tons [6]. With the rapid development of the camellia oil industry, a large area of *C. oleifera* is planted in the red soil hilly region of south China. Jiangxi, Hunan, and Guangxi provinces are the main cultivation areas, accounting for 76% of the national total output [1,7]. At present, however, the key problem in *C. oleifera* cultivation is to accelerate and improve the selection and breeding of different regions and improve the yield and quality of *C. oleifera* [6].

Pollen grains, which are produced in the anthers of higher plants, as sexual reproductive units and carriers of male genetic material, play an important role in plant breeding [8,9]. To support breeding programs, pollen fertility analysis is an indispensable prerequisite for field crossbreeding. Considering that fertilization is achieved by cross-pollination, during which the stigma is in a receptive state, the success of cross-pollination is also

related to the ability of pollen germination [10]. Understanding the floral characteristics of existing germplasm, such as pollen viability and germination ability, is critical to the selection of donors for hybridization [11]. According to Nogueira et al. [12], in order to assess in vivo pollen germination, field hybridization must be performed and the results must be monitored. However, this process is time-consuming and expensive, so to reduce cost and time, as well as to aid decision-making, methods of assessing pollen viability by staining or in vitro germination are effective alternatives.

Polyploidy, also known as genome-wide replication, is a major mechanism of plant adaptation and speciation [13,14]. It is widespread in plant families and an estimated 47–70% of angiosperm species are polyploid [13]. Polyploidy can serve as a bridge for interspecific hybridization between two species previously separated by ploidy differences [15]. It has been reported that ploidy levels affect pollen tube growth and seed viability; ploidy crossing of fertile offspring may produce new, desirable traits in established varieties or allow the transfer of traits between varieties [16]. The species of oil tea not only vary greatly in traits, but also have different ploidy among some varieties [17]. Breeding improvement using mixed ploidy levels requires cytological methods to evaluate the fertility of potential parents and the suitability of potential hybrid combinations [16]. Pollen fertility can be evaluated by pollen viability, which is staining with nuclear stains and in vitro germination [18]. At present, there is no report on the pollen fertility of *C. oleifera* with different ploidy. Therefore, vigor staining and in vitro germination methods were used in this study to evaluate the fertility of some types of *C. oleifera* pollens at four ploidy levels. What is more, studies have shown that, in addition to pollen vitality, the size of pollen grains also has an impact on pollen fertility [19], and pollen size can be influenced by ploidy levels [20], so we measured pollen size in this experiment. The results of this paper will shed light on the study of pollen fertility of new hybrids and guide the hybrid breeding strategies of different varieties for *C. oleifera.*

2. Materials and Methods

2.1. Plant Materials and Pollen Collection

The unopened buds of *C. oleifera* were collected from a planted forest called the state-owned Dongfeng Forest Farm, Liping, Guizhou Province, China. The gathering time was about 10 a.m. every day, and the flowering period did not reach the peak. The anthers of each bud are picked off with tweezers in a small carton that had been folded in advance and placed at room temperature for about 4 h to disperse the pollen. Then, they were placed in a 1.5 mL centrifuge tube and stored in a −80 °C refrigerator for temporary storage.

2.2. Pollen Ploidy

Flow cytometry (Sysmex CyFlow Space, Sysmex, Kobe, Japan) was used to detect the ploidy of oil tea pollens of different types [21–23]. First, we added an appropriate amount of sterile water to the small centrifugal tube containing the pollen and soaked the pollen for about 20 min. The pollen immersed in the bottom of the tube was sucked out into a new small centrifugal tube and the pollen of each sample was incubated in 20 µL of 40% polyvinyl pyrrolidone and 400 µL of nuclei extract buffer (CyStain®UV PRECISE P, Sysmex, Kobe, Japan) for 5 min in the dark. The mixture was then filtered through a 30 µm CellTrics®filter into a sample tube. Then, 1600 µL of staining buffer (CyStain®UV PRECISE P) was added and it was left for 5 min [24]. Finally, flow cytometry analysis was performed using ultraviolet excitation (λ = 355–375 nm) and blue fluorescence emission parameters (λ = 435–500 nm) (rate, nuclei per second). The analysis of each sample was performed with three replications according to the metology requirements [23].

2.3. Pollen Size

Pollen diameter was measured after Carbopol fuchsin dye staining. Appropriate amount of pollens were placed on the slide and stained with 50 uL Carbopol fuchsin dye solution for 10 s, then covered immediately with a cover glass [23], and then respectively

observed and photographed with an Olympus BX51 compound microscope (Olympus Corp., Tokyo, Japan). The polar and equatorial lengths of pollens were observed by scanning electron microscope (SEM-6380LV, JEOL, Tokyo, Japan) [25,26]. Pollen size was measured using image processing software Image J (National Institutes of Health, Bethesda, Rockville, MD, USA) [27].

2.4. Pollen Viability

In this study, we used fluorescein diacetate (FDA) staining to evaluate pollen viability [28]. According to the criteria for the estimation of pollen efficiency given by Rotreklová and Krahulcová [29], pollen that emits yellow-green fluorescence was considered as active. Each treatment consisted of three replicates.

2.5. Pollen Germination

The in vitro germination method was used to determine the germination rate of pollens at four ploidy [30]. The culture medium for in vitro germination of *C. Oleifera* pollens obtained by previous screening was 1% agar + 10% sucrose + 0.01% boric acid [31].

2.6. Statistical Analysis

SPSS 19.0 software (IBM, New York, NY, USA) was used for data analysis and one-way analysis of variance (ANOVA) was used to test the differences in pollen size, pollen viability, and pollen germination rate of different ploidy. When $p \leq 0.05$, we used the Duncan multiple comparison method to evaluate the significant difference between the means. The origin pro8.5 software (origin lab company, Northampton, MA, USA) was used to draw graphs [32].

3. Results

3.1. Pollen Size Measured after Staining and Scanning Electron Microscopy

In this experiment, we first determined the ploidy of 32 types of *C.oleifera* pollen by flow cytometry (Figure S1), and through statistical analysis and comparison, the diameters of the 32 types of pollen grains of four ploidy vary widely, ranging from 35.88 μm (NR-2, diploid) to 58.16 μm (YNYC-3, octaploid). According to the test, the pollen diameters at four ploidy levels were significantly different ($p < 0.001$). The average diameter of *C. oleifera* pollen grains was in the order of octaploid (50.54 μm) > hexaploid (49.40 μm) > tetraploid (41.92 μm) > diploid (37.85 μm) (Table 1 and Figure 1). In terms of diploid, NR-4 had the largest pollen grain diameter (39.30 μm); DF24 (44.24 μm) had the largest pollen grain diameter among the tested tetraploid pollens. For hexaploid, LE38 had the largest pollen diameter (50.87 μm), about 3.38 μm larger than the smallest pollen TXP-14 (47.49 μm). Among the four ploidy pollens of *C. oleifera*, the octoploid pollen had the largest mean diameter (Table 1; Figure 1), and the pollen grain diameter fluctuated between 46.31 μm and 58.16 μm, among which the pollen grain diameter of YNYC-3 was the largest (58.16 μm) (Table 1). The average polar axis lengths of diploid, tetraploid, hexaploidy, and octaploid measured by scanning electron microscopy were 36.68 μm, 44.27 μm, 47.93 μm, and 58.51 μm, respectively. The average equatorial axis lengths of diploid, tetraploid, hexaploid, and octoploid were 22.05 μm, 27.00 μm, 29.97 μm, and 30.84 μm, respectively (Table 2). The pollen size at four ploidy levels was ordered from largest to smallest as follows: octaploid > hexaploid > tetraploid > diploid (Tables 1 and 2; Figures 1 and 2).

Table 1. Average diameters of pollens at four ploidy levels of *C. oleifera* after carbopol fuchsin dye staining.

Ploidy Level	Pollen Type	Pollen Diameter (μm)	Mean Pollen Diameter (μm)	Ploidy Level	Pollen Type	Pollen Diameter	Mean Pollen Diameter (μm)
diploid	NR-1	38.85 ± 3.11 ^{ab}	37.85 ± 3.00 ^{d}	tetraploid	DP43	42.81 ± 2.36 ^{ab}	41.92 ± 2.73 ^{c}
	NR-2	35.88 ± 3.18 ^{d}			DP47	40.89 ± 3.67 ^{bc}	
	NR-3	36.23 ± 3.63 ^{bc}			DB(1)	40.23 ± 2.78 ^{cd}	
	NR-4	39.30 ± 2.92 ^{a}			DF24	44.24 ± 2.56 ^{a}	
	NR-5	39.04 ± 3.41 ^{b}			ZX0907	40.94 ± 1.87 ^{bc}	
	NR-6	38.31 ± 2.38 ^{c}			DBH	42.98 ± 3.70 ^{b}	
	NR-9	37.60 ± 3.09 ^{bc}			MX-5	42.31 ± 2.60 ^{c}	
	NR-10	37.61 ± 2.31 ^{bc}			CJ-03	40.92 ± 2.28 ^{bc}	
hexaploid	CJ-12	48.46 ± 3.71 ^{ab}	49.40 ± 3.42 ^{ab}	octaploid	YNYC-1	49.47 ± 3.19 ^{e}	50.54 ± 3.63 ^{a}
	ASX0902	50.75 ± 2.87 ^{b}			YNYC-2	53.92 ± 3.39 ^{b}	
	TXP-14	47.49 ± 2.77 ^{c}			YNYC-3	58.16 ± 6.19 ^{a}	
	CJ-07	48.68 ± 5.43 ^{ab}			YNYC-4	52.40 ± 2.81 ^{c}	
	XH097	50.06 ± 2.86 ^{ab}			YNYC-5	50.11 ± 3.35 ^{d}	
	LE38	50.87 ± 2.73 ^{a}			YNYC-7	47.64 ± 3.58 ^{de}	
	LE3	48.80 ± 3.37 ^{ab}			YNYC-8	46.31 ± 3.74 ^{ef}	
	GTDB 02	50.06 ± 3.63 ^{ab}			YNYC-9	46.34 ± 2.79 ^{ef}	

Note: Two hundred pollen grains of each pollen type were selected to measure the pollen diameter and calculate their mean value and standard deviation. Different letters in the column of the table indicate significant differences.

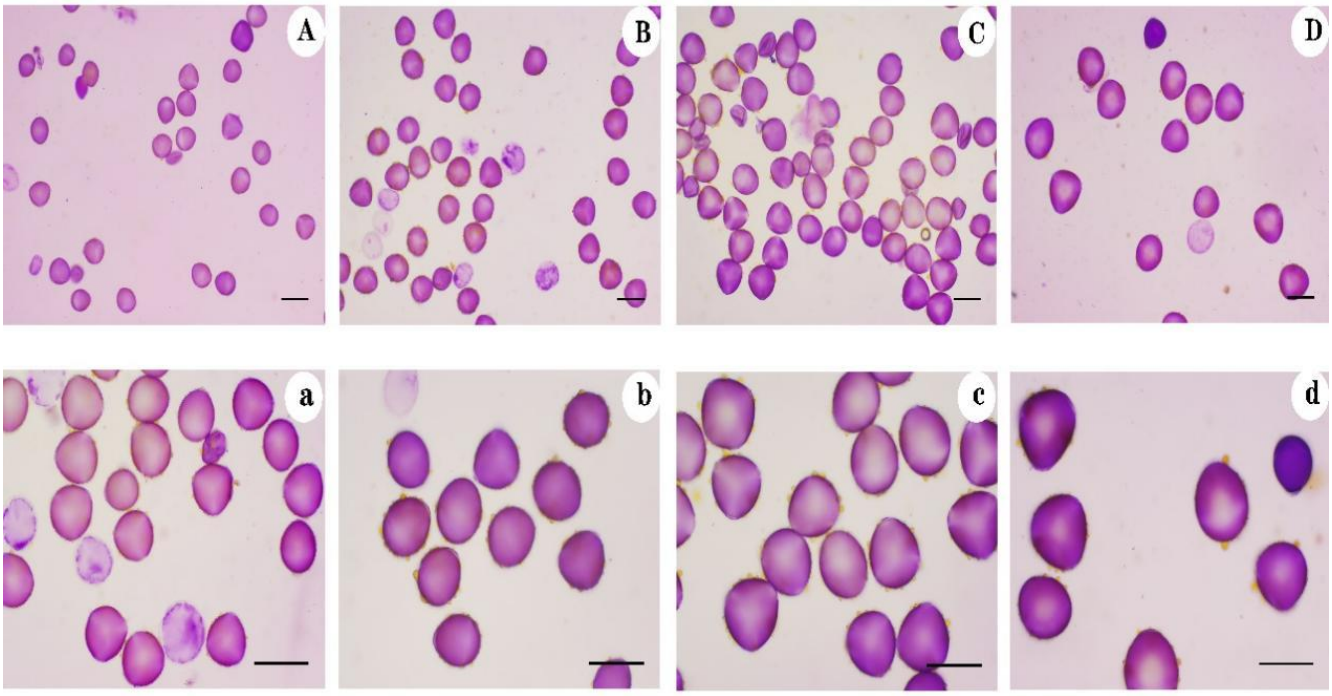

Figure 1. Microscopic observation of *C.oleifera* pollen after carbofuchsin dye solution. Capital letters (**A**–**D**) and lowercase letters (**a**–**d**) represent the microscopic images magnified at 20× and 40× of the dyed pollen with ploidy 2, 4, 6, and 8, respectively. Bar = 50 μm.

Table 2. Average polar and equatorial lengths of *Camellia oleifera* pollen measured by scanning electron microscopy at four ploidy levels.

Ploidy Level	Polar Axis Length (μm)	Equatorial Length (μm)
diploid	36.68 ± 2.87 ^{d}	22.05 ± 1.13 ^{c}
tetraploid	44.27 ± 3.06 ^{c}	27.00 ± 1.42 ^{b}
hexaploid	47.93 ± 2.39 ^{b}	29.97 ± 1.71 ^{ab}
octaploid	58.51 ± 2.88 ^{a}	30.84 ± 2.08 ^{a}

Note: Polar axis lengths and equatorial lengths were determined for 200 pollen grains at each ploidy level. Different letters in the column of the table indicate significant differences.

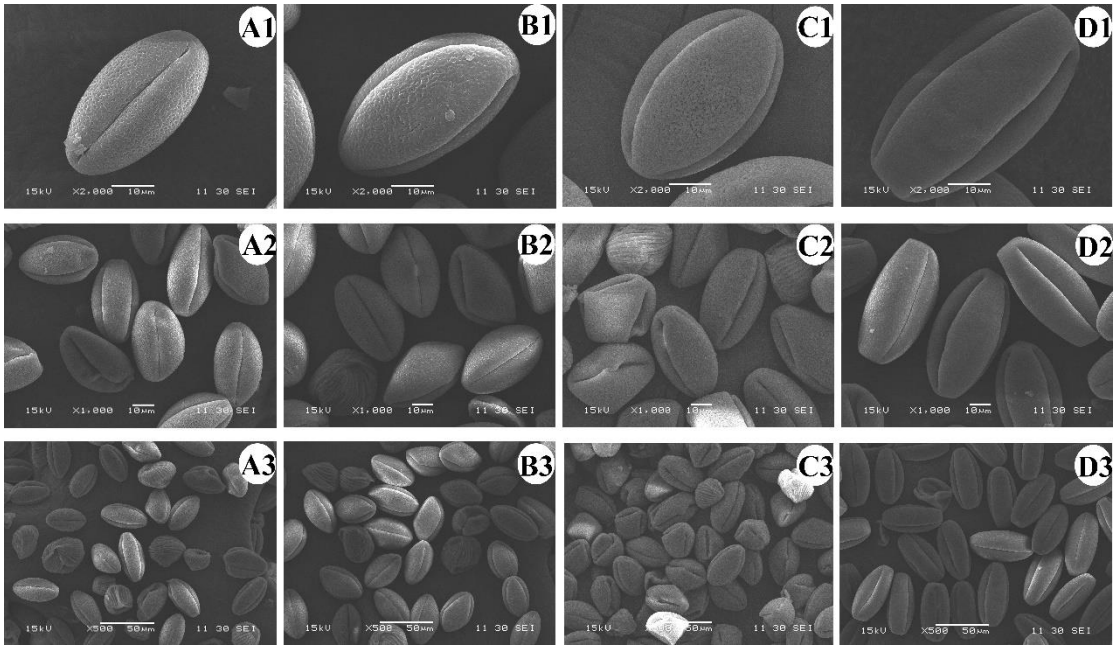

Figure 2. Scanning electron microscopy of *C.oleifera* pollen, capital letters (**A**–**D**) represent "diploid, tetraploid, hexaploid, and octaploid", respectively, and numbers "(**1**–**3**)" represent magnification of "2000× (individual), 1000× (population), and 500× (population)", respectively.

3.2. Pollen Viability Assessed by Staining

In hexaploid, the average pollen viability ranged from 74.28% to 88.24%, among which CJ-12 had the highest pollen viability (88.24%). The pollen vigor of each type was sorted from high to low: CJ-12 (88.24%) > XH097 (85.38%) > TXP-14 (83.95%) > LE3 (79.33%) > LE38 (76.34%) > GTDB02 (75.29%) > CJ07 (74.70%) > ASX0902 (74.28%). As for tetraploid, the pollen vigor ranks were as follows: DP43 (80.25%) > DF24 (78.24%) > DB(1) (75.71%) > DP47 (72.76%) > DBH (71.52%) > MX-5 (67.12%) > CJ-03 (65.60%) > ZX0907 (56.40%). For diploids, NR-3 had the highest pollen viability among all pollens, which was 74.61%, and the NR-1 was the lowest (45.89%). Pollen vitality was sorted as follows: NR-3 (74.61%) > NR-9 (72.04%) > NR-10 (68.91%) > NR-6 (63.77%) > NR-4 (63.56%) > NR-5 (63.17%) > NR-2 (62.83%) > NR-1 (45.89%). Observing octoploids, the pollen vigor of the eight types was quite different, between 12.10% and 77.56%, among which, for pollen vigor, the highest was YNYC-1 and the lowest was YNYC-8. In addition, the pollen vigor of YNYC-7, YNYC-8, and YNYC-9 was not high (<30%). The order of pollen vigor from high to low was as follows: YNYC-1 (77.56%) > YNYC-5 (75.11%) > YNYC-4 (74.76%) > YNYC-2 (65.45%) > YNYC-3 (63.57%) > YNYC-8 (27.87%) > YNYC-7 (24.06%) > YNYC-9 (12.10%) (Table 3). For the pollens of *C. oleifera* at four ploidy levels, the order of average pollen viability was as follows: hexaploid (79.69%) > tetraploid (70.95%) > diploid (64.35%) > octaploid (52.56%) (Table 4; Figure 3).

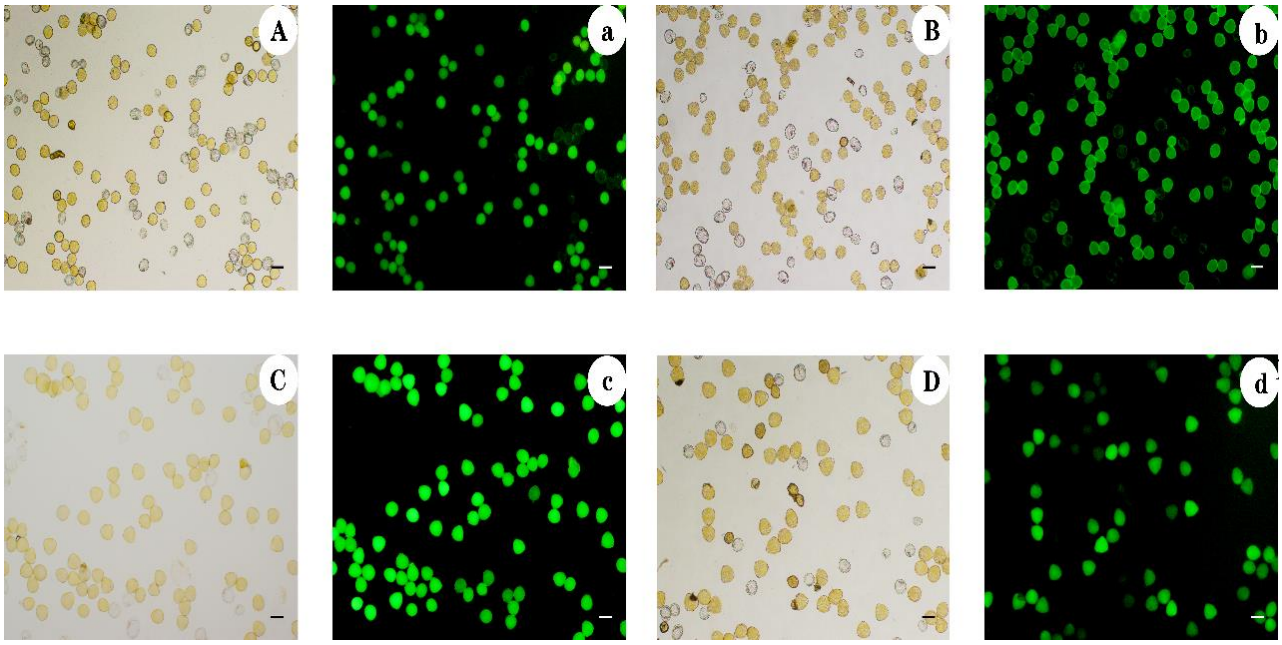

Figure 3. Light field and fluorescence magnification (10×) image of *C.oleifera* after staining with FDA dye. Capital letters (**A**–**D**) and lowercase letters (**a**–**d**) represent microscopic observations of diploid, tetraploid, hexaploid, and octoploid pollens in bright field and fluorescence, respectively. Bar = 50 μm.

Table 3. Pollen viability and germination rate of *C. oleifera* at four ploidy levels.

Ploidy Level	Pollen Type	Pollen Viability (%)	Pollen Germination Rate (%)	Ploidy Level	Pollen Type	Pollen Viability (%)	Pollen Germination Rate (%)
diploid	NR-1	45.89 ^{b}	34.52 ^{d}	tetraploid	DP43	80.25 ^{a}	76.45 ^{a}
	NR-2	62.83 ^{ab}	40.17 ^{c}		DP47	72.76 ^{cd}	72.06 ^{bc}
	NR-3	74.61 ^{a}	53.71 ^{ab}		DB(1)	75.71 ^{c}	74.05 ^{bc}
	NR-4	63.56 ^{ab}	50.65 ^{bc}		DF24	78.24 ^{b}	66.28 ^{bc}
	NR-5	63.17 ^{ab}	50.75 ^{b}		ZX0907	56.40 ^{e}	65.56 ^{c}
	NR-6	63.77 ^{ab}	49.21 ^{bc}		DBH	71.52 ^{cd}	75.47 ^{b}
	NR-9	72.04 ^{ab}	61.00 ^{a}		MX-5	67.12 ^{d}	68.15 ^{bc}
	NR-10	68.91 ^{ab}	55.64 ^{ab}		CJ-03	65.60 ^{de}	66.22 ^{bc}
hexaploid	CJ-12	88.24 ^{a}	84.59 ^{a}	octaploid	YNYC-1	77.56 ^{a}	69.79 ^{a}
	ASX0902	74.28 ^{cd}	77.04 ^{b}		YNYC-2	65.45 ^{b}	63.25 ^{ab}
	TXP-14	83.95 ^{ab}	57.03 ^{d}		YNYC-3	63.57 ^{bc}	25.71 ^{b}
	CJ-07	74.70 ^{cd}	63.58 ^{bc}		YNYC-4	74.76 ^{ab}	65.10 ^{ab}
	XH097	85.38 ^{ab}	83.98 ^{ab}		YNYC-5	75.11 ^{ab}	68.87 ^{ab}
	LE38	76.34 ^{bc}	64.70 ^{c}		YNYC-7	24.06 ^{cd}	14.86 ^{bc}
	LE3	79.33 ^{b}	63.11 ^{bc}		YNYC-8	27.87 ^{c}	19.68 ^{c}
	GTDB 02	75.29 ^{c}	80.12 ^{ab}		YNYC-9	12.10 ^{d}	8.89 ^{cd}

Note: Eight types of pollen grains of *C. oleifera* were selected for each ploidy, and no less than 500 pollen grains of each variety were selected for pollen viability testing. The pollen germination rate was measured 2 h after pollen germination in vitro. Different letters in the column of the table indicate significant differences.

Table 4. Mean pollen diameter, pollen viability, and germination rate of *C. oleifera* at four ploidy levels.

Ploidy Level	Mean Pollen Diameter (μm)	Mean Pollen Viability (%)	Mean Pollen Germination Rate (%)
diploid	37.85 ± 3.00 ^{d}	64.35 ± 8.90 ^{b}	49.46 ± 9.30 ^{b}
tetraploid	41.92 ± 2.73 ^{c}	70.95 ± 8.33 ^{ab}	70.53 ± 7.00 ^{ab}
hexaploid	49.40 ± 3.42 ^{ab}	79.69 ± 8.58 ^{a}	71.78 ± 10.00 ^{a}
octaploid	50.54 ± 3.63 ^{a}	52.56 ± 6.14 ^{c}	42.02 ± 9.00 ^{c}

Note: The average diameter, pollen viability, and pollen germination rate of diploid, tetraploid, hexaploid, and octoploid of *C. oleifera* pollens were determined using eight pollen species at each ploidy level. Different letters in the column of the table indicate significant differences.

3.3. Pollen Germination Rate Assessed by In Vitro Germination

In terms of hexaploid, the pollen germination rate fluctuated from 57.03% to 84.59%, among which CJ-12 had the highest pollen germination rate (84.59%). The order of pollen germination rate was as follows: CJ-12 (84.59%) > XH097 (83.98%) > GTDB02 (80.12%) > ASX0902 (77.04%) > LE38 (64.70%) > CJ-07 (63.58%) > LE3 (63.11%) > TXP-14 (57.03%). With regards to tetraploid, the pollen germination rate fluctuated between 65.56% and 76.45%, while the pollen types with the highest and lowest pollen germination rates were still DP43 and ZX0907. The order of their pollen germination rate was as follows: DP43 (76.45%) > DBH (75.47%) > DB(1) (74.05%) > DP47 (72.06%) > MX-5 (68.15%) > DF24 (66.28%) > CJ-03 (66.22%) > ZX0907 (65.56%). For diploids, NR-9 had the highest pollen germination rate among all pollens, which was 61.00%, and the pollen germination rate of NR-1 was the lowest (34.52%). The pollen germination rate was ranked from high to low as follows: NR-9 (61.00%) > NR-10 (55.64%) > NR-3 (53.71%) > NR-5 (50.75%) > NR-4 (50.65%) > NR-6 (49.21%) > NR-2 (40.17%) > NR-1 (34.52%). Considering octoploids, the pollen germination rate of the eight types tested was quite different, which fluctuated between 8.89% and 69.79%, among which, for pollen germination, the highest rate was YNYC-1 and the lowest was YNYC-8. Besides, the pollen germination rates of YNYC-7, YNYC-8, and YNYC-9 were not high, with all being less than 20%. The germination rates of the eight pollen species were as follows: YNYC-1 (69.79%) > YNYC-5 (68.87%) > YNYC-4 (65.10%) > YNYC-2 (63.25%) > YNYC-3 (25.71%) > YNYC-8 (19.68%) > YNYC-7 (14.86%) > YNYC-9 (8.89%) (Table 3). The order of pollen germination rate from high to low was hexaploid (71.78%) > tetraploid (70.53%) > diploid (49.46%) > octoploid (42.02%) (Table 4; Figures 4 and 5), and the differences were very significant (p = 0.001) (Table 5).

Table 5. Variance analysis for the pollen diameter, pollen viability, and pollen germination rate of *C. oleifera* at four ploidy levels.

Factor	SS	DF	F	P	Significance
pollen diameter	885.142	3	53.936	0.000	*
pollen viability	3136.838	3	4.774	0.008	*
pollen germination rate	5396.710	3	7.704	0.001	*

Note: SS is equal to the sum of squares between groups. DF = degree of freedom. F is equal to mean squared between groups/within groups. The p-value indicates a significant difference. * was significant at the 0.01 level.

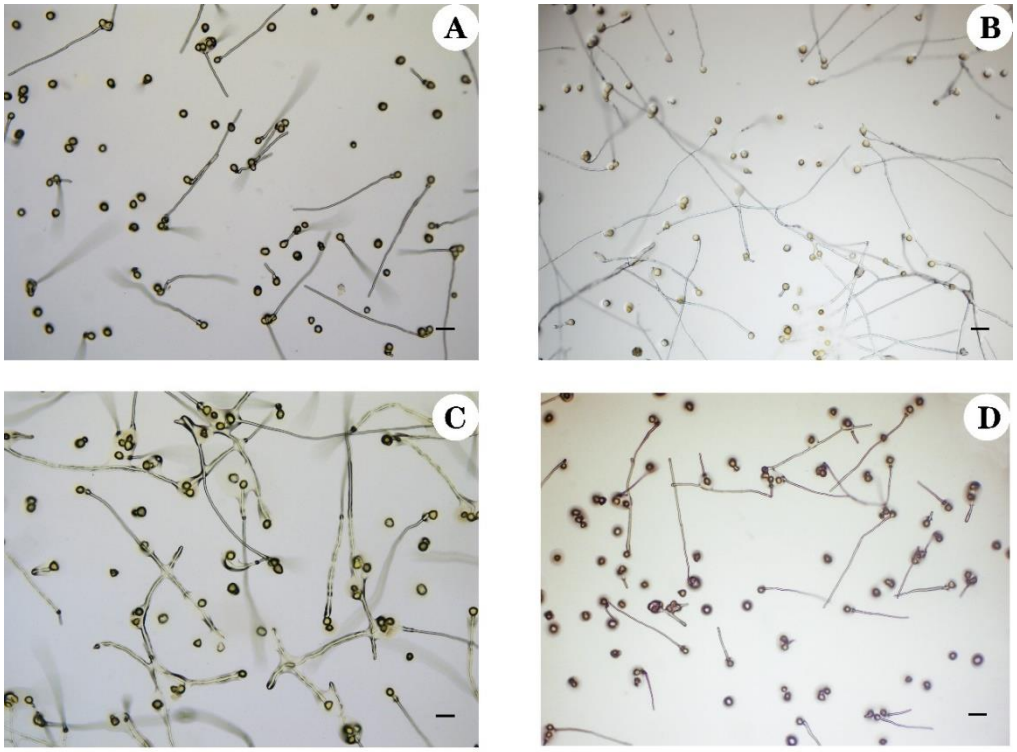

Figure 4. Pollen germination after 2 h in culture medium of *C. oleifera*. (**A**–**D**) represent the pollens of *C. oleifera* at four ploidy levels of 2, 4, 6, and 8, respectively. Bar = 100 μm.

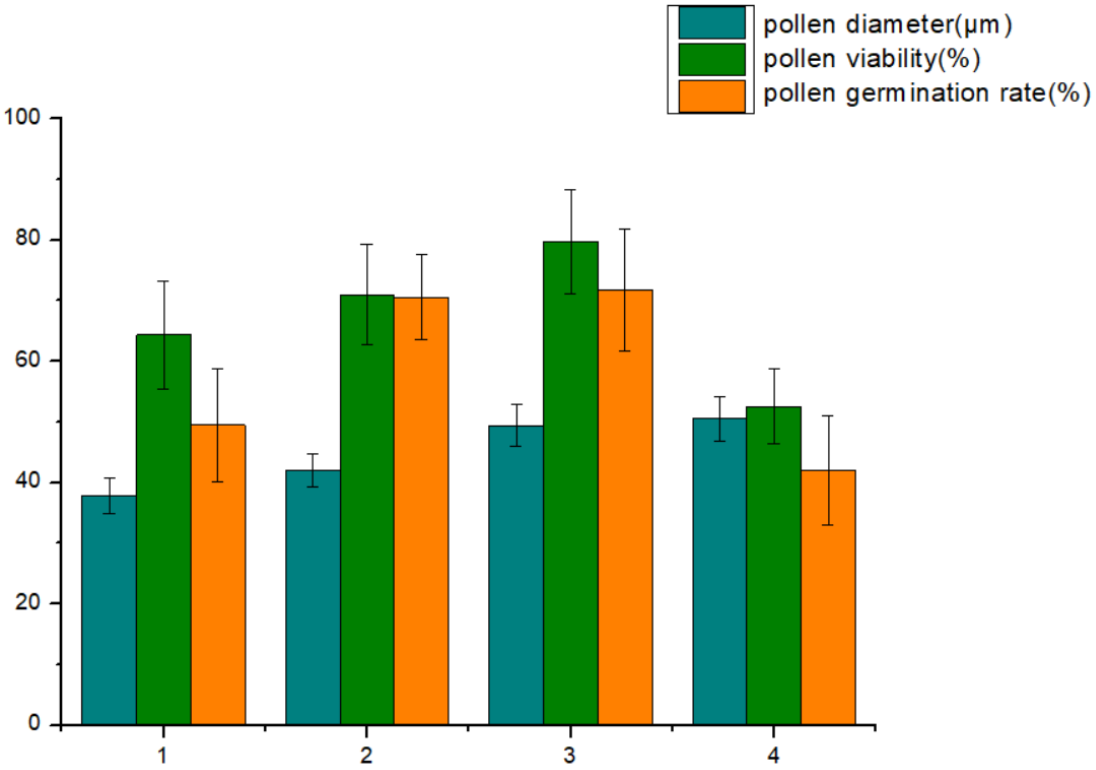

Figure 5. Comparison of the average diameter, pollen viability, and pollen germination rate of four ploidy *C. oleifera* pollens in the column chart; 1, 2, 3, and 4 on the abscissa represent diploid, tetraploid, hexaploid, and octoploid, respectively.

4. Discussion

4.1. Pollen Size Related to the Ploidy Level

Pollen, as the carrier of male heredity, has all the gene types of a variety and is an important material for cross breeding [33]. In higher plants, polyploid plants generally have larger organs compared with diploid plants [34,35] and pollen size is often positively correlated with ploidy [36]. Pollen size is often used as a biological parameter to estimate the ploidy and viability of mature plants. In general, it is quantified by image-based diameter measurements [37]. For example, in the study of *Avena* [38] and *Rumex* [39], ploidy had a significant effect on pollen grain size and pollen grain size increased with the increase in ploidy level. As reported by Jan et al. [40], there is a clear tendency for polyploid pollen grains to be larger than diploid pollen grains. For some species, the size of mature pollen grains has been found to be positively correlated with the level of somatic ploidy in donor plants. In the report of Cui et al. [41], pollen size was positively correlated with ploidy and the size of tetraploid, triploid, and diploid pollens decreased successively. Besides, from Nico et al. [37], for Arabidopsis thaliana diploid-, tetraploid-, and octaploid-times body sequence of observation, they decided on pollen size analysis based on volume at different times to distinguish the level of sex of pollen plants having a very strong discrimination ability, in order to quickly determine the cell ploidy level of the plant or plant group, providing a simple and reliable method. In this experiment, by measuring and analyzing the pollens of *C. oleifera* of diploid, tetraploid, hexaploid, and octoploid, we found that the average pollen size increased with the increase in ploidy; that is, from diploid to octoploid, the average pollen grain size showed an increasing trend and the pollen size of four ploidy showed very significant differences, which is consistent with the results of Souza-Pérez et al. [20], who found that pollen grain size was heterogeneous and the pollen grain size of octoploid plants was larger, thus pollen size can be influenced by ploidy levels.

4.2. Pollen Viability and Pollen Germination at Different Ploidy Levels

Cross breeding is an important way to improve the character of plants and the quality of parents directly affects the efficiency of breeding [42]. Pollen quality is evaluated according to the viability and germination of pollen grains [43]. Pollen viability determines the reproductive success of a species to a certain extent and high viability pollen will increase the rate of seed setting, which will lead to the highest yield [42,44]. In vitro pollen germination tests are used to determine the germination rate of pollen and can also be used to assess pollen viability by monitoring the germination rate over time [45]. There were significant differences in the pollen viability of different cultivars [33]. Studies have shown that the level of ploidy affects pollen viability and pollen viability varies with the change in ploidy [16,41]. In our study, the pollen viability ($p = 0.008$) and germination rate ($p = 0.001$) at four ploidy levels showed significant differences. The average pollen viability fluctuated between 52.56% and 79.69% and the pollen germination rate ranged from 42.02% to 71.78%. Indeed, the vigor and germination rate of the hexaploid were the highest (79.69% and 71.78%, respectively), followed by tetraploid and diploid, and the average vigor and germination rate of the octaploid were the lowest. Specifically observing each pollen type selected and tested for ploidy, we found that pollen viability and pollen germination rate varied in the range of 12.10–88.24% and 8.89–84.59%, respectively, while hexaploid CJ-12 and octoploid YNYC-9 had the highest and lowest pollen viability and pollen germination rate, respectively. The results of Souza-Pérez et al. [20] showed that pollen viability in octoploid was low and there were differences between both intra and ploidies. The effect of pollen morphology on pollen viability depends on plant ploidy. Further, we observed that, in octoploids, not all types had low pollen viability and germination rate. For example, the pollen viability and germination rates of YNYC-7, YNYC-8, and YNYC-9 were less than 30%, while those of YNYC-1 could go up to about 70%. Therefore, we cannot assume that the pollen vigor of all octuploid camellia species is low. To sum up, differences in pollen grain viability and pollen size found in *C. oleifera* suggest that ploidy levels can affect these

properties to varying degrees. Our study on pollen viability is of great significance for interspecific hybridization and variety management of *C. oleifera*.

4.3. Comparison of Two Vigor Measurement Methods

In plant breeding, simple methods are often needed to determine the viability of fresh pollen [46]. The common method for determining pollen viability is staining [47] and the germination test is considered to be the best indicator of pollen viability, which is a very effective and convenient method to study the biological application of pollen [46]. Some studies have suggested that the pollen viability measured by the staining method and germination method is quite different [41]. From their study, the pollen viability of the same variety measured by the germination method in the same time period was lower than that measured by the staining method, and the low degree was related to the variety and the time of pollen collection. In this experiment, we found that, although the pollen viability of YNYC-3 could reach more than 60%, its pollen germination rate was only about 25%. This may be in line with the findings of Cui et al. [41], who found that a small amount of pollen viability could be observed by the staining method, but the germination method showed that they do not have the ability to germinate. We speculated that the reason for this phenomenon may be that more aborted pollens were produced; the stained pollen grains were not necessarily germinable; and though some pollens had vitality, their vigor was not high and they could not germinate further. In addition, we found that the average germination rate of pollen of some types was greater than the percentage of vigor; we also observed such research results in the study of Guo et al. [9]. Cui et al. [41] reported that the germination rate of pollen grains is faster or slower, and the actual germination strength of pollen grains may be greater than the observed value. In a word, although the results measured by the two methods may be different, it is undeniable that both methods are simple and effective methods to measure the pollen viability. Therefore, in this study, we used these two methods to measure the pollen viability of different ploidy levels of *C. oleifera* pollen to improve the accuracy of the results.

5. Conclusions

In our study, the pollen size of *Camellia oleifera* increased with the increase in ploidy. The pollen viability and germination rate of hexaploid were higher than those of diploid, tetraploid, and octoploid, among which the pollen viability and germination rate of hexaploid CJ-12 were both high. The four ploidy pollens showed significant differences in size, pollen viability, and pollen germination rate. The results of this experiment will provide more consistent results within and between laboratories, and will be easily integrated into existing *C. oleifera* breeding programs.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy12112592/s1, Figure S1. Flow cytometric histogram of pollen of *C. oleifera* at four ploidy. "A, B, C, and D" represent diploid, tetraploid, hexaploid, and octaploid, respectively; the X-axis is relative DAPI fluorescence and the Y-axis is the number of cells.

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