Research paper

Pollen Fertility and Cytological Observations of the Interspecific Hybrid Cultivar *Camellia grijsii* × *C. oleifera*

Rui Zhao[1)] Shixin-Xiao[1)] Deyi-Yuan[1)] Joseph Masabni[2)] Feng Zou[1)]

【Summary】

Oil tea (*Camellia oleifera*) is one of the most economically important forest species native to China. Successful pollination requires pollen grains with high fertility, which directly determines the cross-breeding success of oil tea. To better understand pollen fertility, pollen viability and vigor of the interspecific hybrid cultivar 'DY-4' (*Camellia grijsii* 'Youza 2' × *C. oleifera* 'Huashuo') were investigated using staining and *in vitro* germination tests. Results indicated that pollen viability was 51.13% and 66.08% by the TTC and FDA staining methods, respectively. The germination rate was 54.22% when using 1% agar, 100 g/L sucrose, 0.1 g/L H_3BO_3, 0.03 g/L $MgSO_4$, and 0.01 g/L IAA, which was higher than the control with 1% agar (germination rate = 19.09%). Meiosis and chromosome of 'DY-4' pollen mother cells exhibited abnormal behavior including free or lagging chromosomes and abnormal spindle orientation. In addition, scanning electron microscopy showed that 'DY-4' exhibited many deformed pollen grains, with a deformation rate of about 16%. The accumulation of these abnormalities may affect pollen fertility in the cultivar 'DY-4'. Our current cytological observation and fertility analysis of pollen of the cultivar'DY-4' will lay the foundation for cross-breeding of oil tea in the future.

Key words: meiosis, Oil Tea, pollen fertility, pollen germination, pollen morphology.

Zhao R, Xiao S, Yuan D, Joseph Masabni, Zou F. 2022. Pollen fertility and cytological observations of the interspecific hybrid cultivar *Camellia grijsii* × *C. oleifera*. Taiwan J For Sci 37(3):179-91.

[1)] Key Laboratory of Cultivation and Protection for Non-Wood Forest Trees of Ministry of Education, Central South University of Forestry and Technology, Changsha 410004, Hunan, China. 中南林業科技大學教育部經濟林培育與保護重點實驗室，410004湖南省長沙市天心區韶山南路498號。

[2)] Texas A&M AgriLife Research and Extension Center, 17360 Coit Rd, Dallas TX 75252, USA. 美國德克薩斯州奧弗頓市，德克薩斯農工大學，美國德克薩斯州達拉斯Coit路17360號，75252。

[3)] Corresponding author, e-mail:zoufeng06@126.com 通訊作者。

Received May 2022, Accepted August 2022. 2022年5月送審 2022年8月通過。

研究報告

油茶種間雜交品種的花粉育性及細胞學觀察（攸縣油茶×普通油茶）

趙蕊[1)] 肖詩鑫[1)] 袁德義[1)] Joseph Masabni[2)] 鄒鋒[1)]

摘 要

油茶(*Camellia oleifera*)是中國特有且最重要的經濟林樹種之一。花粉粒的高育性，直接決定了油茶雜交育種的授粉成功率。為了解油茶種間雜交品種‘DY-4’ (攸縣油茶‘攸雜2號’×油茶‘華碩’)的花粉育性，採用染色法和離體萌發法對其花粉活力和活勢進行了研究。結果表明TTC法和FDA法測定花粉活力分別為51.13% 和66.08%。1%瓊脂、100 g/L蔗糖、0.1 g/L H_3BO_3、0.03 g/L $MgSO_4$、0.01 g/L IAA處理的萌發率為54.22%，高於1%瓊脂處理的19.09%發芽率。‘DY-4’ 的花粉母細胞減數分裂和染色體表現出游離或滯後染色體和紡錘體方向異常等不正常行為。掃描電鏡顯示位於“DY-4”之間的花粉變形較多，變形率約為16%。這些異常的積累可能會影響‘DY-4’的花粉育性。本研究對油茶品種‘DY-4’花粉的細胞學觀察和育性分析，為今後油茶雜交育種奠定基礎。

關鍵詞：減數分裂，油茶，花粉育性，花粉萌發，花粉形態。

趙蕊、肖詩鑫、袁德義、Joseph Masabni、鄒鋒。2022。油茶種間雜交品種的花粉育性及細胞學觀察(攸縣油茶×普通油茶)。台灣林業科學37(3):179-91。

INTRODUCTION

Camellia oleifera, commonly known as the eastern olive oil tree, has a long history of cultivation with high economic value in China (Ki et al. 2010). Its seeds are the source of edible oil rich in unsaturated fatty acids and various active ingredients (Xiao 2006, Li et al. 2014). Increasing research has developed cross-breeding protocols, usually using artificial pollination methods to improve yield and quality (Alburquerque et al. 2007, Sharafi and Bahmani 2011). One key factor affecting successful pollination is pollen fertility (Sunilkumar et al. 2013), where the use of viable pollen capable of fertilization is vital for a successful breeding program (Russell 1992, Yandovka and Barabanov 2021). Interspecific hybridization is an important approach for germplasm innovation in many plants and is widely used in fruit trees (Li et al. 2016, Wang et al. 2017). After an interspecific hybrid is developed, further backcrossing is needed to carry out cross breeding for new germplasm innovation. Therefore, it is important and necessary to consider the fertility of F1 generation pollen grain.

Pollen grains arise from meiosis (Zortéa et al. 2019), a sexual reproduction process in which diploid progenitor cells produce four haploid gametes (Khah and Verma 2019). In this process, the progenitor cells divide into tetrads through two successive meioses, during which occur chain exchange between homologous chromosomes and precise separation of sister chromatids. The tetrads release four microspores which develop into pollen grains to produce male gametophytes. Therefore, successful meiosis during gamete formation is an important step in the production of

fertile pollen during plant reproduction (Shin et al. 2021). Studies have shown that changes in pollen size and vitality are usually associated with abnormal behavior during meiosis (Wang et al. 2010a, Tian et al. 2015). When the meiosis process of pollen mother cells is normal and harmonious, with normal bivalent formation and cytokinesis, then 100% pollen vitality can be guaranteed (Pagliarini 2000). But any abnormality during meiosis will result in the formation of sterile gametes and reduce pollen viability (Jiang et al. 2011). Tian et al. (2015) reported that interspecific hybrids often produce a large number of gamete variations due to unbalanced segregation and poor chromosome pairing. At different stages of meiosis, pollen will show different abnormal phenomena. The existence of these abnormal phenomena leads to the imbalance of genetic material in most of its gametes, and eventually forms gametes without viability, resulting in reduction in the fertility of pollen (Xi et al. 2015). The abnormal meiosis process often interferes with microspore formation, resulting in genetically different gametes, malformation or infertility of pollen and reproductive disorders, thus affecting the reproductive success of hybrids in the wild (Aparicio and Albaladejo 2003; Singh et al. 2020).

At present, little information is available regarding pollen fertility of the interspecific hybrids of *C. oleifera* complex. Consequently, in the present work, we used TTC and FDA staining, and *in vitro* germination tests to quantify pollen viability and vigor of the interspecific hybrid 'DY-4' (*C. grijsii* 'Youza 2' × *C. oleifera* 'Huashuo'). In addition, we observed the meiosis behavior of hybrid 'DY-4' and pollen morphology using microscopy. The results of this study provide important information on pollen fertility of interspecific hybrids 'DY-4' and will guide future breeding strategies of *C. oleifera*.

MATERIALS AND METHODS

Plant materials

The experimental materials were from *C. oleifera* germplasm nursery of Central South University of Forestry and Technology (Changsha, China). The germplasm nursery is located at 28°06′03″N and 111°56′30″E, which is a subtropical monsoon humid climate with abundant rainfall, distinct seasons, and sufficient sunshine. The annual average precipitation is 1200 mm, the average temperature is 16~18℃, the average sunshine is 1600 h, and the frost free period is 285 days. It is one of the most suitable growing areas for *C. oleifera*.

The interspecific hybrid cultivar 'DY-4'was obtained from the cross breeding of 'Youza 2' × 'Huashuo' in 2016. 'Youza 2' was an excellent individual screened from *C. grijsii* plant selection for its excellent oil quality, high oil content, thin shell characteristics, and high resistance to anthracnose (Weng 1997; Zhuang et al. 2012; Li et al. 2021). *C. oleifera* 'Huashuo' has the characteristics of large fruit, high yield, strong resistance, and high light use efficiency (Tan et al. 2011).

Staining for Pollen viability

In this study, pollen viability was assessed using the Fluorescein diacetate (FDA) fluorescence staining and 2,3,5-Triphenyltetrazolium chloride (TTC) staining methods, according to the methodologies of Li et al. (2021) and Luo et al. (2020), respectively. We placed 0.01 grams of pollen into a small centrifuge tube and added a prepared dye mixture. After a few minutes, we used a pipette to draw 100 μL of the solution containing pollen and placed it on a microscope slide. Images of pollen grains were viewed

at 10X magnification using an Olympus BX51 composite microscope (Olympus Corporation, Tokyo, Japan). When stained with FDA, pollen grains exhibiting yellow-green fluorescence under fluorescence lighting are considered active. If no fluorescence was observed, then the pollen is considered dead. When stained with TTC, pollens appearing red are considered viable, while those that are not dyed red are considered inactive. We randomly selected a microscope view field with more than 50 pollens and counted the number of active or live pollens. A total of six fields were observed to represent 3 replicates for each stain treatment.

Pollen germination

We used the *in vitro* germination test design to determine pollen vigor of the hybrid cultivar 'DY-4'. The control group (CK) was cultured in medium containing 1% agar, while treatment T1 consisted of 1% agar, 100 g/L sucrose, 0.1 g/L H_3BO_3, 0.03 g/L $MgSO_4$, and 0.01 g/L IAA. We incubated the pollen culture medium in the dark at 25℃ for 2 hours. Each treatment consisted of 3 replications. We counted pollen germination using a BX-53 microscope (Olympus, Tokyo, Japan). For each sample count, we randomly selected five visual fields with no less than 50 pollen grains per field (Yuan et al. 2010, Xiong et al. 2016, Deng et al. 2018). Pollen germination rate was calculated as the ratio of the number of germinated pollen grains divided by the total number (Bryhan and Serdar 2008,Tan et al. 2010).

Meiosis observation

In mid-October 2021, flower buds were harvested and fixed in a newly prepared Carnoy's fixing solution (95% ethanol:glacial acetic acid at 3:1 ratio) at 4℃ for 24 h. Samples were then transferred to 70% ethanol for preservation. Afterwards, the fixed flower buds were rinsed several times with distilled water and placed in 1 mol/L hydrochloric acid metal bath and were treated for 10 min at 60 ℃, then the flower buds were rinsed several times with distilled water. The anthers were separated using the anatomic microscope, placed on slides and stained with Carbopol fuchsin dye solution. The anthers were gently squeezed with tweezers to remove excess anther wall and impurities. Finally, we observed and photographed the anthers using the BX-51 microscope (Olympus Corporation, Tokyo, Japan) (Li et al. 2021).

Pollen morphology

The pollen grains were dispersed and placed on an aluminum sample table with double-sided tape. The pollen grains were sprayed with gold by an ion sputtering device. The morphology of pollen grains was observed using a scanning electron microscope (SEM-6380LV, JEOL, Tokyo, Japan) (Taylor et al. 2015; Hu et al. 2020). When taken photographs, representative fields were viewed at 2000X magnification for individual grains, and at 1000X and 500X for pollen population. Using image processing software ImageJ (National Institutes of Health, Bethesda, USA), we measured the various pollen parameters according to Bispo et al. (2018). Normal and deformed pollens (200 of each) were used to measure average polar and equatorial lengths. The average pollen deformation rate was calculated as the number of deformed pollens divided by the total number of pollens. We counted no less than 1500 pollen grains for this test.

RESULTS

Pollen morphology

Using scanning electron microscopy, we

observed that the appearance of pollen grains can be divided into normal or deformed forms. The average pollen grain deformation rate was 16% in 'DY-4'. The average polar and equatorial lengths of normal pollens were 42.60 μm and 23.60 μm, respectively, while the average polar and equatorial lengths of deformed pollens were 33.64 μm and 23.65 μm, respectively. The equatorial length of normal and deformed pollen was similar, but the polar length of the deformed pollen was about 9 μm shorter than that of the normal pollen (Table 1, Fig. 1).

Pollen viability and vigor

Pollen viability of hybrid 'DY-4' was 51.13% and 66.08% for the TTC and FDA staining, respectively. Pollen viability with TTC staining was about 15% lower than with FDA staining. Viewed under the microscope, 'DY-4' pollens had few empty pollen grains which couldn't be stained and showed

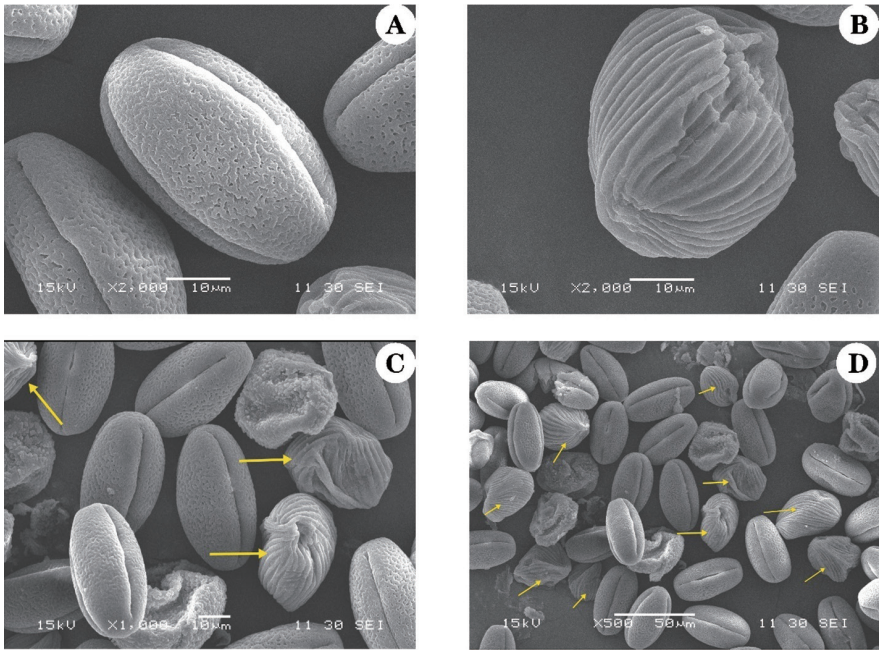

Fig. 1. A: Microscopic images of normal pollen of *Camellia oleifera* 'DY-4'; B: Microscopic images of abnormal pollen of *Camellia oleifera* 'DY-4'; C-D: Pollen grains of *Camellia oleifera* 'DY-4'. The arrows indicate deformed pollens of *Camellia oleifera* 'DY-4'.

Table 1. Average polar and equatorial lengths of normal and deformed pollens and average pollen deformation rate of hybrid cultivar 'DY-4'

C. oleifera 'DY-4'	deformation rate (%)	Normal pollen		Deformed pollen	
		Polar axis length (μm)	equatorial length (μm)	Polar axis length (μm)	equatorial length (μm)
	16.00±9.46	42.60±2.01	23.60±2.04	33.64±4.35	23.65±3.53

Note: 200 pollen grains were respectively selected to measure average polar axis length and equatorial length of normal and deformed pollens. No less than 1500 pollen grains were counted to calculate the deformation rate.

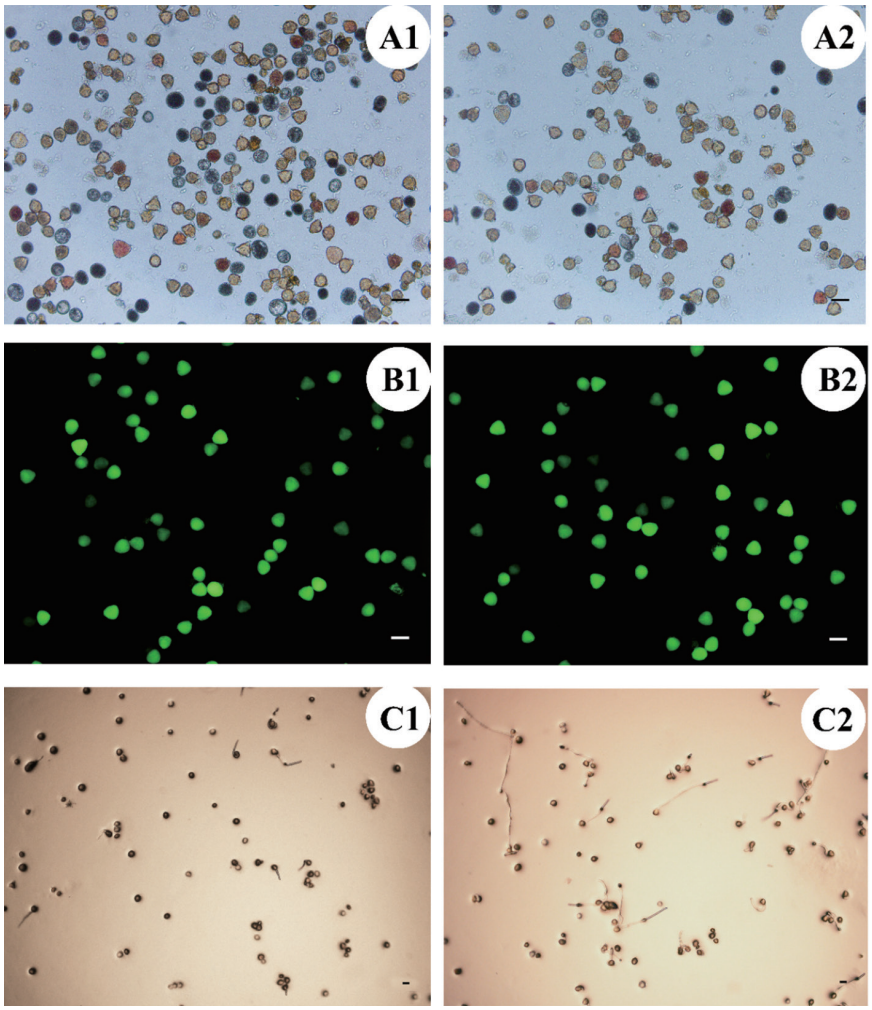

Fig. 2. The capital letters "A" and "B" respectively represent the pollen viability diagram of interspecific hybrid 'DY-4' dyed by TTC and FDA, and the pollen grains were magnified by 10×. "C1" "C2" respectively represent the pollen germination under CK and T1 treatment magnified by 4×. Bar = 50μm.

no pollen viability (Fig. 2). After the *in vitro* pollen germination test, pollen germination rate of 'DY-4' was 19.09% and 54.22% for the CK and T1 treatments, respectively. Compared with the control medium (CK), pollen germination rate in T1 treatment was significantly higher with a significant increase of about 35% in the medium supplemented with nutrients and hormones. Still, pollen germination rate was less than 60% (Table 2, Fig. 2) compared to the average pollen germination rate of parents *C. grijsii* 'Youza 2' and *C. oleifera* 'Huashuo' of about 70-80% (Table S1, Fig.S1).

Abnormal meiotic chromosome behavior in 'DY-4'

In addition to normal chromosome behavior during meiosis, abnormal chromosome behavior and meiosis abnormalities were also

Table 2. Average pollen viability and pollen germination rate of hybrid cultivar 'DY-4'

	Pollen viability (%)		Pollen germination rate (%)	
C. oleifera 'DY-4'	TTC	FDA	CK	T1
	51.13±7.01	66.08±7.10	19.09±5.11	54.22±7.59

Note: Pollen viability was measured by vitality staining, and pollen germination rate was measured by *in vitro* germination. Pollen germination rate was measured after 2h of culture.

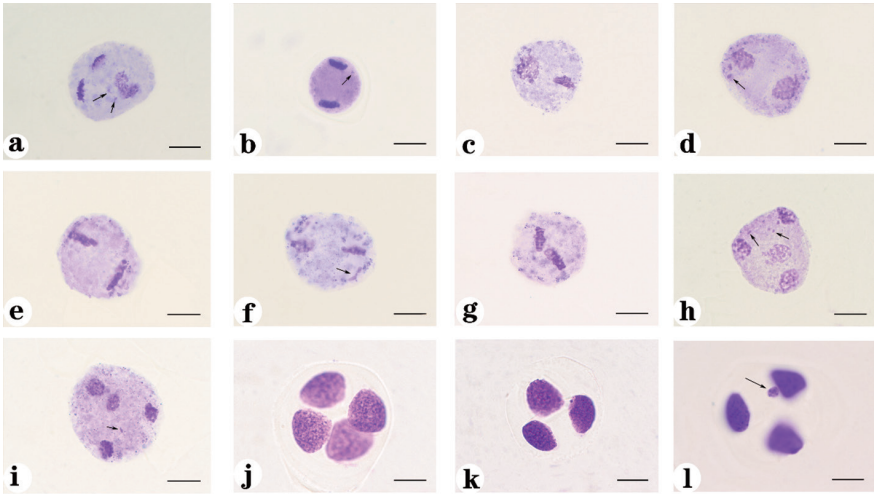

Fig. 3. Observation on meiosis chromosome behavior of hybrid cultiva 'DY-4', the arrow points to some chromosomal abnormalities. a: Lagging chromosome in Anaphase II (arrow); b: Dissociative chromosomes in Anaphase I (arrow); c: Unequal separation in Metaphase I; d: Dissociative chromosomes (arrow) in Metaphase I; e: Perpendicular spindle in Metaphase I; f-g: Abnormal spindle orientation (parallel and tripolar spindles) in Metaphase I; h-i: Dissociative chromosomes (arrow) in Telophase II; j: Tetrad; k: Triad; l: Tetrad with one micronucleus (arrow). Bar = 20μm.

observed in 'DY-4'. Examples of abnormalities include free or lagging chromosomes (Fig. 3a, b, d, f, h, i), and unbalanced chromosome segregation (Fig. 3c). In the middle stage of meiosis, perpendicular spindles were apparent (Fig. 3e), and the spindle direction was abnormal with parallel spindle (Fig. 3f) or tripole spindle (Fig. 3g). In addition to normal tetrad formation (Fig. 3j) by which chromosomes reach the quadrupole, unscrew, undergo cytokinesis, and produce cell wall, abnormal phenomena such as trizygote (Fig. 3k) and micronucleus (Fig. 3l) were also observed during the development stage of tetrads.

DISCUSSION

Pollen viability is a prerequisite for successful pollination and is a valuable and necessary requirement for crop breeding of flowering plants (Silva et al. 2020). In plant breeding, simple tests are often conducted to assess the viability of fresh pollen. Although several indirect testing methods are available, they are often criticized for overestimating pollen quality or viability. At present, the most used methods to quantify pollen viability include staining and in vitro germination. Staining is a popular method to evaluate pollen viability (Atlagić

et al. 2012; Alexander 2019). Usually, staining methods for pollen viability include TTC and FDA fluorescence. TTC staining is used to detect the activity of mitochondrial respiratory products, while FDA fluorescence staining is often used to track pollen viability (Hu and Xia 2022). Studies have shown that the color stability was poor in the results of TTC staining, and the staining boundary between low activity and inactivity was not obvious. Therefore, pollen viability measured by the TTC staining method may be lower than the actual value (Hu and Xia 2022). In this report, pollen viability was calculated at 51.13% for the TTC staining and 66.08% for FDA staining. The TTC staining indicated a 15% lower pollen viability than the FDA method. Compared to TTC and FDA staining which measure the potential viability of pollen, the *in vitro* germination method measures the actual viability. The *in vitro* germination test is considered to be the best indicator of pollen vitality and a very effective and convenient method to study the biological application of pollen (Luo et al. 2020). Studies have indicated that, using the *in vitro* germination test, physiological and biochemical knowledge of pollen viability can be better mastered, which is conducive to the development of hybrids (Pradyut and Subrata 2014). In this experiment, pollen viability of 'DY-4' was evaluated comprehensively using both pollen viability staining and *in vitro* germination, which provided us with an effective basis for the evaluation of pollen fertility.

Staining and *in vitro* germination techniques have achieved useful results in the determination of pollen fertility of *C. oleifera*. Lisa (2019) studied pollen viability of *Hydrangea macrophylla*, *Dichroa febrifuga*, and their hybrids, and observed that the pollen viability of the hybrid was low (about 25%). In this paper, we tested pollen viability of the interspecific hybrid 'DY-4' using FDA and TTC staining techniques and *in vitro* germination test, and we determined conclusively that the fertility of the hybrid progeny was not very high (about 50%) compared to the parents with an average pollen viability of *C. grijsii* 'Youza 2' and *C. oleifera* 'Huashuo' of 70-80%.

Pollen morphology has been proven to be related to pollination, growth, and development (Osborn et al. 2001; Calic et al. 2013). Wang et al. (2010b) studied 10 superior clones of *C. oleifera* and observed that the number of aborted pollen among different clones was various, and that the abortive pollen grains were shriveled and abnormal in shape. Yandovka et al. (2021) reported that small and moderately deformed pollen grains of *Ribes alpinum* were morphologically immature, exhibited a compressed state, and such pollen usually couldn't be stained. Large, deformed pollens were oval in shape and often couldn't be stained, and germinated poorly on artificial nutrient media. In their study, *Ribes alpinum* contained 10% deformed pollen, which could not be stained or germinated on suitable germination media. These results are consistent with our results where we observed an average deformation rate for 'DY-4' at 16%, and that the deformed pollens couldn't be stained nor germinated properly.

Effective variation of genetic material would inevitably lead to corresponding changes in pollen morphology (Mo 1992) and pollen fertility reduction (Shin et al. 2021). In this study, we observed meiosis in hybrid 'DY-4' and found abnormal chromosome behavior. Abnormal phenomena that we observed include free or backward chromosome or chromosome fragments movement, some were located on the equatorial plate, and others moved to the first pole, after most of the normal chromosomes moved to the poles dur-

ing meiosis. These randomly distributed backward chromosomal cycles will result in either a decrease or increase of genetic material, and ultimately affect the fertility of gametes. Koduru and Rao (1981) indicated that some free or lagging chromosomes could form a radiation microtubule system and eventually form micronuclei in the tetrad stage, which would lead to abnormal transmission of genetic material and lead to gamete infertility or abnormal chromosome number in the offspring. Our results of unbalanced chromosome segregation are similar to those observed by Xi et al. (2015) and Kaur and Singhal (2019), who observed that chromosomes were not equally polarized during meiosis. Shin et al. (2021) indicated that the unequal segregation of meiosis chromosomes was responsible for the formation of micronucleus and gamete imbalance, which ultimately leads to low fertility of pollen. Our results are consistent with those observed by Shin et al. (2021). In addition to the above anomalies, we also found perpendicular spindles and abnormal spindle directions such as parallel spindles or tripole spindles. De Storme and Geelen (2013) reported that the unreduced 2n pollen grains were mainly caused by abnormal spindle orientation and cytokinesis. Abnormal phenomena such as trizygote and micronucleus were also evident in our research. Khah and Verma (2019) observed that the accumulation of various chromosomal abnormalities can lead to the production of nonviable gametes and reduced fertility of plants. Studies have also shown that abnormal behavior during meiosis is often accompanied by changes in pollen size (Tian et al. 2015, Wang et al. 2010b). In this study, we also found that the average polar axial length of the deformed pollen was about 9 μm shorter than that of the normal pollen. In brief, we propose that the interspecific hybrid 'DY-4' produced many deformed pollens, which may be caused by many gamete variations, which resulted in reduced fertility of deformed pollens.

CONCLUSIONS

In our study, we examined the pollen viability of the interspecific hybrid 'DY-4' using TTC and FDA staining, and in vitro germination test. 'DY-4' was found to have meiotic chromosomal abnormalities. The pollen deformation rate of the interspecific hybrids 'DY-4' was 16% as determined by scanning electron microscopy. These results suggest that accumulation of these abnormalities may affect pollen fertility in'DY-4'.

ACKNOWLEDGMENTS

The project was partly supported by the Major Scientific and Technological Special Program in Hunan Province (No. 2021NK1007) , the Natural Science Foundation of Hunan Province (No. 2021JJ31157) and the Natural Science Foundation of China (No. 32271841).

LITERATURE CITED

Aparicio A, Albaladejo RG. 2003. Microsporogenesis and meiotic abnormalities in the hybrid complex of *Phlomis composita* (Lamiaceae). Bot J Linn Soc 143:79-85.

Alburquerque N, García Montiel F, Burgos L. 2007. Short communication. Influence of storage temperature on the viability of sweet cherry pollen. Span J Agric Res 5(1):86-90.

Atlagić J, Terzić S, Marjanović-Jeromela A. 2012. Staining and fluorescent microscopy methods for pollen viability determination in sunflower and other plant species. Ind Crops Prod 35: 88-91.

Alexander LW. 2019. Optimizing pollen ger-

mination and pollen viability estimates for *Hydrangea macrophylla*, *Dichroa febrifuga*, and their hybrids. Sci Hortic 246:244-50.

Bryhan N, Serdar U. 2008. Assessment of pollen viability and germinability in some European chestnut genotypes (*Castanea sativa* L.). Hort Sci 35(4):171-8.

Bispo R, Santos L, Santos J, Karsburg I. 2018. Viability estimation and morphological characterization of pollen of Datura suaveolens Willd (Solanaceae) *Datura suaveolens* Willd (Solanaceae). Sci Electron Arch 11(6):61-4.

Calic D, Devrnja N, Kostic I, Kostic M. 2013. Pollen morphology, viability, and germination of Prunus domestica cv. Požegača. Sci Hortic 155:118-22.

De Storme N, Geelen D. 2013. Sexual polyploidization in plants-cytological mechanisms and molecular regulation. New Phytol 198(3):670-84.

Deng ZY, Xiong H, Yuan DY, Niu G, Zou F. 2018. Orthogonal test design for optimizing culture medium for *in vitro* pollen germination of Chinese Chinquapin (*Castanea henryi*). Taiwan J For Sci 33(3):185-95.

Hu Z, Zhao Y, Zhao C, Liu J. 2020. Taxonomic importance of pollen morphology in *Veratrum* L. (Melanthiaceae) using microscopic techniques. Microsc Res Tech 83(1):1-12.

Hu YF, Xia QP. 2022. Comparison of different methods of pollen viability determination and preliminary study on *in vitro* culture-taking *Yulania cylindrica* as an example. Chinese Agric Sci Bull 38(05):43-47. (in Chinese with English abstract).

Jiang Y, Ding C, Yue H, Yang R. 2011. Meiotic behavior and pollen fertility of five species in the genus *Epimedium*. Afr J Biotechnol 10(72):16189-16192.

Koduru PRK, Rao MK. 1981. Cytogenetics of synaptic mutants in higher plants. Theor Appl Genetics 59(4):197-214.

Ki K, Zhou G, Zhang H. 2010. Chemical constituents and biological activities of saponin from the seed of Camellia oleifera. Sci Res Essays 5(25):4088-92.

Khah MA, Verma RC. 2019. Gamma irradiation induced multiple chromosome interchanges in *Hordeum vulgare* L. (Poaceae): meiotic characterization and their implications on pollen fertility. Nucleus Int J Cytol Allied Topics 63(2):1-7.

Kaur D, Singhal VK. 2019. Meiotic abnormalities affect genetic constitution and pollen viability in dicots from Indian cold deserts. BMC Plant Biology 19:10.

Li H, Fang X, Zhong H, Fei X, Luo, F. 2014. Variation of physicochemical properties and nutritional components of oil-tea *Camellia* seeds during riping. For Res 27(1):86-91.(in Chinese with English abstract)

Li GF, Zhang ZK, Yang XH, Qiao YC, He XL, Gao YS, Jiang YY, Lin SQ. 2016. Interspecific and inter-generic hybridization compatibility of *Eriobotrya* species (loquat) and related genera. Hortic Plant J 2(06):315-22.

Lisa WA. 2019. Optimizing pollen germination and pollen viability estimates for *Hydrangea macrophylla*, *Dichroa febrifuga*, and their hybrids. Sci Hortic 246:244-50.

Luo S, Zhang K, Zhong WP, Chen P, FanXM, YuanDY. 2020. Optimization of *in vitro* pollen germination and pollen viability tests for *Castanea mollissima* and *Castanea henryi*. Sci Hortic 271(8):109481.

Li YM, Ye TW, Han CX, Ye ZH, Zhang J, Xiao SX, Yuan DY. 2021. Cytogenetic analysis of interspecific hybridization in oil-tea (*Camellia oleifera*). Euphytica 217(2):1-12.

Mo R. 1992. On significances of intraspecific variation in pollen morphology. J Inner Mongolia Univ (Natural Science Edition) (01):133-7. (in Chinese with English abstract).

Osborn J, El-Ghazaly G, Cooper R. 2001. Development of the exineless pollen wall in *Callitriche truncata* (Callitrichaceae) and the

evolution of underwater pollination. Plant Syst Evol 228(1): 81-7.

Pagliarini MS. 2000. Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. Genet Mol Biol 23:997-1002.

Pradyut B, Subrata M. 2014. Role of some nutrients on *in vitro* pollen germination and tube development of *Luffa cylindrica* (L.) Roem. Ann Plant Sci 3: (09), 813-821.

Russell S. 1992. Double fertilization. Internat Rev Cytol 140:357-88.

Sharafi Y, Bahmani A. 2011. Pollen germination, tube growth and longevity in some cultivars of *Vitis vinifera* L. Afr J Microbio Res 5(9):1102-7.

Sunilkumar K, Mathur R, Sparjanbabu D, Reddy A. 2013. Pollen viability and vigour in interspecific hybrids (*E. guineensis* × *E. oleifera*) of oil palm. J Plant Crops 41(1):91-4.

Silva D, Zambon C, Techio V, PioR. 2020. Floral characterization and pollen germination protocol for *Castanea crenata* Siebold & Zucc. S Afr J Bot 130(1):389-95.

Singh H, Singh J, Kumar P, Singhal VK, Kholia BS, Tewari LM. 2020. Chromosome count, male meiotic behaviour and pollen fertility analysis in *Agropyronthomsonii* Hook.f. and *Elymus nutans* Griseb. (Triticeae: Poaceae) from Western Himalaya, India. Caryologia, 73(2):89-98.

Shin H, Park HR, Park JE, Yu SH, Yi G, Kim JH, Koh WJ, Kim HH, Lee SS, Huh JH. 2021. Reduced fertility caused by meiotic defects and micronuclei formation during microsporogenesis in *xBrassicoraphanus*. Genes Genomics 43:251-8.

Tan XF, Yuan DY, Yuan J, Liao T. 2010. Pollen germination in *Camellia oleifera* with ascorbic acid and plant growth regulators. J Zhejiang For Coll 27(6):941-4. (in Chinese with English abstract)

Tan XF, Yuan DY, Yuan J, Zou F, Xie P, Su Y, Yang D, Peng J. 2011. An elite variety: *Camellia oleifera* 'Huashuo'. Sci Silv Sin 47(12):184. (in Chinese with English abstract)

Taylor M, Cooper R, Schneider E, Osborn J. 2015. Pollen structure and development in Nymphaeales: Insights into character evolution in an ancient angiosperm lineage. Amer J Bot 102(10):1685-702.

Tian J, Wang J, Dong L, Dai F, Wang J. 2015. Pollen variation as a response to hybridisation in *Populus* L. section *Aigeiros* Duby. Euphytica: Int J Plant Breed 206(2):433-43.

Weng Y. 1997. Studies of *Camellia grijsii* Hance. Commonwealth Forestry Review, 76(2):132-133. (in Chinese with English abstract).

Wang X, Chen Y, Jiang L, Liu Z, Peng S, Wang R, Ma L, Yang X. 2010a. Electron microscopic scanning on pollen-morphology characters of *Camellia oleifera* superior clones. J Cent South Univ For Tech 30(05):67-71+90. (in Chinese with English abstract).

Wang J, Kang XY, Zhu Q. 2010b. Variation in pollen formation and its cytological mechanism in an allotriploid white poplar. Tree Genet Genomes 6(2):281-90.

Wang Y, Du K, Deng Q, Zeng J, Zhang J. 2017. Identification of interspecific hybrids between loquat (*Eriobotrya japonica* Lindl.) and Bengal loquat (*E. bengalensis* hook.). Pakistan J Bot 49(3):897-902.

Xiao Y. 2006. Investigation and prospect of bio-active components in vegetable oil. Cereal and Food Indust 13:1-5.

Xi XJ, Zha Q, Jiang AL, Tian YH. 2015. Meiotic chromosome behavior of pollen mother cells and gamete fertility in triploid grape 'Summer Black'. Acta Botanica Boreali-Occidentalia Sinica,35(12):2422-7. (in Chinese with English abstract)

Xiong H, Zou F, Yuan D, Zhang X, Tan X. 2016. Orthogonal test design for optimizing the culture medium for *in vitro* pollen germi-

nation of feijoa (*Acca sellowiana* cv. Unique). New Zeal J Crop Hort Sci 44(3):192-202.

Yuan D, Wang R, Yuan J, Liao T, Cui X, Cai L. 2010. Effects of different nutrient elements and ratios on pollen germination rate of *Camellia oleifera*. J Fujian Agri For Univ (Natural Science Edition), 39(5):471-4. (in Chinese with English abstract)

Yandovka L, Barabanov I. 2021. Pollination and hybridisation systems in genus Ribes L. (Grossulariaceae). IOP Conference Series: Earth Environ Sci 677(4):042026.

Zhuang R, Yao X, Zhou Q. 2012. *Camellia oleifera* in China, 2nd edition. China Forestry Press, Beijing, China. (in Chinese with English abstract)

Zortéa KVM, Rossi A, Bispo RB. 2019. Meiotic behavior and pollen viability of *b* L.: native fruit species of the Amazon. Floresta Ambiente 26(3):e20180375.

Supplementary Material

Table S1. Average pollen viability and pollen germination rate of *Camellia grijsii* 'Youza 2' and *Camellia oleifera* 'Huashuo'

cultivar	Pollen viability (%)		Pollen germination rate (%)
	TTC	FDA	T1
C.grijsii 'Youza 2'	66.03±9.03	77.06±7.68	64.77±8.11
C.oleifera 'Huashuo'	71.47±7.39	81.74±8.63	74.29±6.11

Note: Pollen viability was measured by vitality staining, and pollen germination rate was measured by *in vitro* germination. Pollen germination rate was measured after 2h of culture.

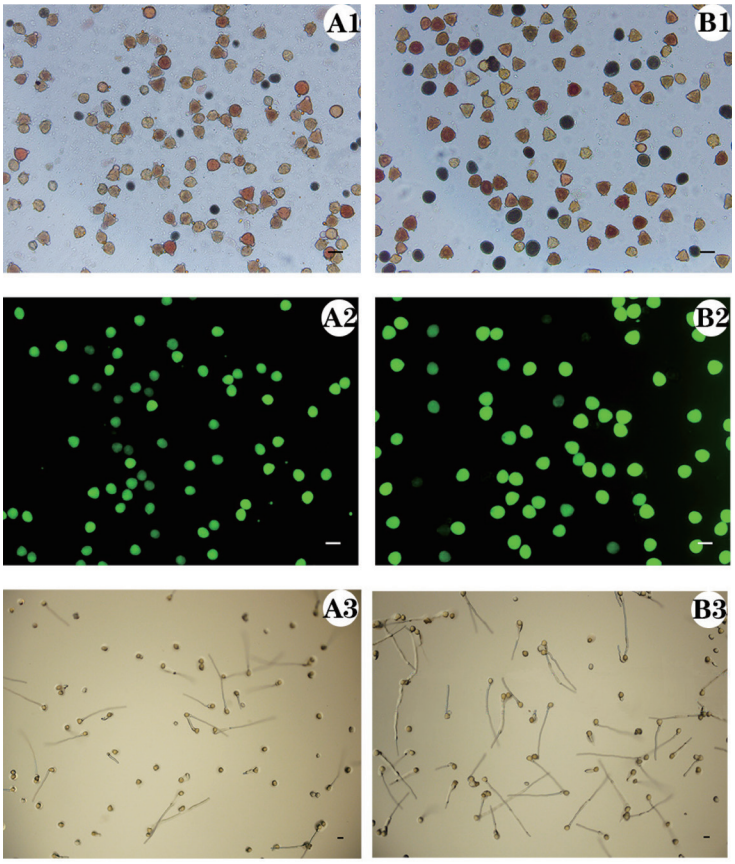

Fig. S1. The capital letters "A" and "B" respectively represent *Camellia grijsii* 'Youza 2' and *Camellia oleifera* 'Huashuo' . Numbers "1,2,3" respectively represent pollen viability after TTC, FDA staining and *in vitro* germination. Bar = 50μm.