Fertility and Progeny Ploidy of Triploids in China Rosa Germplasm

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Abstract

Background

Although triploid plants are often assumed to be sterile, in *Rosa*, triploids appear to have been important in both species evolution and breeding. A better understanding of the fertility of China *Rosa* triploids and their behavior when used in interploidy crosses will not only improve gene introgression from China triploid roses to modern roses, but also enhance our understanding of male and female fertility mechanism with triploids. The objective of this study was to evaluate the usefulness of five triploid China roses for breeding by measuring the pollen size, quantifying the fertility and following the ploidy transmission of interploidy crosses.

Results

In the five triploids, the pollen grain size of *Rosa multiflora* var. *cathayensis* Rehd 'Fen Tuan Qiang Wei' followed a normal distribution, suggesting that 1 mainly ploidy level pollen grain were produced, while the pollen size of other 4 triploids followed skewed, flattened, and slightly bimodal distributions, indicating a wide range of chromosome numbers. Although none of the 5 triploids produced hips as females, 3 gave good hip and seed production on the tetraploid mother plant when used as the pollen parents. Based on the ploidy level analysis of their progeny, 'Fen Tuan Qiang Wei' produced 1n viable pollen whereas 'Chun Shui Lü Bo' and 'Yu Shi Zhuang' produced 2n viable pollen. In the meiosis of triploids, the triads, pentads and microcyte in the meiosis products indicated the objective triploid production of euploid pollen grains.

Conclusions

In this study we reported 1 China triploid rose produces 1n viable pollen, and 2 China triploid roses produce 2n viable pollen, and no aneuploid progeny was produced. Our results indicate that the triploids contribute significantly to tetraploids formation in the crossing system of Genus *Rosa*, and it is possible for breeders to create novel rose types with valuable triploids in the future. With the special triploid resources found in this experiment, Genus *Rosa* will be an excellent material to study the mechanism of triploids in the future.

Background

Polyploidy (whole-genome duplication, WGD) is a major force in the evolution of higher plants (Simillion et al., 2002; Blanc and Wolfe, 2004). Most natural polyploids are thought to origin from sexual mechanism. As the fertilization of two unreduced gametes (female gamete and male gamete) seems to be an unlikely event, natural tetraploids are most likely to origin in two steps, via a triploid intermediary. Some studies about the mechanism of polyploid origin have been reported (Ramsey and Schemske 1998; Burton and Husband, 2001; Husband, 2004). Most triploid are assumed to be sterile because their gametes are aneuploid, but some triploids do have fertility in breeding (Zhang, 2009; Wang, 2017; Zhang, 2017). The greater variability for pollen size in triploids had been observed, which reflected different ploidy levels or aneuploid gametes

(Jacob and Pierret 2000; leus 2005; Crespel et al. 2006; Zlesak, 2009). Irregular chromosome pairing and abnormal meiotic division are the reasons for variations in pollen size and fertility, as has been reported in triploid *Populus* (Wang et al. 2010) and lily (Zhang, 2017). The aberrations during chromosome pairing, segregation, spindle formation, or cytokinesis might produce triads, dyads or monads, which are probably to produce unbalanced gametes (Bosco et al. 1999; Risso-Pascotto et al. 2005; Wang et al. 2010; Zhang, 2017).

Roses are very valuable ornamental plants around the world and thousands of cultivars have been developed. Most botanical species are diploid, while most commercially valuable modern rose cultivars are tetraploid. The hybridization among European tetraploids and Asian diploids produced modern rose cultivars (Zlesak, 2009). It is most likely that triploid roses have played a very important role in this long process as a ploidy bridge. About eight to eleven wild species had been used in the breeding history of modern commercial roses (Mac Phail and Kevan 2009). There are still quantity of diploid wild species and some triploid germplasm, which have not been introduced into modern rose breeding programs. Inter-specific or intergeneric hybrids can improve not only the qualitative but also quantitative traits of growth vigor or ornamental value (Van Tuyl and Lim 2003; Bao et al. 2012). Interploidy crosses with triploids have been reported, but the ploidy levels of the progeny were not consistent. In the crosses between tetraploid females and triploid males, Leus (2005) reported about 98% (123/125) tetraploid offspring, but Zlesak (2009) obtained about half triploid (23/43) offspring and half tetraploid offspring (20/43). In the crosses between diploid females and triploid males, Barden got diploid, triploid, and tetraploid offspring (Barden and Zlesak, 2004).

Rosa resources from China, such as *Rosa chinensis* and Chinese Old Garden Roses, have contributed recurrent flowering and tea scent to modern roses, and played very important roles in the formation history of modern roses. In China *Rosa* germplasm, there are some triploids that have not been used in breeding program but possess valuable traits. A better understanding of the fertility of China *Rosa* triploids and their behavior when used in interploidy crosses will not only improve gene introgression from China triploid roses to modern roses, but also enhance our understanding of male and female fertility mechanism with triploids. This study will also help us clarify the 'triploid bridge' in modern roses formation history, and make it possible for breeders to create novel rose types in the future. The objectives of this study were to evaluate the usefulness of triploid China rose for breeding by measuring the pollen size, quantifying the fertility and following the ploidy transmission of interploidy crosses.

Methods

Plant materials

Five triploid China roses, including 4 Chinese old garden rose cultivars 'Chun Shui Lü Bo', 'Yu Shi Zhuang', 'Hu Zhong Yue', 'Si Mian Jing' and 1 species *R. multiflora* var. *cathayensis* Rehd 'Fen Tuan Qiang Wei' were used as triploid material (Table 1). Eight tetraploid modern roses and one diploid Chinese old garden rose were used as hybrid parents. Crosses between the triploids and tetraploids or diploids were conducted to produce populations with ploidy level segregation. Ploidy level analysis was conducted on the 190 randomly selected seedlings from the hybrid population.

Table 1
Ploidy and rose class of the parents used in this experiment.

Cultivar name	Classi- fication	Ploidy level	Cultivar name	Classi- fication	Ploidy level
Old Blush	Chinese Old Garden Rose	2x	Chun Chao	Shrub	4x
<i>R. multiflora</i> 'Fen Tuan Qiang Wei'	Species	3x	Queen Elizabeth	Hybrid Tea	4x
Chun Shui Lü Bo	Chinese Old Garden Rose	3x	Hongwu Yue	Shrub	4x
Hu Zhong Yue	Chinese Old Garden Rose	3x	Carefree Beauty	Shrub	4x
Qing Lian Xue Shi	Chinese Old Garden Rose	3x	Gold Mary	Floribunda	4x
Yu Shi Zhuang	Chinese Old Garden Rose	3x	Compassion	Climber	4x
Tutti Frutti	Shrub	4x	Dee Dee Bridgewater	Climber	4x

Note: *Rosa multiflora* var. *cathayensis* 'Fen Tuan Qiang Wei' is described in <Flora of China >, vol.9, Rosaceae, Rosa 60b, 2003. Old Blush, 'Chun Shui Lü Bo', 'Hu Zhong Yue', 'Qing Lian Xue Shi', 'Yu Shi Zhuang' are all described in <China Old Garden Rose>, published by China Science Press, 2015. The other modern roses are all described in <Modern Rose Ⅹ> (2013). All the plant material are commercial available. The authors bought them and identified them according to the books above, and they are all planted in Beijing Institute of Landscape Architecture now.

Pollen viability and size observation

Fresh pollen sample was collected (Zlesak, 2009), then stored in refrigerator at -20°C. The sample was then mounted in a drop of 1% aceto-carmin on a microscope slide and viewed under the Olympus microscope (DP74, Japan). Empty and shrunken pollen grains were scored. The diameter of 500 pollen grains were measured using the attached calibrated micrometer of Olympus microscope.

Pollen viability was evaluated using the method proposed by Feng et al. (2017). A pollen grain was considered to have germinated if the pollen tube length was equal to or greater than the pollen diameter. Five microscopic field areas were randomly selected, and 5 plant samples were observed.

Pollen morphological observation

Pollen morphology was studied with scanning electron microscopy (Hitachi S-3400, Tokyo, Japan) following Zhang's method (Zhang, et al. 2017).

Crossing experiment

Interploidy crosses were conducted by hand in May 2013–2016 as proposed by Gao (2018), and hips were collected in October. Seeds were placed in wet sand storage at 1–4 °C for 2–3 months, after which they were sown in a greenhouse at 25 °C. F₁ seedlings were planted in an open field. The plant materials in the experiment were collected from and approved by the Beijing Institute of Landscape Architecture.

Observation of Pollen Tube Growth

Pollinated pistils of *Rosa* ‘Carefree Beauty’ were excised 8 h, 12 h, 24 h, 48 h and 72 h after hand pollination with pollens from triploid ‘Chun Shui Lü Bo’. Pollen tube growth was observed according to Michael’s protocol (Michael et al, 2015). Visualization was performed with an Olympus epifluorescence microscope (DP74) using an excitation wavelength of 450/90 nm and an emission wave length of 520 nm.

Stigma receptivity observation

Stigma receptivity was studied by hydrogen peroxide test under microscope in a laboratory according to Monika’s protocol (Monika, 2017). The flowers were taken at the first day the petals reveal the stigmatic surface and the observations were recorded 1hour intervals from 8 a.m. to 8 p.m. The receptivity of stigma was estimated by the bubbling quantity from stigma.

Analysis of ploidy levels

Counts of somatic chromosome number was done in shoot tip tissue following Wang’s method (Wang, 2017). The preparation was observed by using an Olympus DP75 microscope.

The ploidy level of progeny was also determined by flow cytometry (Feng et al., 2017). Samples were analyzed with a BD Company Accuri C6 flow cytometer. 5000–10,000 particles were detected and collected, ‘Old Blush’ was used as diploid standard. The somatic chromosome numbers of some seedlings were also checked by counting chromosomes of cells from shoot tips as described above.

Cytological observations of microsporogenesis

Anthers of ‘Fen Tuan Qiang Wei’ and ‘Chun Shui Lü Bo’ undergoing meiotic development were collected and immediately fixed in freshly prepared Carnoy’s solution (3:1 ethanol-acetic acid) for 24 h at room temperature and then stored in 70% ethanol at 4°C until analysis. Microscopic observations and image acquisition were conducted as described by Zhang et al. (2017).

Results

Pollen morphology, size-frequency distribution and viability

Pollen grains of the five triploids had 3 colporates that annularly distributed in equal space, and extended to the two poles. They were long elliptic shaped, and their outer wall was coarse, microperforate, and decorated with streaks (Fig. 1; N3P4C5 type, see Erdtman, 1971). Some anomalous pollens were found. The pollen grain size of the 5 triploids were analyzed by using 1 diploid and 1 tetraploid as controls (Table 2). According to P/E value, compared with ‘Old Blush’, the five triploids had more long spherical shaped pollens, and less

extra long spherical shaped pollens. Compared with the tetraploid ‘Dee Dee Bridgwater’, ‘Yu Shi Zhuang’ and ‘QingliangXueshi’ had similar quantity of long spherical shaped pollens, and the other 3 triploids had fewer long spherical shaped pollen.

Table 2
Analysis of rose pollen morphology.

Cultivar name	Polar axis length(μm) ±SE	Equatorial axis length(μm)±SE	Near spherical shape	Long spherical shape	Extra long spherical shape	Coefficient of variation
			(P/E= 0.75~1.33)	(P/E= 1.33~2)	(P/E > 2)	
Old Blush	40.23 ± 0.27b	19.00 ± 0.23c	0	16.7%	83.3%	11.6
Dee Dee Bridge water	42.80 ± 0.37a	30.01 ± 0.55a	25.5%	72.6%	2.0%	15.0%
Fen Tuan Qiang Wei	41.27 ± 0.32b	20.87 ± 0.19b	2.0%	48.0%	50.0%	13.3%
Chun Shui Lü Bo	36.85 ± 0.41c	18.93 ± 0.19c	2.7%	52.2%	45.2%	19.3%
Yu Shi Zhuang	31.38 ± 0.37d	18.45 ± 0.17c	11.2%	72.5%	16.4%	20.8%
Qing Lian Xue Shi	33.24 ± 0.36d	18.78 ± 0.14c	3.7%	75.8%	20.6%	18.8%
Hu Zhong Yue	31.96 ± 1.25d	20.45 ± 0.67b	23.7%	60.5%	15.8%	24.3%
Note: Different lower case letters represent significant differences at p < 0.05.						

Multiple comparisons analysis was conducted on the pollen polar axis length of 'Old Blush', 'Dee Dee Bridgewater' and five triploids (Table 2). 'Dee Dee Bridgewater' pollen was the largest, followed by 'Old Blush' and 'Fen Tuan Qiang Wei'. The pollens of other 4 triploids were smaller than 'Old Blush'. The pollen size variation coefficient of diploid 'Old Blush' and tetraploid 'DEE DEE Bridgewater ' was 11.6% and 15.0% respectively. The variation coefficient of triploid 'Fen Tuan Qiang Wei' was 13.3%, whereas the variation coefficient of the other 4 triploids were from 18.8%to 24.3%, higher than the diploid and tetraploid.

Gaussian distribution tests were conducted on frequency distribution with polar axis length of pollen grains (Table 3). Only 'Old Blush', 'Dee Dee Bridgewater ' and 'Fen Tuan Qiang Wei' showed a normal distribution of pollen size, indicating only one ploidy level pollens were produced. The other four triploids had skewed, flattened, and slightly bimodal distributions, indicating the wide range of pollen size in triploid (Fig. 1). Pollen germination rate of 5 triploids on artificial liquid medium were very different, with 'Fen Tuan Qiang Wei' had the highest germination rate of 32.00%, and 'Huzhong Yue' has the smallest germination rate of 4.00% (Table 4).

Table 3
Gaussian distribution tests on frequency distribution with pollen grain size.

Cultivar or species	Ploid level	Gaussian distribution tests
Old Blush	2x	0.566
Dee Dee Bridgewater	4x	0.399
Fen Tuan Qiang Wei	3x	0.230
Chun Shui Lü Bo	3x	0.091
Yu Shi Zhuang	3x	0*
Qing Lian Xue Shi	3x	0*
Hu Zhong Yue	3x	0.089
Note: * indicate a significant difference at $P \leq 0.05$.		

Table 4
Pollen germination rates of five triploid roses.

Cultivar or species	Pollen amount/flower	Pollen germination Rate(%)
Chun Shui Lü Bo	+++	10.0
Yu Shi Zhuang	++	6.0
Hu Zhong Yue	+	4.0
Qing Lian Xue Shi'	+	5.0
Fen Tuan Qiang Wei	++++	32.0
Note: From '++++' to '+' means the amount of pollen from maximum to minimum.		

Pollen tube growth observation and male fertility of five triploids

The pollen of 'Chun Shui Lü Bo' on the stigma of 'Carefree Beauty' germinated 8–12 hours after pollination (Fig. 3a, b, c). By 24 hours the pollen tubes grew through the tissue of the stigma and reached the middle or the upper part of the style (Fig. 3d), by 48 hours the pollen tubes reached the lower middle of the style (Fig. 3e), and by 72 hours after pollination, a few pollen tubes reached the ovary (Fig. 3f).

Higher fruit set and number of seeds per hip were observed in interploidy crosses with the tetraploid 'Dee Dee Bridgewater' as a seed parent and triploid 'Chun Shui Lü Bo', 'Yu Shi Zhuang' and 'Fen Tuan Qiang Wei' as pollen parents (Table 5). The cross between diploid 'Old Blush' (♀) and triploid 'Fen Tuan Qiang Wei' (♂) had lower fruit set rate and germination rate than the cross between tetraploid 'Dee Dee Bridgewater' (♀) and

triploid 'Fen Tuan Qiang Wei' (馥团强威). Crosses with triploid 'Qin Lian Xue Shi' and 'Hu Zhong Yue' as pollen parents did not set any hips.

Table 5
Results of hybridization with rose triploids as male parents.

Seed parents	Ploidy level	Pollen parents	Ploidy level	No. of pollinated flowers	No. of hips	Hip set (%)	No of seeds	No of seedings	Germination (%)
Dee Dee Bridgewater	4x	Chun Shui Lü Bo	3x	40	18	45	275	115	42
Tutti Frutti	4x	Chun Shui Lü Bo	3x	12	7	58	60	20	33
Chun Chao	4x	Chun Shui Lü Bo	3x	43	19	44	111	14	13
Queen Elizabeth	4x	Chun Shui Lü Bo	3x	25	19	76	140	38	27
Compassion	4x	Chun Shui Lü Bo	3x	22	1	5	3	2	67
Carefree Beauty	4x	Chun Shui Lü Bo	3x	48	14	29	33	13	39
Old Blush	2x	Fen Tuan Qiang Wei	3x	20	10	50	126	14	12
Gold Mary	4x	Fen Tuan Qiang Wei	3x	45	21	47	295	44	14
Compassion	4x	Fen Tuan Qiang Wei	3x	40	17	43	257	34	14
Carefree Beauty	4x	Fen Tuan Qiang Wei	3x	15	6	40	91	16	18
Carefree Beauty	4x	Hu Zhong Yue	3x	11	0	0	-	-	-
Dee Dee Bridgewater	4x	Qing Lian Xue Shi	3x	10	0	0	-	-	-

Seed parents	Ploidy level	Pollen parents	Ploidy level	No. of pollinated flowers	No. of hips	Hip set (%)	No of seeds	No of seedings	Germination (%)
Carefree Beauty	4x	Qing Lian Xue Shi	3x	3	0	0	-	-	-
Dee Dee Bridgewater	4x	Yu Shi Zhuang	3x	21	7	33	168	50	30
Queen Elizabeth	4x	Yu Shi Zhuang	3x	6	0	0	-	-	-
Total	-	-	-	364	139	38	1559	306	20

Stigma receptivity analysis and female fertility of triploids

‘Carefree Beauty’ stigmas turned to blue with high bubbling and ‘Old Blush’ stigmas turned yellow with high bubbling indicating high and moderate pistil receptivity respectively. In contrast, the stigmas of the five triploids showed little bubbling indicating low pistil receptivity (Fig. 4c, d, e, f, g). This agrees with the lack of set among the triploids when used as females (Table 6).

Table 6
Results of hybridization by using triploid as female parents

Seed parents	Ploidy level	Pollen parents	Ploidy level	No. of pollinated flowers	No. of hips
Chun Shui Lü Bo	3x	Chun Chao	4x	10	0
Huzhong Yue	3x	Chun Chao	4x	20	0
Huzhong Yue	3x	Hongwu Yue	4x	10	0
Qinglian Xueshi	3x	Hongwu Yue	4x	23	0
Yu Shi Zhuang	3x	Chun chao	4x	10	0
Yu Shi Zhuang	3x	Hong wuYue	4x	10	0
Yu Shi Zhuang	3x	Huang shou Pa	4x	10	0
Yu Shi Zhuang	3x	Carefree Beauty	4x	10	0
Yu Shi Zhuang	3x	Old Blush	2x	11	0
Fen Tuan Qiang Wei	3x	Old Blush	2x	20	0
Fen Tuan Qiang Wei	3x	Carefree Beauty	4x	20	0

Progeny ploidy levels screening by flow cytometric analysis and chromosome counting

Flow cytometric analyses of the 138 progenies, which were from the crosses with ‘Chun Shui Lü Bo’ or ‘Yu Shi Zhuang’ as male parents and tetraploids as female parents, indicated that 129 seedlings were tetraploid, 6 seedlings were pentaploid and 3 seedlings were triploid (Table 7). Chromosome counts of the pentaploid and triploid seedlings showed that these were tetraploids with 28 chromosomes (Fig. 5). Other check of the flow cytometric results with chromosome counts confirmed the ploidy designation.

Table 7
Ploidy level evaluation of rose F₁ interploidy progeny by flow cytometry analysis.

Seed parents	Pollen parents	No. of progeny	Progeny Ploid Level			
			2x	3x	4x	5x
Chun Chao	Chun Shui Lü Bo	8	0	0	8	0
Dee Dee Bridgewater	Chun Shui Lü Bo	56	0	0	56	0
Queen Elizabeth	Chun Shui Lü Bo	17	0	1	13	2
Carefree Beauty	Chun Shui Lü Bo	18	0	0	18	0
Tutti Frutti	Chun Shui Lü Bo	12	0	0	9	3
Dee Dee Bridgewater	Yu Shi Zhuang	28	0	2	25	1
Old Blush	Fen Tuan Qiang Wei	7	7	0	0	0
Carefree Beauty	Fen Tuan Qiang Wei	6	0	6	0	0
Compassion	Fen Tuan Qiang Wei	17	0	17	0	0
Gold Mary	Fen Tuan Qiang Wei	22	0	22	0	0

Flow cytometric analyses of the 45 progenies from the crosses with ‘Fen Tuan Qiang Wei’ as male parents and tetraploids as female parents were all triploids, and the 7 seedlings from the cross of ‘Old Blush’ and ‘Fen Tuan Qiang Wei’ were diploid (Table 7). Chromosome counting were also conducted on 6 seedlings which were randomly selected from the cross of ‘Gold Mary’ and ‘Fen Tuan Qiang Wei’, and the results showed they were all triploid (Fig. 5), coincident with the flow cytometric analysis.

Abnormal meiotic chromosome behaviors of ‘Chun Shui Lü Bo’ and ‘Fen Tuan Qiang Wei’

Abnormal chromosomal behaviors were recorded (Table 8) in all stages of meiosis, indicating complex chromosome pairing and unbalanced chromosome segregation in the triploid rose genotypes examined. During metaphase I and anaphase I phases, chromosomes bridges (15.8%; Fig. 6E), lagging chromosomes (38.2%; Fig. 6F) and chromosomes that did not align on the equatorial plate were observed (Fig. 6C, E, F, H). During meatphase II, parallel spindles (56.3%; Fig. 6J), 120° spindles (18.8%; Fig. 6K) and lagging

chromosomes(16.7%; Fig. 6L) were observed. Lagging chromosomes and unequal chromosome distributions were also observed later during anaphase II and telophase II. The most normal meiosis among the triploids was seen in ‘Fen Tuan Qiang Wei’, with only 29.5% of the cells in anaphase I stage showing lagging chromosomes. Unbalanced chromosome segregation and triads with two micronuclei were also observed (Fig. 7). For triploids, meiotic products were mostly tetrads with some pentads (24.2%; Fig. 6Q), a few triads (6.7%; Fig. 6P) as well as a few tetrads with 1 or 2 microcytes (Fig. 6R, 6T), triads with 1 or 2 microcytes (Fig. 6S)

Table 8
Meiotic abnormalities of the triploid rose ‘Chun Shui Lü Bo’

Phases	Number of observed cells	Abnormalities	No. of abnormal cells	Abnormal cells (%)
Prophase I	254	-	0	0
Metaphase I	2216	Lagging chromosome	165	7.5%
Anaphase I	424	Lagging chromosome Bridge	162	38.2%
			67	15.8%
Telophase I	84	Chromosome not align on the equatorial plate	18	21.4%
Metaphase II	48	Parallel spindles	27	56.3%
		120° spindles	9	18.8%
		Lagging chromosome	8	16.7%
Anaphase II	16	Lagging chromosome	4	25.0%
		Unequal distribution	1	6.3%
Telophase II	43	Lagging chromosome	1	2.3%
Meiotic product	1429	Triad	95	6.7%
		Triads with microcytes	18	1.3%
		Triads with two microcytes	6	0.4%
		Tetrads with microcytes	150	10.5%
		Tetrads with 2 microcytes	6	0.4%
		Penads	346	24.2%
		Penads with microcytes	28	2.0%

Table 9
Meiotic abnormalities of triploid 'Fen Tuan Qiang Wei'

Phases	Number of observed cells	Abnormalities	Abnormal cells (%)
Anaphase $\square$	536	Lagging chromosome	29.5%
Meiotic product	610	Triads	12.5%

Discussion

The positive correlation between DNA content and pollen size makes it possible to decide the ploidy level by measuring the pollen size (Dewitte et al. 2012). For normal diploid plants, the frequency distribution of pollen grain size is unimodal, but for unreduced pollen producers, it is bimodal (Tondini et al.1993). In this research, pollen grains size of 'Old Blush'(diploid) 'Dee Dee Bridgewater ' (tetraploid) and 'Fen Tuan Qiang Wei' (triploid) followed a unimodal distribution, suggesting that 1 ploidy level pollen grain were produced. For triploid 'Chun Shui Lü Bo' and 'Yu Shi Zhuang', the size of pollen grains had higher variation coefficient (from 18.8%to 24.3%) and followed a skewed, flattened, and slightly bimodal distributions, suggesting the wide range of pollen size in triploid, which may represent the various chromosome numbers of pollen grains due to irregular chromosome pairing and unbalanced chromosome segregation.

For the 5 triploid China rose cultivars used as pollen parents in this study, 2 of them did not set any hips, 3 of them gave good hip and seed production on the tetraploid mother plant. This result indicated that some of the triploid China *Rosa* germplasm do have good fertility ability, and can be used as gene introgression bridge in future rose breeding program.

In this study, when triploid 'Fen Tuan Qiang Wei' was used as male parent, it produced 1n pollen as indicated by the unimodal pollen size distribution, the triploid seedlings from the 4x ($\square$) $\times$ 3x ($\square$) crosses, and the diploid seedlings from the 2x ($\square$) $\times$ 3x ($\square$) crosses. This result is different from Leus's, Zlesak's and Barden's work (Leus, 2005; Zlesak, 2009; Barden and Zlesak, 2004), and also different from the crosses in current study when 'Chun Shui Lü Bo' or 'Yu Shi Zhuang' were used as male parents. This is the first report that triploid rose producing mostly 1n viable pollen, and 1n pollen is more vigor than 2n pollen. It is a special result. To my knowledge, the only similar report I can find is about *Hieraciumechioides*, Peckert reported he got about 80% triploids in 4x $\times$ 3x crosses (Peckert, 2006).

When 'Chun Shui Lü Bo' and 'Yu Shi Zhuang' were used as male parents and crossed with a tetraploid female, 2n pollen grains were favored for fertilization as all of the seedlings from these crosses are tetraploid. This result is similar to Leus's report, he (2005) obtained about 98% (123/125) tetraploid offspring in the 4x ($\square$) $\times$ 3x ($\square$) crosses but different from Zlesak's report, who obtained about half triploid (23/43) offspring and half tetraploid offspring (20/43) in the 4x ($\square$) $\times$ 3x ($\square$) crosses. Greater vigor of 2n pollen was the reason for most offspring were tetraploid in this research and in Leus's research.

In current study, when triploids were used as male parents, only euploid progeny were obtained, the same as Leus's and Zlesak's work. It seems aneuploid progeny are rarely produced when triploid *Rosa* are used as

male parents. This is different from the studies about apple (Zhang, 2009), lily (Zhang, 2017) and populus (Wang, 2017), in which more aneuploid than euploid progeny were obtained. This is also different from Ramsey's report. In Ramsey's summary, the most common pollen chromosome number was $3x/2$, followed by $3x/2 - 1$, and the lowest pollen chromosome number was $1 \times$ (3%) and diploid $2 \times$ (2%) (Ramsey, 1998). In current study, the wide range of pollen size of 'Chun Shui Lü Bo' and 'Yu Shi Zhuang' suggested the various chromosome numbers of pollen grains. So probably some of the pollens were aneuploid. The reason why all the progeny obtained in this study were euploid may be that euploid gametes have greater vigor than the aneuploidy gametes in fertility. Though the aneuploidy pollen is not vigorous, in crosses with triploid *Rosa* as male parents, the fruit set and the average number of seeds per hip were higher compared with most other triploids (Zhang, 2017; Zhang, 2009; Wang, 2017). This result indicates that the *Rosa* triploids have some kind of mechanism to make sure they can produce much more vigorous euploid progeny than other triploids.

In this research, the triads, pentads and microcyte in the meiosis products indicated the objective triploid production of euploid pollen grains. Microcyte formation likely resulted in the loss of chromosomes from microspores, which may increase the variation in pollen size. According to Ramsey, euploid gametes are very rare, because haploid-diploid chromosome assortments are only produced when the separation of multivalents and unpaired chromosomes at the first meiotic division in a spore mother cell is very unequal (Ramsey and Schemske 1998). Few studies had been done about the unique mechanism of meiosis in triploid roses. The reasons why triploid roses can produce so many euploid gametes, and why 'Fen Tuan Qiang Wei' produced $1n$ viable pollen whereas 'Chun Shui Lü Bo' and 'Yu Shi Zhuang' produced $2n$ viable pollen are still uncertain. More researches need to be done to clarify the mechanism in the future.

Conclusion

In this study we reported 1 China triploid rose produces $1n$ viable pollen, and 2 China triploid roses produce $2n$ viable pollen, and no aneuploid progeny was produced. Our results indicate that the triploids contribute significantly to tetraploids formation in the crossing system of Genus *Rosa*, and it is possible for breeders to create novel rose types with valuable triploids in the future. With the special triploid resources found in this experiment, Genus *Rosa* will be an excellent material to study the mechanism of triploids in the future.

Abbreviations

Not applicable.

Declarations

Ethics approval and consent to participate: Not applicable.

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Availability of data and material: All the data can be available, and it is according to China law that if the material can be available.

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Figures

Figure 1

Scanning electron micrographs of pollen grains from five triploid roses in polar and equatorial view. 1-4, Polar view, equatorial view, external surface, and anomalous pollens of 'Fen Tuan Qiang Wei'; 5-8, Polar view, equatorial view, external surface, and anomalous pollens of 'Chun Shui Lü Bo'; 9-12, Polar view, equatorial view, external surface, and anomalous pollens of 'Yu Shi Zhuang'; 13-16, Polar view, equatorial view, external surface, and anomalous pollens of 'Qing Lian Xue Sshi'; 17-20, Polar view, equatorial view, external surface, and anomalous pollens of 'Hu Zhong Yue'.

Figure 2

Frequency distribution with polar axis length of pollen grains from five triploid roses, 'Old Blush' (diploid) and 'Dee Dee Bridgewater' (tetraploid).

Figure 3

Pollens from 'Chun Shui Lü Bo' germination and pollen tube growth in pistils of 'Carefree Beauty', viewed using fluorescence microscopy. a. Germinating pollen grains on surface of stigma, 8 h after cross-pollination $\times 10$. b. Germinating pollen grains on surface of stigma, 8 h after cross-pollination, magnified a $\times 40$. c. Pollen tube elongation, 12 h after pollination $\times 20$. d. Pollen tube reaching upper part of style, 24 h after pollination $\times 20$. e. Pollen tube reaching lower part of style, 48 h after pollination $\times 40$. f. Pollen tube entering ovules, 72 h after pollination $\times 40$.

Figure 4

Stigma receptivity of five triploid roses, 'Old Blush' (diploid) and 'Carefree Beauty' (tetraploid). a: 'Carefree Beauty' ; b: 'Old Blush'; c: 'Fen Tuan Qiang Wei'; d: 'Chun Shui Lü Bo'; e: 'Hu Zhong Yue'; f: 'Qing Lian Xue Shi'; g: 'Yu Shi Zhuang'.

Figure 5

Mitotic chromosome numbers in root tip cells from interploidy crosses with triploids as male parents, a-g from the cross between 'Dee Dee Bridgewater' (4x, ♀) and 'Yu Shi Zhuang' (3x, ♂), $2n=4x=28$; h-k from the cross between 'Queen Elizabeth' (4x, ♀) and 'Chunshui Lübo' (3x, ♂), $2n=4x=28$; l-o from the cross between 'Tutti Frutti' (4x, ♀) and 'Chunshui Lübo' (3x, ♂), $2n=4x=28$; p-u from the cross between 'Gold Mary' (4x, ♀) and 'Fen Tuan Qiang Wei' (3x, ♂), $2n=3x=21$.

Figure 6

Meiosis chromosomal behavior of 'Chun Shui Lü Bo' pollen mother cells A-B metaphase, C metaphase (arrow showing lagging chromosome), D anaphase, E anaphase (arrow showing chromosome bridge), F anaphase (arrow showing lagging chromosome), G telophase, H telophase (arrow showing chromosome outside metaphase plate), I prophase, J metaphase (parallel chromosomes), K metaphase (120° chromosome), L metaphase (arrow showing lagging chromosome), M anaphase, N telophase, O

tetrad, P triad, Q quartet, R quadrant with microspores, S Triad with two microcytes, T quadrant with two microcytes.

Figure 7

Meiosis chromosomal behavior of *R. multiflora* ‘Fen Tuan Qiang Wei’ pollen mother cells, a Prophase, b metaphase, c anaphase (arrow showing lagging chromosome), d telophase, e telophase, g anaphase, h tripolar spindles at telophase, showing 3 nuclei were produced, i tetrads, j triads with 2 micronuclei