Meiotic behaviour and pollen fertility of F_1 , F_2 and BC_1 progenies of *Iris dichotoma* and *I. domestica*

Wenji Xu^{1,2}, Gangjun Luo^{1,2}, Xiaoyin Lian¹, Fengyang Yu^{1,3}, Yang Zheng¹,
Jiajun Lei¹, Xiaoying Bi^{1,*}

¹ College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China

² School of Advanced Agriculture and Bioengineering, Yangtze Normal University, Fuling 408100, China

³ Shanghai Botanical Garden, Shanghai 200231, China

ABSTRACT

Pollen characteristics are very important for *Iris* interspecific hybridisation. In this study, the pollen viability and male meiosis were studied in yellow-flowered *Iris dichotoma* (Y2), *I. domestica* (S3) and their hybrids F_1 , F_2 and BC_1 (BC_1 -Y and BC_1 -S). The BC_1 -Y hybrids showed higher pollen viability than that of F_1 , F_2 and BC_1 -S hybrids, which were between *I. dichotoma* (26.1%) and *I. domestica* (35.1%). Two sterile hybrids, F_2 -1 and BC_1 -S-1, exhibited more meiotic abnormalities (57.3% and 58.7%) than other individuals. During the first meiotic division, a diffuse diplotene stage was observed for the first time in the genus *Iris*. The meiotic abnormalities included non-congressed chromosomes, chromosome bridges, lagging chromosomes, unequal division, abnormally oriented spindle fibres, nonsynchronous division and polyad, and resulted in reduced pollen fertility. The relatively high frequency of 2n pollen grains was found in hybrids of BC_1 -Y-2, BC_1 -Y-1, BC_1 -S-2, BC_1 -S-3 and BC_1 -S-4. Our research provides a new resource for meiotic behaviour and pollen fertility of the genus *Iris*.

Keywords: 2n gametes, abnormal meiosis, interspecific hybrid, *Iris*, male

INTRODUCTION

Iris dichotoma and *I. domestica* are herbaceous perennials belonging to the family Iridaceae, with a diploid chromosome number of $2n=2x=32$ native to China, and have a long flowering period in summer and high resistance to cold, drought and barren conditions, which make them excellent materials for iris breeding (Zheng et al., 2017). Therefore, *I. dichotoma* and *I. domestica* have been attracting the attention of plant breeders (Bi et al., 2012). In nature, *I. dichotoma* is found with violet, yellow and white flowers, but no red or orange flowers. Its individual flowers stay open for about 4 h in form the late afternoon to the early evening (Luo et al., 2016). To improve its flower colour and flowering

time, interspecific crosses were conducted to introgress useful genes from *I. domestica* to *I. dichotoma* and this has resulted in some novel varieties (Yang et al., 2013; Lian et al., 2016; Ruan et al., 2017; Xu et al., 2017; Gao et al., 2020). Previous studies concentrated on violet and white-flowered *I. dichotoma* plants (Chimphamba, 1973; Ruan et al., 2017), whereas *I. dichotoma* with yellow flowers has not been comprehensively investigated. The F_2 generation of *I. dichotoma* (violet-flowered) and *I. domestica* shows great variation in flower colours, but not with yellow (Lenz, 1972). Therefore, creating new germplasm with yellow-flowered *I. dichotoma* is a significant goal of *Iris*-breeding programs.

*Corresponding author.
e-mail: xiaoyingbi@syau.edu.cn (Xiaoying Bi).

Pollen fertility is very important for plant development and reproduction (Consolaro et al., 1996; Conterato et al., 2006). Variations in pollen fertility are usually attributed to abnormal chromosome pairing and abnormal meiotic division (Yabuya, 1991). Abnormal meiosis, such as early chromosome migration, lagging chromosomes, bridge, unequal separation, micronuclei, multiple spindles, nonsynchronous division, unequal separation and so on, was observed in many plants, especially in interspecific hybrids (Wang et al., 2010; Hu et al., 2021). Compared with their parents, interspecific hybrids of *Dendranthema* had a higher frequency of chromosome bridges and lagging chromosomes (Cui et al., 2007). Approximately 56.5% of the cells of allotriploid lily ‘Cocossa’ demonstrated meiotic abnormalities at telophase II (Zhang et al., 2017). Sometimes, the high frequency of abnormal meiosis was also found in intraspecific hybrids. The hybrids of *Hydrangea aspera* subsp. *aspera* Kawakami group $\times$ subsp. *sargentiana* showed a higher frequency of meiotic abnormalities (48.2%) compared with the two parents and resulted in reduced pollen fertility (Crespel and Morel, 2014). Generally, the fertility of interspecific/intraspecific hybrid could be restored by successive backcrosses. So far, there is no information on pollen viability and meiosis of *I. dichotoma*, *I. domestica* and their hybrids. Therefore, meiotic behaviour and pollen fertility of yellow-flowered *I. dichotoma*, *I. domestica* and their F_1 , F_2 and BC_1 hybrid progenies were investigated in this

study. Furthermore, the relationships between meiotic abnormalities and crossbreeding were discussed. The knowledge acquired will contribute to the rational use of these genetic resources.

MATERIALS AND METHODS

Plant materials

Yellow-flowered *I. dichotoma* (Y2) was collected from Pinglu, Shanxi Province, and *I. domestica* (S3) was obtained from the Shenyang Botanical Garden, Shenyang, Liaoning Province, China, in 2007. Both of them flower from July to late August, and their flowering periods overlap extensively. The populations of F_1 (Y2 $\times$ S3), F_2 [(Y2 $\times$ S3) $\otimes$], BC_1 -Y [(Y2 $\times$ S3) $\times$ Y2] and BC_1 -S [(Y2 $\times$ S3) $\times$ S3] were established from 2009 to 2014, and 242, 126, 130 and 121 individuals were obtained, respectively. According to their fertility, we selected some of them for this study (Figure 1). Meiosis behaviour and pollen viability were studied in two parents, one F_1 hybrid (F_1 -5), three F_2 hybrids (F_2 -1, F_2 -2 and F_2 -3), four BC_1 -S hybrids (BC_1 -S-1, BC_1 -S-2, BC_1 -S-3 and BC_1 -S-4) and two BC_1 -Y hybrids (BC_1 -Y-1 and BC_1 -Y-2).

Analysis of male meiosis

For analyses of meiosis, 2 mm, 4 mm, 6 mm, 8 mm and 10 mm length of young flower buds were collected between 8:00 am and 10:00 am in the middle of July

Figure 1. The flower of *I. dichotoma* (Y2), *I. domestica* (S3) and their hybrid progenies: A – *I. dichotoma* (Y2); B – *I. domestica* (S3); C – F_1 -5; D – F_2 -1; E – F_2 -2; F – F_2 -3; G – BC_1 -S-1; H – BC_1 -S-2; I – BC_1 -S-3; J – BC_1 -S-4; K – BC_1 -Y-1; L – BC_1 -Y-2.

2015 and 2016 (field temperature about 29–31°C), fixed in a mixture of absolute alcohol and glacial acetic acid (3:1) for 24 h and then stored in 70% alcohol at 4°C. The anthers were squashed in 5% carbol fuchsin and examined under a microscope (Motic BA400, China).

Pollen viability and size observation

Flowers were collected at anther dehiscence and the pollens were dispersed in the laboratory for 3 h (relative humidity <30%; temperature 24°C). The pollens were cultured on a solid medium (150 g · L⁻¹ sucrose + 100 mg · L⁻¹ H₃BO₃ + 100 mg · L⁻¹ CaCl₂ + 3 g · L⁻¹ agar, pH 5.8) at 25°C in an incubator (RLD-350D-4, China) for 2 h. At least 200 pollen grains per individual were observed under a microscope (Motic BA400, China). A pollen grain was considered to have germinated if the length of the pollen tube was equal to or longer than the pollen diameter.

Pollen size and morphological observation

Pollen grains were directly attached to double-sided adhesive tape and coated with gold. For pollen diameter, at least 1,000 pollen grains were measured for each accession. Microscopic observation and image acquisition were conducted using a scanning electron microscope at a magnification of 500× (TM3030, Japan).

RESULTS

Abnormal meiotic chromosome behaviours

During normal microsporogenesis, simultaneous meiosis was observed in *I. dichotoma*, *I. domestica* and their hybrid progenies: cytokinesis started only after telophase II (after the division of the four nuclei) and resulted in four microspores (tetrad), each containing one nucleus. Figure 2A–2T shows photomicrographs of successive meiotic stages, from interphase I to the pollen stage. One of the striking observations of the interphase I stage in this study was the diffuse appearance of diplotene (Figure 2E, 2F). The diffuse stage in diplotene was observed in *I. dichotoma*, *I. domestica* and all of their hybrids. At the beginning of the diplotene, nuclei were filled with extended and fuzzy single chromosome threads, corresponding to the diffuse stage described in many plants, and they stained only faintly or not wholly. Following the diffuse stage, the chromosomes contracted markedly (Figure 2G).

In each stage of meiosis, several frequent meiotic abnormalities were recorded. Across all cells analysed, the abnormal meiosis rates in most hybrid progenies were much higher than that of their parents. Over 50.0% of the cells exhibited abnormalities in F₂-1 (57.3%), BC₁-S-1 (58.7%), BC₁-S-2 (54.5%) and BC₁-S-3

Figure 2. Normal meiosis process of pollen mother cells in *I. dichotoma* (Y2), *I. domestica* (S3) and their hybrid progenies: A – pollen mother cell (BC₁-S-2); B – leptotene (BC₁-S-2); C – zygotene (BC₁-S-2); D – pachytene (BC₁-S-4); E – the diffuse appearance of diplotene (F₁-5); F – late diplotene (BC₁-Y-1); G – late diplotene (F₂-3); H – diakinesis (BC₁-S-4); I – metaphase I (BC₁-S-2); J – anaphase I (BC₁-S-4); K – telophase I (BC₁-S-2); L – prophase II (BC₁-S-2); M – metaphase II (BC₁-Y-2); N – anaphase II (F₁-5); O – telophase II (BC₁-S-4); P – telophase II (BC₁-S-2); Q, R – tetrad (BC₁-S-4); S – tetrad spores (BC₁-S-4); T – pollen (Y2).

Table 1. The meiosis abnormalities rate (%) in *I. dichotoma*, *I. domestica* and their hybrid progenies.

Phase abnormalities	Plant materials											
	Y2	S3	F ₁ -5	F ₂ -1	F ₂ -2	F ₂ -3	BC ₁ -S-1	BC ₁ -S-2	BC ₁ -S-3	BC ₁ -S-4	BC ₁ -Y-1	BC ₁ -Y-2
Metaphase I	288	241	200	130	184	210	162	207	165	80	142	150
Non-congression	114 (39.6%)	94 (39.0%)	83 (41.5%)	57 (43.8%)	80 (43.5%)	85 (40.5%)	98 (60.5%)	126 (60.9%)	78 (42.3%)	30 (37.5%)	40 (28.2%)	27 (18.0%)
Anaphase I	216	170	156	104	155	140	113	120	150	126	115	143
Lagging chromosomes	82 (37.9%)	35 (20.6%)	53 (34.0%)	11 (10.6%)	42 (27.1%)	31 (22.1%)	46 (40.7%)	50 (41.7%)	60 (40.0%)	41 (32.5%)	24 (20.9%)	14 (9.8%)
Bridge	19 (8.7%)	0 (0.0%)	28 (17.9%)	12 (11.5%)	5 (3.2%)	18 (12.9%)	3 (2.7%)	6 (5.0%)	11 (7.3%)	15 (11.9%)	5 (4.3%)	10 (7.0%)
Telophase I	195	152	152	123	121	127	107	102	109	88	120	167
Unequal separation	22 (11.3%)	8 (5.3%)	35 (23.0%)	64 (52.0%)	13 (10.7%)	9 (7.1%)	47 (43.9%)	24 (23.5%)	0 (0.0%)	25 (28.4%)	27 (22.5%)	45 (26.9%)
Micronuclei	7 (3.6%)	0 (0.0%)	3 (2.0%)	0 (0.0)	9 (7.4%)	12 (9.4%)	10 (9.3%)	4 (3.9%)	19 (17.4%)	5 (5.7%)	3 (2.5%)	0 (0.0%)
Metaphase II	160	160	154	230	131	140	101	142	164	142	150	150
Non-congression	1 (0.6%)	14 (8.8%)	24 (15.6%)	67 (29.1%)	28 (21.4%)	19 (13.6%)	26 (25.7%)	24 (16.9%)	30 (18.3%)	22 (15.5%)	10 (6.7%)	5 (3.33%)
Abnormal spindle	3 (1.9)	2 (1.3%)	41 (26.6%)	76 (33.0%)	6 (4.6%)	17 (12.1%)	14 (13.9%)	54 (38.0%)	61 (37.2%)	44 (31.0%)	25 (16.7%)	34 (22.7%)
Anaphase II	220	188	202	221	194	200	214	281	310	235	250	269
Lagging chromosomes	31 (14.1%)	26 (13.8%)	12 (5.9%)	42 (19.0%)	51 (26.3%)	26 (13.0%)	14 (6.5%)	51 (18.1%)	43 (13.9%)	54 (23.0%)	22 (8.8%)	46 (17.1%)
Bridge	6 (2.7%)	0 (0.0%)	14 (6.9%)	8 (3.6%)	11 (5.7%)	10 (5.0%)	0 (0.0%)	0 (0.0%)	17 (5.5%)	6 (2.6%)	0 (0.0%)	3 (1.1%)
Abnormal spindle	5 (2.3%)	6 (3.2%)	17 (8.4%)	46 (20.8%)	27 (13.9%)	11 (5.5%)	64 (29.9%)	30 (10.7%)	71 (22.9%)	16 (6.8%)	6 (2.4%)	2 (0.7%)
Not synchronous division	14 (6.4%)	11 (5.9%)	33 (16.3%)	74 (33.5%)	39 (20.1%)	23 (11.5%)	70 (32.7%)	90 (32.0%)	65 (21.0%)	75 (31.9%)	29 (11.6%)	14 (5.2%)
Telophase II	223	172	200	150	125	183	151	138	152	295	166	121
Unequal separation	43 (19.3%)	42 (24.4%)	76 (38.0)	91 (60.7%)	62 (49.6%)	67 (36.6%)	82 (54.3%)	72 (56.5%)	96 (63.2%)	98 (33.2%)	33 (19.9%)	21 (17.4%)
Micronuclei	8 (3.6%)	0 (0.0%)	1 (4.5%)	1 (0.7%)	4 (3.2%)	12 (6.6%)	24 (15.9%)	6 (4.3%)	2 (1.3%)	30 (10.2%)	19 (11.4%)	12 (9.9%)
Total	1,302	1,083	1,064	958	910	1,000	848	990	1,050	966	943	1,000
Abnormalities rate	26.2%	22.0%	39.5%	57.3%	41.4%	34.2%	58.7%	54.5%	52.7%	47.7%	25.8%	23.3%

(52.7%), in contrast to 26.2% in *I. dichotoma*, 22.0% in *I. domestica*, 39.5% in F_1 -5, 41.4% in F_2 -2, 34.2% in F_2 -3, 47.7% in BC_1 -S-4, 25.8% in BC_1 -Y-1 and 23.3% in BC_1 -Y-2 (Table 1).

During the first meiotic division, the presence of non-congressed chromosomes that did not align on the equatorial plate was observed at metaphase I in all 12 accessions (Figure 3A, 3B). The consequent behaviour of these univalents depended on the genotype and resulted in retarded movement to the poles and the formation of micronuclei. At anaphase I, lagging chromosomes were found in all 12 accessions. In BC_1 -Y-1 and BC_1 -Y-2, the univalents joined the large chromosome sets at the poles, although they were retarded in their movement (Figure 3C). In contrast, lagging chromosomes formed new micronuclei in BC_1 -S-3. In this phase, chromosomal single and double bridges were found (Figure 3D, 3E). In BC_1 -S-1, chromosome stickiness resulted in thick chromosomal bridges, restricting

chromosome separation (Figure 3F). Chromosome bridges were completely absent in *I. domestica* and appeared only rarely in F_2 -2 and BC_1 -Y-1. At telophase I, micronuclei derived from lagging chromosomes and/or acentric fragments were found (Figure 3H). F_2 -1 and BC_1 -S-1 showed relatively high numbers (52.0% and 43.9%, respectively) of cells with unequal chromosome segregation (Figure 3G).

During the second meiotic division, abnormalities related to the non-congressing chromosomes were observed in *I. dichotoma*, BC_1 -Y-1 and BC_1 -Y-2 with a low frequency at metaphase II. In other genotypes, this percentage varied between 8.5% and 29.1% (Figure 3I, 3J). Apart from normal spindles that had a linear alignment, abnormal spindles in meiosis II were also found. When abnormal organisation occurred, the most common conformations were a tripolar spindle (Figure 3K), followed by the perpendicular formation of the convergent zone (Figure 3L). At anaphase II,

Figure 3. Abnormal meiosis phenomena in pollen mother cells of F_1 , F_2 and BC_1 plants from the cross *I. dichotoma* (Y2) $\times$ *I. domestica* (S3): A, B – non-congression in metaphase I of F_2 -2 and F_1 -5, respectively; C – laggard chromosomes in anaphase I of BC_1 -Y-1; D – bridge in anaphase I of BC_1 -S-2; E – double bridges in anaphase I of F_2 -3; F – thick bridge in anaphase I of BC_1 -S-1; G – unequal separation in telophase I of F_2 -1; H – micronuclei in telophase I of BC_1 -S-2; I – non-congression in metaphase II of BC_1 -S-3; J – early separation in metaphase II of BC_1 -S-1; K – tripolar spindle fibres in metaphase II of F_2 -1; L – perpendicular spindle fibres in metaphase II of BC_1 -S-1; M – laggard chromosomes in anaphase II of BC_1 -S-2; N – bridge in anaphase II of F_2 -3; O – tripolar spindle fibres in anaphase II of F_2 -1; P, Q – perpendicular spindle fibres in anaphase II of F_1 -5 and BC_1 -S-2, respectively; R – multiple spindle fibres of BC_1 -S-3; S, T – not synchronous division in anaphase II of F_1 -5 and F_2 -3, respectively; U, V – unequal separation in telophase II of BC_1 -S-4; W – unequal separation in telophase II of BC_1 -S-4; X – unequal separation in telophase II of BC_1 -S-2; Y – two micronuclei in telophase II of BC_1 -S-2.

lagging chromosomes (Figure 3M), chromosome bridges (Figure 3N) and tripolar and perpendicular spindles were observed again (Figure 3O–3Q). Another spindle abnormality was the occurrence of multiple spindles in BC₁-S-3 (Figure 3R). In F₁-5 and F₂-3, chromosome separation was asynchronous at anaphase II (Figure 3S, 3T). Chromosome disequilibrium segregation was also found at anaphase II and telophase II (Figure 3U, 3V). New micronuclei formed at telophase II, which resulted in the formation of polyads (Figure 3Y).

At the tetrad formation, the second meiotic division mostly ran perpendicular to the first, causing the four microspores to be orientated as shown in Figure 4G. Rarely, the resulting four microspores were arranged in a row during this stage. One of the striking observations of the tetrad stage in this study was the sporad configurations in hybrids (Table 2 and Figure 4) including monads, dyads, triads, tetrads and polyads, reflecting low pollen fertility. Monads were mainly observed in F₂-1, but not viable (Figure 4A). Dyads

Table 2. Distribution (%) of sporad configurations in *I. dichotoma*, *I. domestica* and their hybrid progenies.

Materials	No. of cells studied	Monad Rate (%)	Dyad Rate (%)	Triad Rate (%)	Tetrad Rate (%)	Polyad Rate (%)
Y2	245	1.9	4.3	10.6	83.1	0.0
S3	213	0.0	3.8	13.6	78.4	4.2
F ₁ -5	253	0.4	5.1	5.5	50.2	38.7
F ₂ -1	208	10.1	36.5	16.4	35.1	1.9
F ₂ -2	201	6.0	0.0	16.0	72.1	6.0
F ₂ -3	260	0.0	7.7	30.8	57.7	3.9
BC ₁ -S-1	432	2.3	14.6	33.8	45.4	3.9
BC ₁ -S-2	254	1.2	7.1	29.1	51.2	11.4
BC ₁ -S-3	271	5.5	11.1	33.2	46.1	4.1
BC ₁ -S-4	373	0.0	4.6	37.0	53.1	5.4
BC ₁ -Y-1	363	1.7	10.5	34.2	47.9	5.8
BC ₁ -Y-2	319	1.3	15.7	34.5	44.5	4.1

Figure 4. The meiosis sporad in *I. dichotoma* (Y2), *I. domestica* (S3) and their hybrid progenies: A – monad of F₂-1; B – dyad of BC₁-S-3; C – dyad of F₂-2; D – triad of BC₁-Y-1; E – triad of BC₁-S-3; F – triad of F₂-3; G – tetrad of F₁-5; H – tetrad of BC₁-S-2; I – tetrad of BC₁-S-1; J – pentad of BC₁-S-2; K – pentad of BC₁-S-4; L – polyad of F₂-3; M – polyad of BC₁-S-3; N – polyad of BC₁-S-1; O – polyad of BC₁-S-2.

and triads were found in most of the individuals at the end of microsporogenesis, indicating the production of unreduced gametes. Triads (composing of one 2n microspore and two n microspores) might result from the formation of disoriented (tripolar) spindles. However, the size of microspores in BC₁-S-2 and BC₁-S-3 was very variable and several pentads, hexads or abnormal tetrads (containing three large and one small microspores) were observed (Figure 4I–4O).

Pollen viability and size variation

The analyses of pollen viability and size in *I. dichotoma*, *I. domestica* and their hybrids are shown in Table 3. F₂-1 and BC₁-S-1 were male-sterile and did not have any pollen. The average pollen fertility of F₁-5 (10.1%) was much lower than that of its parents, whereas F₂-2 produced few pollen grains, and they rarely germinated. Pollen abortion rates of *I. dichotoma* and *I. domestica* (1.9% and 14.5%, respectively) were lower than that of

the interspecific hybrids except for BC₁-S-4 (13.3%). The mean pollen fertility of BC₁-S-2 (1.6%) was the lowest besides F₂-1 and BC₁-S-1 without pollen. The pollen germination rates of F₁-5 (10.1%), F₂-2 (2.4%) and F₂-3 (21.5%) were lower than that of *I. dichotoma* (26.1%) and *I. domestica* (35.1%). In BC₁-S-2 (1.6%) and BC₁-S-3 (4.8%), almost no pollen grains germinated. BC₁-S-4 pollen appeared normal but germinated poorly with only 7.2% of pollen germination. However, BC₁-Y-1 and BC₁-Y-2 demonstrated higher pollen germination rates than others except for *I. domestica* (Figure 5).

The normal-sized pollen grain diameters of *I. dichotoma*, *I. domestica*, F₁-5, F₂-2, F₂-3, BC₁-S-2, BC₁-S-3, BC₁-S-4, BC₁-Y-1 and BC₁-Y-2 were 65–75 µm, 60–70 µm, 60–70 µm, 63–73 µm, 60–70 µm, 58–68 µm, 61–71 µm, 62–72 µm, 65–75 µm and 63–73 µm, respectively. In addition to single pollen grains, including regular smaller ellipsoidal and larger spherical pollen grains, a few adherent pollen grains

Table 3. Pollen germination rates of *I. dichotoma* (Y2), *I. domestica* (S3) and their hybrid progenies.

Materials	Pollen in plant	Normal pollen size (µm)	Large pollen (%)	No. of pollens observed	No. of pollens germinated	No. of abort pollens	Abortion rate (%)	Germination rate (%)
Y2	More	65–75	0.5	1,713	447	33	1.9	26.1
S3	More	60–70	0.2	2,706	951	392	14.5	35.1
F ₁ -5	More	60–70	0.8	2,039	205	738	36.2	10.1
F ₂ -1	None	–	–	–	–	–	–	–
F ₂ -2	Little	63–73	0.7	1,354	32	628	46.4	2.4
F ₂ -3	More	60–70	1.1	1,014	218	323	31.9	21.5
BC ₁ -S-1	None	–	–	–	–	–	–	–
BC ₁ -S-2	Little	61–71	1.7	582	9	311	53.4	1.6
BC ₁ -S-3	Little	62–72	1.7	673	32	334	49.6	4.8
BC ₁ -S-4	More	58–68	1.9	1,049	76	139	13.3	7.2
BC ₁ -Y-1	More	65–75	2.0	1,899	526	696	36.7	27.7
BC ₁ -Y-2	More	63–73	2.3	1,916	597	631	32.9	31.2

Large pollen grains: >1.3× diameter of normal-sized pollen.

Figure 5. Pollen germination of *I. dichotoma* (Y2), *I. domestica* (S3) and their hybrid progenies: A – *I. dichotoma* (Y2); B – *I. domestica* (S3); C – F₁-5; D – F₂-2; E – F₂-3; F – BC₁-S-2; G – BC₁-S-3; H – BC₁-S-4; I – BC₁-Y-1; J – BC₁-Y-2.

were viable. Furthermore, some large pollen grains (>1.3 times the diameter of normal-sized pollen grain) were observed in *I. dichotoma* (0.5%), *I. domestica* (0.2%), F_1 -5 (0.8%), F_2 -2 (0.7%), F_2 -3 (1.1%), BC_1 -S-2 (1.9%), BC_1 -S-3 (1.7%), BC_1 -S-4 (1.7%), BC_1 -Y-1 (2.0%) and BC_1 -Y-2 (2.3%). As large grains are an indication of $2n$ pollen production, $2n$ gametes might be present in these plants (Figure 6C–6F), which have potential for polyploid breeding, especially in BC_1 -Y-2, BC_1 -Y-1, BC_1 -S-2, BC_1 -S-3 and BC_1 -S-4. Although meiotic aberrations in these large pollen producers resulted mainly in triads, monads, dyads and polyads were also observed.

DISCUSSION

The most striking form of the diffuse stage that we observed in these hybrids was the diffuse diplotene stage. To our knowledge, this phenomenon was found in the *Iris* species for the first time. The same phenomenon has also been reported in *Rosa* and large genome cereals (Colas et al., 2017) as well as in *Larix* (Kołowierz-Lubnau et al., 2015). Especially in interspecific hybrid plants, the diffuse stage has been proposed to be widespread in plant meiosis, affecting restitution from diffuse to fully contracted chromosomes, with chromosomes often entering metaphase before fully contracting (Klásterská and Natarajan, 1974). In this study, it occurred in summer with high temperatures and drought stress. In *Larix* species, meiosis calmed during the autumn and pollen mother cells remained in the diffuse stage for several months to resist the cold (Ekberg et al., 1968). This possibly reflects that the diffuse stage is influenced by some extraordinary environmental conditions.

The high frequency of meiosis abnormalities was observed in BC_1 -S-1 (58.7%), BC_1 -S-2 (54.5%),

BC_1 -S-3 (52.7%), BC_1 -S-4 (47.7%) and F_2 -1 (57.3%), with low pollen-germination rates (0, 1.6%, 4.8%, 7.2% and 0, respectively). Pollen fertility analysis of different cross combinations in this study indicated that meiotic abnormalities, such as non-congression, lagging chromosomes, unequal separation and abnormal spindle fibres orientation, led to low pollen fertility. As previously reported, pollen viability is closely associated with meiosis of pollen mother cells, and meiotic abnormalities affect pollen development (Boff and Chifino-wittmann, 2002; Zha et al., 2007; Farco and Dematteis, 2014; Usandizaga et al., 2020). The relationship between meiotic abnormalities and fertility has been studied in many plant species, including *Musa* and *Brassica* (Adeleke et al., 2004; Yang et al., 2020). Meiotic abnormalities are shown to be negatively correlated with pollen fertility (Devi and Borua, 1997).

The pollen fertility of F_1 -5 (10.1%) was much lower than that of its parents *I. dichotoma* (26.1%) and *I. domestica* (35.1%). Backcrossing F_1 -5 to *I. dichotoma* resulted in rapid restoration of fertility, and the pollen fertility of BC_1 -Y-1 and BC_1 -Y-2 was higher than that of F_1 -5. However, when backcrossing F_1 -5 to *I. domestica*, the BC_1 hybrids had a high total meiosis abnormality rate (exceeding 50.0%) and produced no or shrunken pollen with relatively low pollen fertility (0 to 4.8%) compared with F_1 -5 (10.1%). Low fertility of BC_1 plants was also reported in *Agropyron junceum* (Charpentier et al., 1986) although backcrossing can restore the fertility of interspecific hybrids (Yushkina et al., 2009). F_1 -5 was male fertile and presented only 1–4 unpaired chromosomes. Partial fertility of the interspecific hybrid could be associated with relatively high chromosome association frequency, indicating partial genomic

Figure 6. Scanning electron micrographs of different pollen grains: A – Y2; B – S3; C – F_1 -5; D – F_2 -3; E – BC_1 -S-3; F – BC_1 -S-4. C–F – occurrence of large pollen grains. Scale bar = 50 μ m (magnification 500 $\times$).

homology between the parental species (Asano, 1984). The abnormal segregation of chromosomes in meiosis I and II can lead to the formation of micronuclei, which either may remain in microspore tetrads or may be eliminated in the form of microcytes for additional cytokinesis. In this case, the formed tetrads will be genetically unbalanced, and the resulting pollen will become dysfunctional or shrunken. Although tetrads were found in F_2 -1 and BC_1 -Y-1, they did not grow cell walls and form pollen grains. Therefore, the frequency of 2n pollen was low in *I. dichotoma*, *I. domestica* and their hybrids which exhibited a high frequency of dyad and triad. Additionally, microspores in tetrads could originate from an unequal chromosome division, usually leading to sterile pollen (Do Nascimento et al., 2014).

In this study, the lagging chromosomes, unequal separation and nonsynchronous division led to chromosomes slowly moving to cell poles, forming the micronucleus or disappearing in the cytoplasm, and finally led to the production of unbalanced tetrads or polyad and abnormal pollen grains. Similar meiotic aberrations have been reported in *Brachiaria* (Gallo et al., 2007), *Begonia* (Dewitte et al., 2010), *Hydrangea aspera* (Crespel and Morel, 2014) and allotriploid lily 'Cocossa' (Zhang et al., 2017). They can be contributed to chromosome pairing problems due to their lack of homology and are the expression of the typical behaviour of univalent or non-paired chromosomes. In some rare cases, unequal and incomplete nuclei separation resulted in monads, such as asymmetric cytokinesis in pollen mother cells in which the entire nucleus was included in one of the cells (Barba-Gonzalez et al., 2005). In this study, the presence of dyads and/or triads at the tetrad stage imply the production of 2n pollen grains. Abnormal spindle fibres orientation is associated with the production of 2n gametes. The formation of disoriented spindle fibres led to a dyad (composed of two 2n microspores) in the case of fused or parallel spindle fibres, and to a triad (composed of one 2n microspore and two n microspores) in the case of tripolar spindle fibres. The production of 2n gametes was observed in interspecific hybrids of potato (Conicella, 1991), *Populus* (Dong et al., 2015) and strawberry (Luo et al., 2018).

The spontaneous occurrence of unreduced gametes can produce unexpected polyploids such as triploids and tetraploids in both natural and artificial crosses in various plants such as *Lilium* (Van Tuyl et al., 2005), *Tulip* (Marasek-Ciolakowska, 2012) and *Primula* (Kato et al., 2008). When they occur in interspecific hybrids with some extent of viability, 2n gametes can be used to produce sexual progeny through either crossing or selfing (Ramanna and Jacobsen, 2003; Van Tuyl and Lim, 2003). In the case of meiotic polyploidisation, polyploid progenies are raised by crossing with 2n gamete-producing genotypes. The most important advantage of meiotic polyploidisation is that homologous recombination occurs at a high frequency between parental chromosomes during meiosis.

Therefore, a large amount of heterozygosity can be transmitted to the polyploid offspring. Because of the function of unreduced gametes, a considerable number of *Iris* garden varieties with large flowers, improved form and plant habit, and fully fertile were produced through intercrossing by meiotic polyploidisation (Bee et al., 1986). In this study, the selection of individuals producing 2n gametes allowed manipulating the ploidy level of the offspring. Because unreduced pollen is not always viable, as previously demonstrated in *Begonia* (Dewitte et al., 2010), screening for viable unreduced gametes should be undertaken in the future, as these cells present an interesting tool for polyploidisation and can be used as pollen donors to produce polyploid iris individuals exhibiting larger flowers as well as higher pollen and seed fertility (Barba-Gonzalez et al., 2004; Nikoloudakis et al., 2018).

CONCLUSIONS

In summary, this study analysed the relationship between meiotic abnormalities and pollen fertility in 12 *Iris* individuals comprising parents and hybrids resulting from their crosses and backcrosses. Meiotic abnormalities negatively affected pollen fertility. The diffuse diplotene stage was observed for the first time in the genus *Iris*. Poor pollen fertility of BC_1 -S presented difficulties for further breeding. As all BC_1 -Y individuals displayed relatively similar flower colour (yellow) and higher pollen fertility than other cross combinations, they are excellent plant material for yellow-flowered iris breeding. In addition, BC_1 -Y-2, BC_1 -Y-1, BC_1 -S-2, BC_1 -S-3 and BC_1 -S-4 have potential in polyploid breeding.

FUNDING

This work was supported by the Educational Department of Liaoning Province (L2010491) and Shanghai Landscaping and City Administration Bureau (F122424).

AUTHOR CONTRIBUTIONS

X.B. and W.X. designed experiments and performed analytical measurements. W.X., G.L., X.L., Y.Z. and F.Y. performed statistical analysis. W.X., G.L. and J.L. performed manuscript writing.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Received: February 7, 2021; accepted: April 9, 2021.