F1 distant hybrids between two edible lilies (Lilium brownii var. viridulum and L. davidii var. unicolor) produce more n than 2n functional eggs with more recombinant chromosomes

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Abstract

Lilium brownii var. *viridulum* (*BB*) and *L. davidii* var. *unicolor* (A^dA^d) are grown for edible in China, but their breeding lags far more than other ornamental lily breeding. To improve the situation, F_1 BA hybrids were used as seed parents to cross with A^dA^d and other diploid Asiatic lilies (AA). The results showed that the F_1 BA hybrids could produce BC_1 progenies. The average seedlings obtained from the combinations per fruits were very variable from 1.7 to 22.5, indicating that their compatibilities are different depending on their males. Among the 18 progenies with genomic in situ hybridization (GISH), ten of them were diploid (2n = 2*x* = 24), two are aneuploid - near diploid (2n = 2*x* ± 1), and other six were allotriploid (2n = 3*x* = 36); and all of them contain much more recombinant chromosomes and breakpoints than other distant lily hybrids. We concluded that F_1 LA hybrids produced much more *n* functional egg cells than *2n* functional egg cells and this would pay a new way to realize lily ingression breeding at diploid ploidy level.

Introduction

The genus *Lilium* of the family Liliaceae has 111 species according to World Flora Online retrieved July 2022 (www.wikepedia.org). They are classified into seven sections, Archelirion, Sinomartagon, Leucolirion, Martagon, Pseudolirium, Liriotypus and Daurolirion (De Jong 1974). Numerous hybridizations indicate that interspecific hybridizations within each section are usually easy successful with normal pollination and their hybrids are highly fertile, and the modern lily groups, Asiatic (A), Orienal (O) and Longiflorum (L), are bred from hybridizations within Sinomartagon, Archelirion and Leucolirion, respectively (Van Tuyl et al. 2000, 2018; Van Tuyl and Arens 2011; Marasek-Ciolakowska et al. 2018). These groups are usually diploid, except that Asiatic lily also has some tretraploids through chromosome doubling and triploids by diploid × tetraploid or reciprocal crosses (Li et al. 2011; Zhang et al. 2012). Meanwhile, the interspecific hybridizations between different sections are hard; however, some hybrids are obtained by cut style pollination and embryo rescue (Asano and Asano 1977a, 1977b; Asano and Myodo 1980; Van Tuyl et al. 1991, 1997). These F_1 hybrids have abnormal meiosis – some of their homoeologous chromosomes can pair each other and form bivalents while some not and remain univalents, and genomic in situ hybridization (GISH) are used to distinguish their parental genomes (Karlov et al. 1999; Lim et al. 2000, 2001; Van Tuyl et al. 2005; Barba-Gonzalez et al. 2004, 2005a; Zhou et al. 2008a), thus, these hybrids are doubtless distant hybrids or intersectional hybrids. Interestingly, the F_1 distant hybrids, such as LA, OA or OT, are highly sterile, but a few of them produce small part of functional *2n* pollen and most of them produce small part of functional *2n* eggs, and thus allotriploid triploid BC_1 progenies, such as LAA, OAO, AOA, OTO, can be obtained (Van Tuyl et al. 2005; Lim et al. 2001; Zhou 2007; Zhou et al. 2008b; Barba-Gonzalez et al. 2005b; Khan et al. 2009a, 2009b, 2010; Luo et al. 2012; Chung et al. 2013; Liu et al. 2021). These allotriploid lilies are caused from unilateral sexual polyploidization (reviewed by Ramanna and Jacobsen 2003). To date, the LA, OA and OT lily cultivars are usually allotriploids and they are mainly from unilateral sexual polyploidization (Zhang et al. 2012).

These results unveil that the functional *2n* gametes are predominantly from first division restitution (FDR) and they contain zero or a few recombinant chromosomes.

Apart from ornamental values, many lily bulbs are edible. However, most of them are not popular for food because they are usually bitter, except that *L. brownii* var. *viridulum* and *L. davidii* var. *unicolor* are traditionally welcome in China. Compared with ornamental lilies, the breeding reports on edible lilies are rare and so new lily varieties for food are urgently needed at least in China. It is known that unilateral sexual polyploidization is crucial to breeding allotriploid ornamental lily cultivars, therefore, using the similar way to breed allotriploid edible lily would be a short cut to breed new varieties for edible or for both edible and ornamental. It has been proved that *L. brownii* var. *viridulum* (*BB*) and *L. davidii* var. *unicolor* (A^dA^d) could be discriminated in F_1 BA hybrids with FISH and GISH (Wu et al. 2021). According to the previous findings in ornamental lily breeding, it is expected that the progenies would be triploid if the F_1 BA hybrids are backcrossed with A^dA^d or other diploid Asiatic lilies (AA). To prove this point and breed allotriploid edible lily cultivars, we made backcrosses using F_1 hybrids of two edible lilies with Asiatic lilies and analyzed the genome composition of their BC_1 progenies, and then discussed the significance on lily breeding.

Materials and methods

Plant materials

The F_1 BA hybrids between *Lilium brownii* var. *viridulum* (*BB*) × *L. davidii* var. *unicolor* (A^dA^d), created in 2018, was male sterile, but used as female parents. A^dA^d and other seven Asiatic lilies - J2115, J1707, J1811,16110, 'Black Magic', J2013, and 15109, have two common Asiatic lily genomes (AA: 2n = 2*x* = 24) because they belong to or originated from section Sinomartagon (Table 1). They were male fertile and used as males. *BB* is different from A^dA^d because GISH can discriminate their genomes in BA (Wu et al. 2021). All the lilies were grown in a greenhouse of Jiangxi Agricultural University, China.

Table 1
The results of the hybridizations between F_1 BA hybrids and Asiatic lilies (AA). 'BM' means Asiatic lily cultivar 'Black Magic'.

Cross code	Female (BA)	Male (AA)	Flowers pollinated	Fruits harvested	Embryo sacs rescued	Seedlings	Seedlings per fruits
21061	18001	J2115	2	1	9	3	3
21063	18001	J1707	5	3	23	6	2
21064	18001	J1811	3	3	33	5	1.7
21065	18001	16110	4	3	55	17	5.7
21100	18001	'BM'	16	9	168	74	8.2
21101	18001	J2013	7	4	152	90	22.5
21102	18001	15109	10	5	134	69	13.8
21113	18001	A^dA^d	10	7	143	54	7.7
Total			57	35	717	318	9.1

Pollination and embryo rescue

The pollination and embryo rescue were referred to Zhong et al. (2022). To avoid being contaminated with unnecessary pollen, stigma was wrapped with aluminum foil after pollination. The number of pollenated flowers and the backcrossing combination were listed in Table 1. The fruits were harvested when they become yellow or soft. In a fume hood, their embryo sacs or developed ovules were isolated with knife and forceps and put on lily embryo rescue medium, containing 2.2 $g{\cdot}L^{-1}$ MS (Duchefa Biochemie), 60 $g{\cdot}L^{-1}$ sucrose and 4 $g{\cdot}L^{-1}$ gelrite (Duchefa Biochemie) (pH = 5.8). They were cultured in a dark chamber at 25 ℃ for 40–60 d, and then, the germinated seeds were transferred to lily propagation medium, containing 2.2 $g{\cdot}L^{-1}$ MS, 50 $g{\cdot}L^{-1}$ sucrose and 4 $g{\cdot}L^{-1}$ gelrite (pH = 5.8) at 25 ℃ and 2000 l×light intensity for about 10 weeks.

Mitotic chromosome preparation

The mitotic chromosomes were prepared according to Zhong et al. (2022). When roots were 0.5-1 cm long, they were cut off and incubated in 0.7 mM cycloheximide (Duchefa Biochemie) at room temperature for 4 h, and then stored in a fixative at least 30 min. To prepare chromosome slides, root tips were washed with distilled water and softened in an enzyme mix, containing 1% (w/v) cellulase RS (Duchefa Biochemie) and 1% (w/v) pectinase Y23 (Duchefa Biochemie), at 37 ℃ for about 1 h. Their meristem was mixed with 16 µL 45% acetic acid on a glass slide, covered with a cover glass and then gently squashed. The chromosome slides were checked with a phase contrast microscope (Zeisis A1)

Genomic in situ hybridization (GISH)

BB DNA, isolated with plant genome DNA extraction kit (CW05535, CoWin Biotech), was labeled with biotin as probe according to protocol of nick translation (Roche 11745824910). HS (herring sperm) DNA, as block, was treated at 100℃ for 30 min. GISH protocol is according to Xiao et al. (2019). The signals of biotin-probe were detected with Streptavidin-CY3 (a red fluorescence dye), together with DAPI (a blue-purple fluorescence dye) for counterstaining chromosomes. When checked with a fluorescence microscope (ZEISS Scope. A1), *BB* chromosomes were red and AA chromosomes were blue.

Results

Hybridization of BA × AA

The F_1 BA hybrids had very low pollen germination, ranging from 0 to 6.3%, meaning highly male sterile. However, when they were used as seed parents and backcrossed with eight diploid Asiatic lilies, all the combinations were successful (Table 1). The results indicated that the F_1 BA hybrids, regardless male sterility, could be female to be backcrossed with appropriate males. However, the average seedlings obtained from the combinations per fruits were very variable from 1.7 to 22.5, indicating that their compatibilities are different depending on their males. This means that Asiatic lilies share common genomes, but their genomes have some difference. This is reasonable because the Asiatic cultivars or lines are from hybridization between different taxonomic species of Sinomartagon.

Genomic composition and ploidy levels of BC_1 progenies

18 BC_1 seedlings were analyzed with GISH analysis (Table 2). All of them true hybrids, because they were composed of B, A and B/A chromosomes (Figs. 1 & 2). Ten of them were diploid ($2n = 2x = 24$) (Fig. 1), two are aneuploid - near diploid ($2n = 2x \pm 1$) (Fig. 2k-l), and other six were allotriploid ($2n = 3x = 36$) (Fig. 3m-r). In addition, 21101-5 had an extra very small chromosome (Fig. 1f).

Table 2
Genome compositions of 18 progenies based on GISH analysis, indicating their total chromosome numbers (CH), the chromosomes numbers contributed by egg (Egg^{ch}) and pollen ($Pollen^{ch}$), Chromosome numbers originate from L. *Lilium brownii* var. *viridulum* (B^{ch}) and AA (A^{ch}), recombinant chromosome numbers (B/A^{ch}), total break points (BP) of all recombinant chromosomes and average break points per recombinant chromosome (Av./R).

Seedlings	Female	Male	CH	Egg^{ch}	$Pollen^{ch}$	B^{ch}	A^{ch}	B/A^{ch}	BP	
	BA	AA							total	Av./R
21061-1	18001	J2115	24	12	12	0	13	11	29	2.6
21063-2	18001	J1707	24	12	12	0	13	11	28	2.5
21065-3	18001	16110	24	12	12	1	16	7	21	3.0
21100-3	18001	'BM'	24	12	12	2	12	10	20	2.0
21101-1	18001	J2013	24	12	12	0	13	11	24	2.2
21101-5	18001	J2013	24	12	12	1	13	10	26	2.6
21102-2	18001	15019	24	12	12	1	13	10	23	2.3
21113-3	18001	A^dA^d	24	12	12	1	13	11	31	2.8
21113-8	18001	A^dA^d	24	12	12	3	13	8	15	1.9
21113-10	18001	A^dA^d	24	12	12	0	15	9	24	2.7
21063-3	18001	J1707	23	12	12	0	14	9	20	2.2
21064-2	18001	J1811	25	12	12	1	14	10	26	2.6
21100-2	18001	'BM'	36	24	12	4	13	19	49	2.6
21100-7	18001	'BM'	36	24	12	3	15	18	40	2.2
21100-10	18001	'BM'	36	24	12	5	14	17	46	2.7
21100-11	18001	'BM'	36	24	12	0	20	16	36	2.3
21100-13	18001	'BM'	36	24	12	6	13	17	24	1.4
21100-18	18001	'BM'	36	24	12	1	16	19	45	2.4

Variation of functional eggs of BA

Since the diploid Asiatic lilies (AA) contributed one set of A chromosomes in the BC_1 progenies, then, the GISH results demonstrated that the F_1 BA hybrids produced functional *n*, aneuploid (near *n*) and *2n* eggs. For convenience and clarity to describe the relationship between F_1 BA hybrids and their functional gametes, their karyotypes were made according to chromosomal morphology and GISH signals (Fig. 3).

Regarding the ten functional *n* eggs (Fig. 2a-j), their 12 chromosomes contributed by F_1 BA hybrids contained 7 to 11 recombinant chromosomes with up to six break points, indicating that all homoeologous chromosomes of the BA hybrids should have associated each other and different types of crossovers have occurred during their megasporogenesis. The more breakpoints on the recombinant chromosomes meant more crossovers or chiasmata formed by chromatids of homoeologous chromosomes during synapsis; the chromosomes without breakpoints implied that they did not pair with their homoeologous chromosomes or crossover did not occur during synapsis.

As for the two functional aneuploid eggs (Fig. 2k-l), they were similar to *n* eggs except that one of them missed a chromosome (n = $x - 1$) and the other had an extra chromosome (n = $x + 1$). The aneuploid eggs high possibly resulted from chromatid nondisjunction at anaphase I or II.

For the six functional *2n* egg (Fig. 3m-r), they contained 16–19 recombinant chromosomes. So many recombinant chromosomes in *2n* gemmates were rarely reported before. It was hard judge these *2n* egg were from first division restitution (FDR), second division resetitution (SDR), or indeterminate meiotic restitution (IMR), because their centromeres were not so clear (see detail in Discussion).

Discussion

L. brownii var. *viridulum* (*BB*) and *L. davidii* var. *unicolor* (A^dA^d) taxonomically belong to different sections - Archelirion and Sinomartagon respectively (De Jong 1974), suggesting their relationship is not close taxonomically or morphologically. To obtain their hybrids successfully, cut style pollination and embryo rescue are prerequisite, and GISH could distinguish their genomes in F_1 BA hybrids (Wu et al. 2021) as in other reported distant lily hybrids (Asano and Asano 1977a, b; Asano 1978; Van Tuyl et al. 1991, 1977, 2000; Karlov et al. 1999), indicating that *BA* are undoubtful distant. They show some common and unique characteristics with other distant hybrids.

Ploidy levels of BC_1 s or Functional gametes of F_1 distant lily hybrids

Based on previous publications, F_1 distant hybrids usually produce allotriploid BC_1 progenies whether the F_1 hybrids are used as female or male (Karlov et al. 1999; Van Tuyl et al. 2002, 2005; Lim et al. 2001; Zhou 2007; Barba-Gonzalez et al. 2005b; Zhou et al. 2008b; Khan et al. 2009a, 2009b, 2010; Luo et al. 2012; Chung et al. 2013; Liu et al. 2021). As shown in Fig. 4, among near 187 BC_1 progenies of LA, OA, OH and OT F_1 hybrids, they are predominantly allotriploids except with a few aneuploid ($3x \pm 1$) (Zhou et al. 2008b; Khan et al. 2009b, 2010; Liu et al. 2021). So far, only a few LA and OT genotypes produced very small amount of diploid or near so progenies (Zhou 2007; Khan et al. 2009a; Luo et al. 2012). On the contrast, in the present research, out of 18 BC_1 progenies of F_1 BA hybrids, ten are diploid and two are aneuploid ($2n = 2x \pm 1$) while other six are allotriploid. In other words, F_1 BA hybrids mainly produce functional *n* eggs while LA, OA and OT predominantly *2n* functional gametes. How to explain the difference? Possibly, B genome (*L. brownii* var. *viridulum*) and A genome (*L. davidii* var. *unicolor*) are not so distant as L and A, O and A, O and H or T and O. The reasons for this are understandable: 1) More

diploid BC_1s of BA are survived diploid, suggesting that their homoeologous chromosomes could pair easily and form bivalent at meiosis, i.e., the meiosis is normal; 2) The fruits of the crosses between BB and AA develop better than those between LL and AA, and the former is more successful (no data available).

Types of 2n gametes

2n gametes are mainly classified into three types -- FDR, SDR and IMR according to the stage at which meiosis undergoes erroneously and result to unreduced gametes or somatic gametes, i.e., FDR *2n* gametes is caused by absence of meiosis I, SDR is by absence of meiosis II, IMR is involved both aberrant meiosis I and aberrant meiosis II (Lim et al. 2001; Ramanna and Jacobson 2003). In addition, the survived *2n* gametes of LA, OA, OH and OT F_1 hybrids are usually from FDR mechanism and only a few from IMR (Lim et al. 2001; Barba-Gonzalez et al. 2005b; Zhou et al. 2008b; Khan et al. 2009b, 2010; Chung et al. 2013; Liu et al. 2021). GISH analysis on F_1 LA hybrids' abnormal meiosis shows that lily LA hybrids have more possibility to produce more aneuploid gametes or IMR *2n* gametes than FDR *2n* gametes, indicating that FDR *2n* gametes are more viable than aneuploid gametes or IMR *2n* gametes because FDR *2n* gametes have balanced chromosomes or genes (Zhou et al. 2008a). However, which mechanism for the *2n* eggs of BA in the present study are hard to judge. GISH signals or genome composition of BC_1s are the criterion to recognize FDR or IMR *2n* gametes. Because these allotriploid BC_1 progenies have no or only a few recombinant chromosomes, it is easy to compare the genome compositions of functional *2n* gametes with their corresponding F_1 distant hybrid. In the present study, recombinant chromosomes in BC_1 plants ranged from 16 to 19 and their centromeres are not clear, so it is hard to judge which mechanism they belong to. Referring to the previous way, it is high possible that the *2n* gametes in the present study are from IMR mechanism.

Recombinant chromosomes

Recombinant chromosomes are crucial for plant introgression breeding (Ramanna and Jacobson 2003). The functional *n* eggs produced by F_1 BA hybrids contain many recombinant chromosomes and variable breakpoints, suggesting their related meioses are normal and different types of crossover or chiasmata occur. Some diploid BC_1s produced by a few F_1 LA genotypes have many recombinant chromosomes and breakpoints; however, some has few L or LA chromosomes (Zhou 2007; Khan et al. 2009a), suggesting that crossovers or chiasmata should seldom occur or homoeologous chromosomes could pair loosely.

It is intriguing that the functional *2n* eggs of BA F_1 hybrids also contain much more recombinant chromosomes than those of LA, OA, OT and OH F_1 hybrids. According to previous reports, the functional *2n* eggs or pollen grains usually contain no or a few recombinant chromosomes (Karlov et al. 1999; Lim et al. 2001; Barba-Gonzalez et al. 2005b; Zhou et al. 2008b; Khan et al. 2009b, 2010; Chung et al. 2013; Liu et al. 2021). The six triploid BC_1s of BAA in the present research contain recombinant chromosomes from 16 to 22, much more than previous BC_1 progenies. As shown in Fig. 4, among 187 triploid BC_1s of

F_1 LA, OA, OH and OT hybrids, 30 (16%) have no recombinant chromosomes, 102 (54.5%) contain 1–4 chromosomes, 53 (28.3%) have 5–14 recombinant chromosomes, and only two (1%) have more than 16 recombinant chromosomes. Besides, the B/A recombinant chromosomes have much more breakpoints than L/A, L/A, O/A, O/H or O/T breakpoints. The so many recombinant chromosomes and breakpoints in BAA could suggest that B and A could easily pair and form different types of single and multiple crossovers, because the recombinant chromosomes and their breakpoints are correlated with crossovers or chiasmata during homoeologous chromosome synapsis. This point needs to be conformed with analyzing the microsporogenesis or megasporogenesis of F_1 BA hybrids.

Partial Female fertility of F $_1$ distant lily hybrids

Modern lily allotriploid intersectional cultivars, like LAA, OTO, and AOA, are mainly selected from BC_1 progenies of F_1 LA, OT and OA, respectively (Zhang et al. 2012). Most F_1 distant lily hybrids are highly male sterile, only minority of them produce some amount of *2n* pollen grains and they used as male to produce allotriploid BC_1 progenies (Van Tuyl et al. 2005; Lim et al. 2001; Barba-Gonzalez et al. 2004). Zhou (2007) used 30 F_1 LA genotypes, most of which are highly male sterile, as seed parents to cross with AA and nearly the crosses were more or less successful, indicating the F_1 LA hybrids are invariably partial female fertile; and the phenomenon is well explained through analysis of lily embryo sac – Fritillaria embryo sac. Such female partial fertilities of F_1 distant hybrid are further confirmed in OT (Luo et al. 2012; Liu et al. 2021) and BA in the present study.

The significance on lily breeding

The functional *n* and *2n* gametes would play different roles from in lily breeding. It has been known that *2n* gametes are the main source for allotriploid lilies, such as LAA, OTO, AOA (Zhang et al. 2012). However, such allotriploid are not so ideal to do further recurrent backcrosses for introgression, because they are highly male sterile or they, as seed parents, produce weak aneuploid lilies (Zhou et al. 2012, 2014). Functional *n* gametes produced by distant lily F_1 are very rare and was reported only in a few F_1 LA hybrids (Zhou 2007; Khan et al. 2009a). So far, it has been hard to realize introgression breeding at diploid ploidy levels in ornamental lily breeding. The present results show that F_1 distant edible lily hybrids produce more functional *n* eggs and *2n* eggs, indicating that not only allotriploid edible lilies can be obtained, but also introgression breeding could be realized at diploid ploidy levels in edible lily breeding.

In fact, most lilies are both ornamental and edible or even medical. It is not intentional or possible to draw a line between them. *L. brownii* var. *viridulum* and *L. davidii* var. *unicolor* are popularly grown for food; however, the scenery is also astonishing when they bloom, though they have not been used as source for modern ornamental lilies. Regard with the modern ornamental lilies, like many Asiatic lilies, their fresh scales or petals also taste delicious. Since *L. davidii* var. *unicolor* taxonomically belongs to section Sinomartagon and it is easily hybridized with Asiatic lilies, then, it is expected that more and more triploid or diploid cultivars with both ornamental and edible or medical values would be released into market.

Conclusion

The present result showed that F_1 hybrids between *L. brownii* var. *viridulum* × *L. davidii* var. *unicolor*, which were similar to other distant lily hybrids, were male sterile but can be seed parents to produce BC_1 progenies; and the BC_1 progenies, which are different from those of F_1 distant lily hybrids, are more diploid than triploid. We concluded that F_1 LA hybrids produced much more *n* functional egg cells than *2n* functional egg cells. Together with discussion, the F_1 LA hybrids would pay a new way to realize lily ingression breeding at diploid ploidy level.

Declarations

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Figures

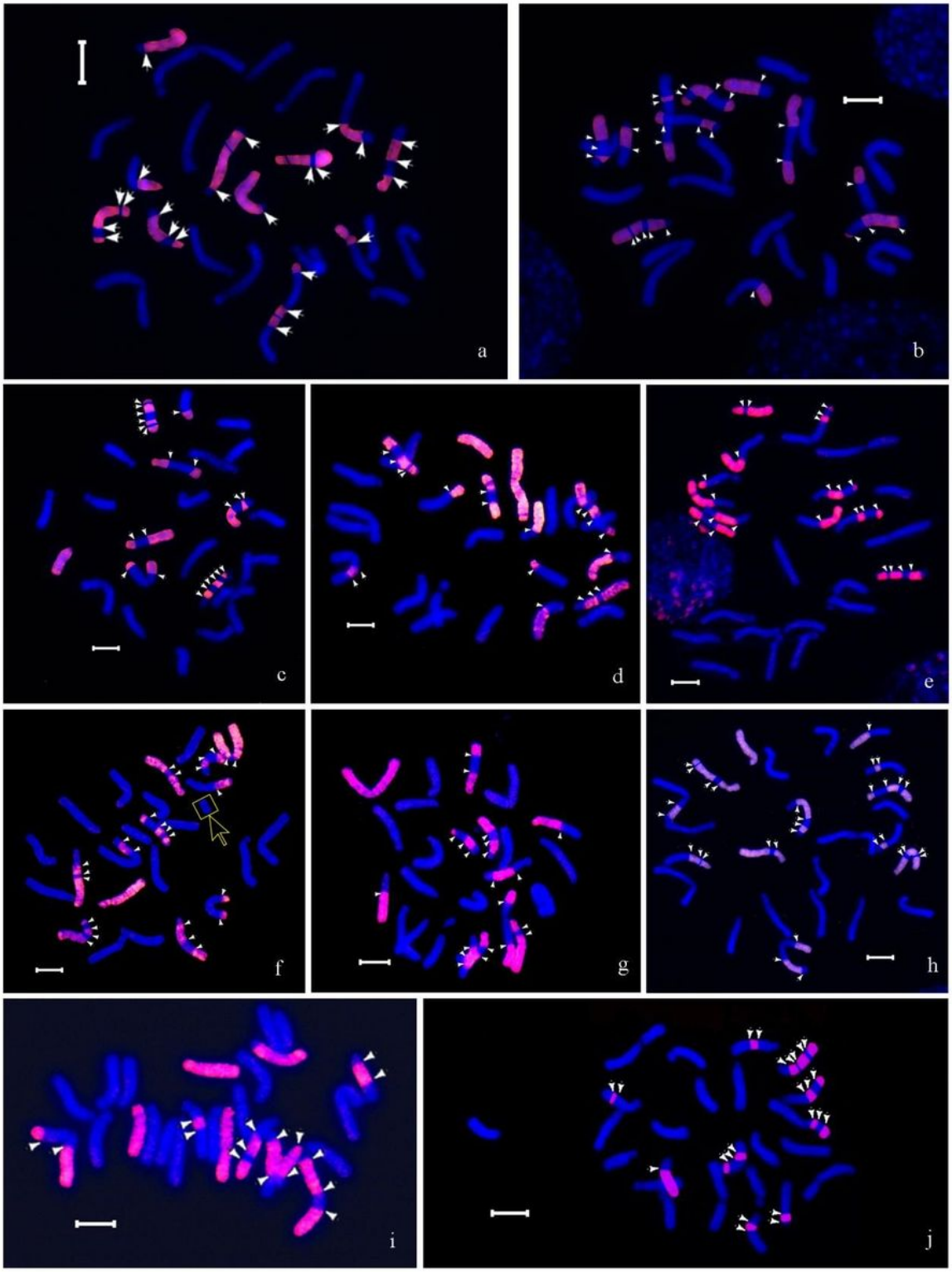

Figure 1

Diploid (2n = 2*x* = 24) GISH results on the metaphase chromosomes of root tips of seedlings obtained from BA × AA. The white arrow heads indicate recombinant break points. The black arrow indicates an extra small chromosome, which may be a detached chromosomal segment. a: 21061-1; b: 21063-2; c: 21065-3, d: 21100-3; e: 21101-1; f: 21101-5; g: 21102-2; h: 21113-3; i: 21113-8; j: 21113-10. Bar = 20 μm

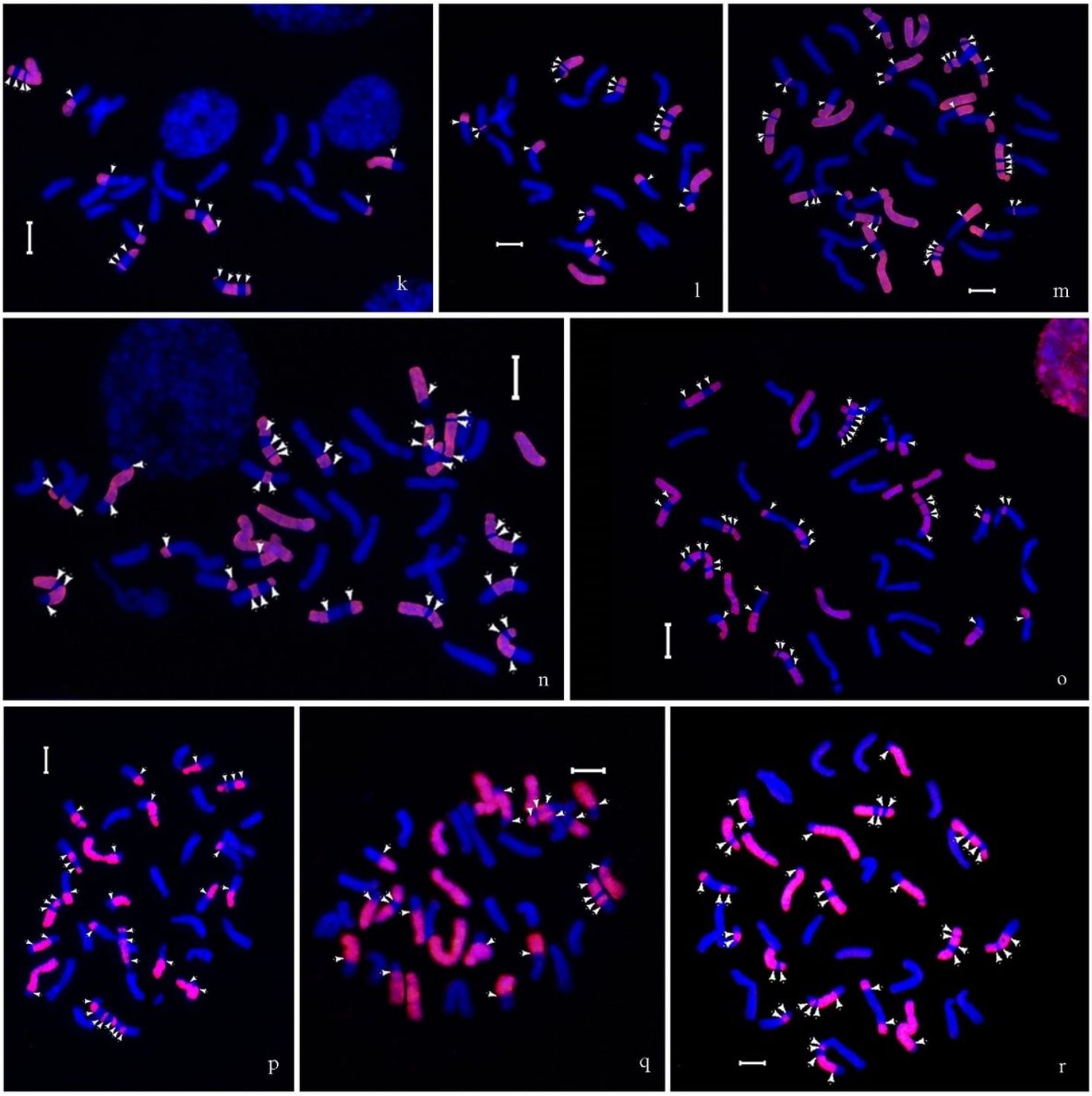

Figure 2

Triploid (2n = 3*x* = 36) and aneuploid (2n = 2*x* - 1, 2n = 2*x* + 1) GISH results on the metaphase chromosomes of root tips of seedlings obtained from BA × AA. The arrow heads indicate recombinant break points. k: 21063-3; l: 21064-2; m: 21100-2, n: 21100-7; o: 21100-10; p: 21100-11; q: 21100-13; r: 21100-18. Bar = 20 μm

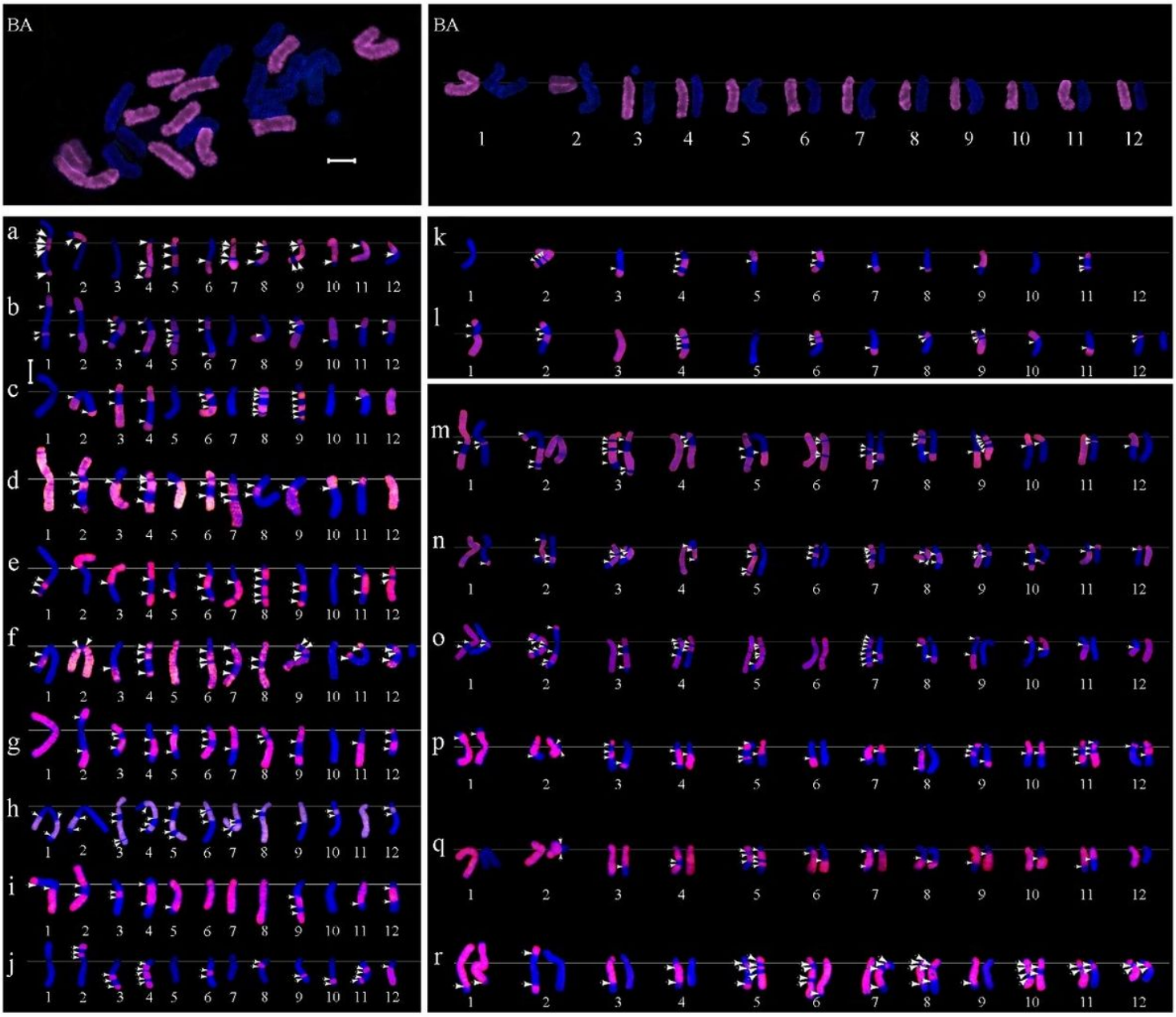

Figure 3

The karyotypes of F_1 LA hybrid and its functional n, *n* ± 1 and 2*n* gametes. BA: Somatic metaphase chromosomes (left) and karyotype of root tips of F_1 LA hybrids; a-j: karyotypes of *n* eggs; k-l: karyotypes of *n* ± 1 eggs; and k-m: karyotypes of 2*n* eggs. The chromosomes of the karyotypes are extracted from Figs. 1 and 2, and a-r here represent the same code numbers as those in Figs. 1 and 2, respectively. Bar = 20 μm

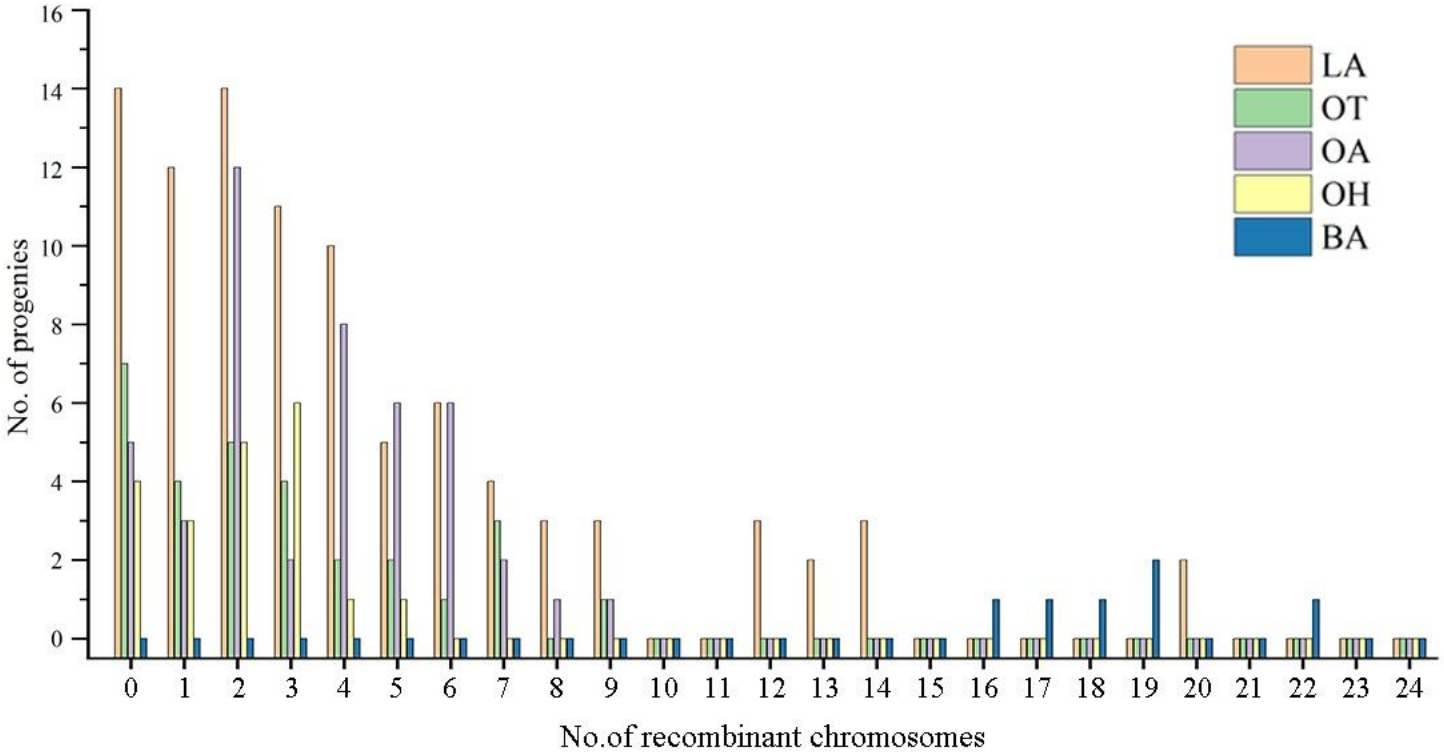

Figure 4

Numbers of the BC_1 progenies of LA, OT, OA, OH and BA (vertical) and their contained recombinants numbers (horizontal).