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RESEARCH ARTICLE

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Cytogenetic comparison of restorers TP-4 and Dminghui63 with maintainer D46B of autotetraploid rice

Abstract Cytogenetical comparison was made between high seed setting restorers TP-4 and Dminghui63 with eminent maintainer line D46B of autotetraploid rice. The meiosis observation demonstrated that the genomes of our autotetraploid materials were all $2n = 48$, which was the same as those in mitosis observation. Low percentages of univalent and trivalent in diakinesis-metaphase I (MI) of restorers TP-4 and Dminghui63 as well as maintainer line D46B of autotetraploid rice were observed. And the percentages of chromosome pairing were all over 99%, showing eminent cytological character. The frequency of TP-4 and Dminghui63 in diakinesis-metaphase I was 2.00/PMC (pollen mother cell) and 2.26/PMC, respectively. However, the frequency of D46B was 6.00/PMC, significantly higher than those of TP-4 and Dminghui63. It indicated that the maintainer D46B had better chromosome pairing capability in diakinesis-metaphase. While, the frequency of lagging chromosomes of the maintainer D46B in anaphase I (AI) was 10.62%, significantly lower than that of TP-4 (19.44%) or Dminghui63 (23.14%), and close to the level of diploid control (7.30%). In telophase I (TI), maintainer D46B exhibited lower frequency of microkernel, and in telophase II (TII) the frequency of normal quartered microspore of maintainer D46B was not only higher than that of TP-4 or Dminghui63 but also than that of diploid control. The percentage of the cell observed chromosome lagging in AI and the percentage of abnormal cell in TI showed a greatly significant positive correlation. That may demonstrate that chromosome separation in AI and microkernel formation in TI are controlled by the same dominant single gene or the major gene of a QTL.

Keywords autotetraploid rice, restorer, maintainer, meiosis, *U*-test, correlation analysis

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1 Introduction

Polyploid rice is a new germplasm and its breeding project is always reckoned as an available strategy to augment crop output (Guo et al., 2002). Polyploid rice, especially autotetraploid rice and allotetraploid rice, has eminent characters and a good application prospect. A study craze had occurred in the world since Nakamori first introduced tetraploid rice in 1933. India International Rice Research Institute (IRRI) studied genetics and the utilization of both autotetraploid and allotetraploid rice before the 1960s. But their studies had to stop for the problem of low seed set (Nayar, 1973). Now the research of autotetraploid rice has been conducted mainly in China since 1951. Through the studies of autotetraploid rice for decades, Chinese scholars have made great progress in germplasm selection, cytological study and so on. But a more substantive progress has not been made because of the same cause of low seed set.

Nowadays, the main constraint of autotetraploid utilization is the problem of low seed set. To solve the problem, the study of reproductive characteristics of autotetraploid rice should be conducted first because the trait of seed set is actually a final result of reproductive expression. Predecessors have done much work to explore the problem of low seed set. OKA (1953, 1954a, 1954b, 1954c) and OKA et al. (1954) systematically studied the autotetraploid rice and discovered many diverse variations in autotetraploid strains, which revealed that autotetraploid rice tended to be in low seed set although pollen fertility was comparatively high. So OKH stated that the low seed set was not caused by the male gamete. Bao and Yan (1956) observed that autotatraploid rice could produce enough fertile pollens but other factors constrained seed set such as failed fertilization after pollination, abnormal development of seed after fertilization and consequent withered seed formation, which revealed that these phenomena were related to abnormal nutrition supply, abnormal reproduction, double fertilization and abnormal seed development after fertilization. Bouharmont (Nayar, 1973) did cytological study on autotetraploid rice and stated that chromatid abnormal separation would occur in about 15%

cells in anaphase I in meiosis. That might finally result in aneuploid formation. Huang et al. (1999) suggested that the percentage of normal polygonum-type embryo sac in autotetraploid rice was lower than that of the diploid control but the percentage of embryo sac degeneration was always opposite. Female infertility seems to be universal in autotetraploid rice and that may be the main cause of low seed set. However, systematically cytological study and analysis in autotetraploid rice are yet scarce.

Many scholars have made many cytological studies on some other autotetraploid materials, such as *Sorghum bicolor* (L.) Moench (Zhang et al., 1997), *Dactylis glomerata* (Shuai et al., 1997), *Fagopyrum esculentum* (Zhang and Chen, 2000) and so on, but their results are not consistent with many issues. Comparatively, few cytological studies on autotetraploid rice have been conducted, and furthermore, eminent germplasms urgently needed are all low seed set strains. No report referred cytological characteristics or the relationships among meiosis and pollen fertility and seed set in the three lines of autotetraploid rice.

In this paper, a systematically cytological study was conducted, and the relationships among meiosis and pollen fertility and seed set were analyzed, furthermore, the genetic characteristics in autotetraploid rice were explored by using the restorers TP-4 and Dminghui63 and the maintainer D46B.

2 Materials and methods

2.1 Materials

All the autotetraploid strains were cultivated by Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS) at Wenjiang Rice Breeding Base. High seed set restorers TP-4 and Dminghui63, maintainer line D46B, and diploid control Minghui63 are shown in Tables 1 and 2.

Table 1 Materials and their origin

material	chromosome number of genome	conformation of genome	origin of material
TP-4	48	AAAA	CIB CAS
Dminghui63	48	AAAA	CIB CAS
D46B	48	AAAA	CIB CAS
Minghui63(control)	24	AA	CIB CAS

2.2 Methods

2.2.1 Field experiment

Autotetraploid strains were planted at Wenjiang Rice Breeding Base in Sichuan Province, China from March 25, 2002 to October 20, 2005. Thirty plants (5×6) were in a plot and each plot area was 1 m^2, every strain was replicated three times and randomly planted in the field. Five plants in every plot were randomly sampled to be studied and analyzed. Theoretic output = 1000–seed weight × Productive panicles per plant × Productive grains per panicle × 0.0003 (t/hm^2). Materials for pollen fertility test were sampled in the plots on June 22, 2005. Fresh pollens were tested by using I^2-IK method (Huang et al., 1999) and partly further confirmed by Dumas and Knox (1983) method: pollens were pollinated onto the stigma, and two to three days later, the stigma was treated in 6 mol/L NaOH for 24 h, and then stained by 0.1% aniline blue for 24 h after being rinsed with water. ZEISS AXIOPLAN 2 microscope was used to observe and photo the fluorescence. It revealed that there was no difference between Dumas and Knox (1983) method and I^2-IK method. In Table 2, the result was done by I^2-IK method.

2.2.2 Mitosis of root tip cells

After the removal of the old roots of the autotetraploid rice, the plants were cultured in the rhizogenic liquid medium for one to three days when new roots grew to 1–2 cm. Healthy root tips were cut off, treated with saturated dichlorobenzene solution for 1–3 h, rinsed with distilled water for three times, and fixed in the solution of methanol and glacial acetic acid at 3 : 1 for 24 h. The roots were then enzymolyzed with 1% cellulose and 1% pectinase for about 5 min at 37°C, then rinsed with distilled water and stained with 1/40 Giemsa at pH 7.2. Finally the specimens were ready for microscopic examination and micrograph at ZEISS AXIOPLAN 2 microscope.

2.2.3 Pollen Mother Cell (PMC) meiosis

Young panicles in meiosis were gathered in field and fixed in Carnoy's II solution (alcohol : aceticacid = 3 : 1) for approximately 24 h, then transferred into 70% alcohol solution and stored at 4°C in a refrigerator. The disposed panicles in 1 mol/L HCl at 60°C for about 8–10 min, stained with

Table 2 Agronomic characteristics of the autotetraploid rice and the diploid control

material	pollen fertility /%	productive panicles per plant[a)]	panicle length /cm	total grains per panicle	productive grains per panicle	seed set /%	1000-grain weight /g	theoretic output /t·hm^{-2}
TP-4	81.78	9.07	24.57	99.14	72.86	73.40	39.71	7.87
Dminghui63	80.76	11.13	23.40	107.00	75.87	71.00	35.40	7.91
D46B	81.04	9.70	20.10	112.12	81.06	72.30	31.05	7.32
Minghui63(control)	83.29	15.40	20.67	146.73	117.87	80.00	27.40	14.88

Note: a) indicates the panicle with at least five productive grains.

improved phenol fuchsine and then the chromosome meiosis was observed (Liu et al., 1995; Liu and Ding, 1996) at a ZEISS AXIOPLAN 2 microscope. The data were disposed by SPSS 11.5 software.

2.2.4 Data analysis

All data were processed with SPSS 11.5 software and *U*-test was done to study the significance of difference.

3 Results and analysis

3.1 Chromosome observation in the meiosis of autotetraploid rice

3.1.1 Chromosome observation in the prophase of meiosis of autotetraploid rice

Chromosomes of restorers TP-4 and Dminghui63 and maintainer D46B of autotetraploid rice tended to show delicate dyeing lines in leptotene, zygotene and pachytene in meiosis. These seemed almost the same as those of diploid control. In diplotene, chromosomes contracted heavily and the chromosome pairing was able to be observed. It revealed that chromosome abundance in autotetraploid rice in diplotene was significantly higher than that of diploid control. However, it was hard to number the chromosomes in diplotene yet.

3.1.2 Chromosome observation in diakinesis-metaphase I (MI) in meiosis of autotetraploid rice

Chromosome contracted more heavily in diakinesis-metaphase I (MI) in meiosis and consequently univalent, bivalent, trivalent and quatrivalent and sometimes multivalent were observed. Significant difference was observed between autotetraploid rice and the diploid control, and between the two restorers and the maintainer (Table 3) as well.

Bivalent could be observed and numbered in diakinesis-metaphase I (MI) for the chromosome had contracted to a tight structure. Univalent, bivalent, trivalent and quatrivalent and multivalent were observed and numbered and the cross of PMC was calculated: cross by univalent signed 0, cross by rod bivalent signed 1, cross by ring bivalent signed 2, cross by chain quatrivalent signed 3 and cross by ring quatrivalent signed 4. Furthermore, cross by ring hexavalent signed 6 and ring octavalent signed 8.

In diakinesis-metaphase I (MI), the diploid control was observed having normal chromosome behavior except two univalents and a quadrivalent. 97% PMCs of the observed 106 cells formed twelve bivalents in diakinesis-metaphase I (MI).

High seed setting restorers TP-4 and Dminghui63 were observed having a few univalents and trivalents in diakinesis-metaphase I (MI). In TP-4, five univalents were observed in two PMCs (2.75%) and the univalent frequency per PMC was 0.05. Only a single PMC was observed having a trivalent (0.92%) and the trivalent frequency per PMC was 0.01. In Dminghui63, ten univalents were observed dispersing in six PMCs in MI (6.38%) and the univalent frequency per PMC was 0.12 (Fig. 1(f)). Four PMCs were observed having trivalents (4.26%) with a trivalent frequency 0.09/PMC. Quadrivalents were observed both in restorers and the maintainer, with the quadrivalent frequencies 2.00/PMC (0–6) in restorers and 2.26/PMC (0–8) in the maintainer, respectively. The maximum number in a PMC was 8. The bivalent frequencies in restorers were 19.96/PMC (9.89 rod + 10.07 ring) and 16.64/PMC (8.90 rod + 10.37 ring), respectively. And the maximum number in a PMC was 24. No multivalent was observed in TP-4 but a ring hexavalent in Dminghui63 (1.06%). Configuration of chromosome in a PMC was calculated as follows:

TP-4: $2n = 48 = 0.046\text{I} + 19.96\text{II}\,(9.890\ \text{rod} + 10.07\ \text{ring}) + 0.009\text{III} + 2.200\text{IV}$ (Fig. 1(d)), and the average cross number was 37.67;

Dminghui63: $2n = 48 = 0.106 + 16.64\text{II}\quad(8.904\ \text{rod} + 10.37\ \text{ring}) + 0.085\text{III} + 2.255\text{IV} + 0.011\text{VI}$, and the average cross number was 37.04 (Table 3).

Table 3 Chromosomes pairing of the PMCs of autotetraploid rice in meiotic diakinesis-metaphase I (MI)

material	total cell number	chromosome pairing							cross number
		univalent	bivalent			trivalent	quadrivalent	multivalent	
			total number	rod	ring				
TP-4	109	0.05	19.96	9.89	10.07	0.01	2.00	0	37.47
		(0–2)	(17–23)	(2–19)	(3–22)	(0–1)	(0–6)	(0)	(29–46)
Dminghui63	94	0.11	19.28	10.37	8.90	0.11	2.26	0.01	37.04
		(0–3)	(8–24)	(1–21)	(3–22)	(0–3)	(0–8)	(0–1)	(27–46)
D46B	85	0.07	11.86	7.20	4.65	0.10	6.00	0	39.51
		(0–1)	(2–20)	(0–14)	(1–12)	(0–2)	(2–11)	(0)	(33–46)
Minghui63(CK)	106	0.06	11.95	5.42	6.54	0	0.01	0	18.53
		(0–2)	(10–12)	(4–10)	(3–11)		(0–1)		(15–24)
$LSD_{0.05}$		0.10	0.88	1.92	0.82	0.07	0.44	0.02	0.86

Note: *LSD* is the short form for least significant difference.

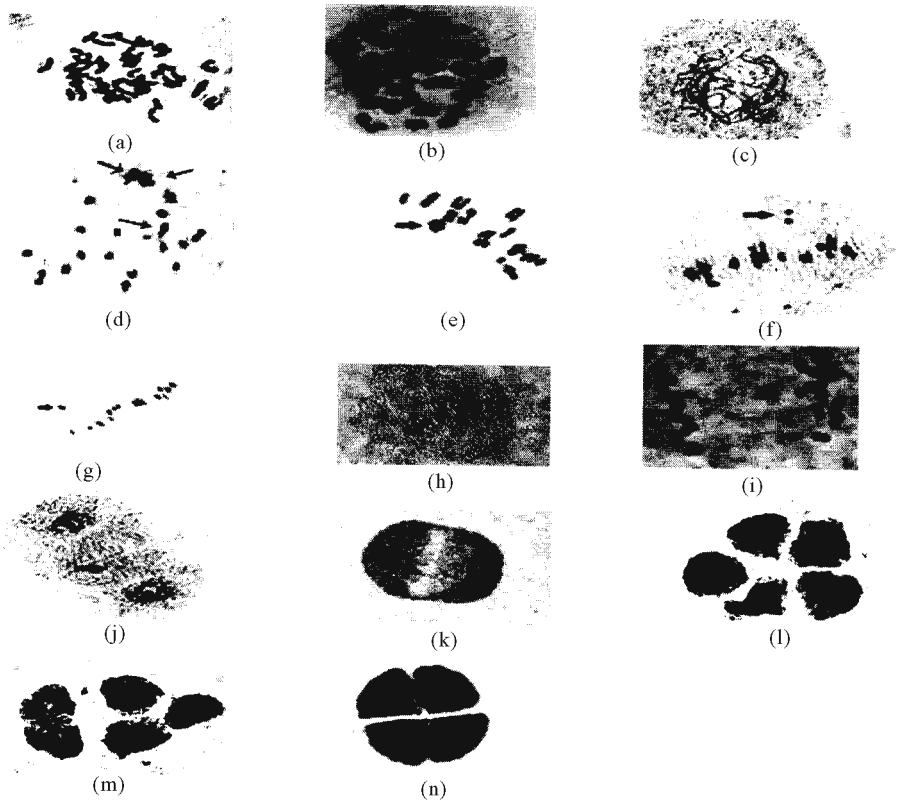

Fig. 1 Observation in meiosis of restorers TP-4 and Dminghui63 and maintainer line D46B of autotetraploid rice. Note: (a) Mitosis of TP-4 in MI $2n = 4X = 48$; (b) mitosis of Dminghui63 in MI, $2n = 4X = 48$; (c) meiosis of TP-4 in zygotene stage; (d) meiosis of TP-4 in diakinesis-metaphase I, $2n = 4X = 18$II (13II ring + 5II rod) + 3IV ring (arrowheads); (e) meiosis of D46B in MI; (f) meiosis of Dminghui63 in MI, with two univalents lagging (arrowheads); (g) meiosis of Dminghui63 in MI with a bivalent lagging (arrowhead); (h) meiosis of TP-4 in MI; (i) meiosis of D46B in AI; (j) meiosis of Dminghui63 in TI with a chromosome lagging (arrowhead); (k) meiosis of D46B in TI; (l) quinary microspore of Dminghui63 in TII; (m) quinary microspore of TP-4 in TII; and (n) normal quartered microspore of D46B in TII

The univalent frequency of the maintainer D46B was 0.07/PMC, which was not significantly different from that of the two restorers. However, the bivalent frequency of D46B (maximum: 20) was significantly lower than that of the two restorers. For trivalent, the frequency of D46B was similar to that of Dminghui63 but significantly lower than that of TP-4. The quadrivalent frequency of D46B was up to 6.00/PMC, which was significantly higher than those of the restorers (Fig. 1(e)). The configuration of chromosome in a PMC of D46B was calculated as follows (Tables 3 and 4):

$2n = 48 = 0.07\text{I} + 11.86\text{II}$ (5.42 rod + 6.54 ring) + 0.10III + 6.00IV), and the average cross number was 39.51 (higher than restorers).

3.1.3 Chromosome observation in AI in meiosis of autotetraploid rice

In AI, chromosomes lagging dispersing in eight PMCs (7.30%) was observed and the frequency was 0.08 (Table 5).

Table 4 *U*-test of statistical analysis of autotetraploid rice in diakinesis-metaphase I

material	univalent		bivalent		trivalent		quadrivalent		multivalent		cross number	
	percentage of the cell with univalent /%	*U*	percentage of the cell with bivalent /%	*U*	percentage of the cell with trivalent /%	*U*	percentage of the cell with quadrivalent /%	*U*	percentage of the cell with multivalent /%	*U*	cross number	*U*
TP-4 versus D46B	2.75 7.14	4439.00	100.00 100.00	461.00**	0.92 4.76	4293.00*	83.49 100.00	461.00**	0.00 0.00	4632.50	37.67 39.51	3605.50**
Dminghui63 versus D46B	6.38 7.14	4737.50	100.00 100.00	598.50**	4.26 4.76	3842.00	85.11 100.00	560.00**	1.06 0.00	3952.50	37.04 39.51	2610.00**

Note: *and ** indicate significant difference at $P < 0.05$ and $P < 0.01$ levels, respectively.

Table 5 Chromosome behavior in AI, the percentage of cells with microkernel in TI and the frequency of cells forming abnormal quartered microspore in TII of autotetraploid rice and diploid control

material	total cell number in AI	the number of cell with chromosome lagging	percentage of the cell with chromosome lagging	lagging frequency per PMC	total cell number in TI	total cell number with microkernel in TI	percentage of abnormal cell in TI/%	microkernel number /PMC in TI	total cell number in TII	number of abnormal quartered microspore	frequency of abnormal cell in TII/%
TP-4	108	21	19.44	0.41	100	4	4.00	0.05	180	6	3.30
Dminghui63	121	28	23.14	0.45	100	6	6.00	0.08	156	12	7.70
D46B	226	24	10.62	0.11	100	1	1.00	0.01	152	2	1.32
Minghui63	110	8	7.30	0.08	100	0	0	0.00	120	2	1.70

In AI, lagging chromosomes, including univalents, bivalents and quadrivalents, were observed in TP-4 and Dminghui63, and the frequencies were 28.74% and 23.67%, respectively. The frequency of TP-4 was significantly higher than that of D46B, and for Dminghui63, it was significantly higher. For D46B, the frequency of lagging chromosomes was 10.62%, a little higher than that of the diploid control. Consequently, lagging chromosomes per PMC of D46B were significantly lower than those of the two restorers (Fig. 1(i)), but a little higher than the diploid control (Tables 5 and 6).

3.1.4 Telophase I (TI)

Normal chromosome behavior was observed in TI in meiosis of the diploid control and no microkernel was observed in that period.

For TP-4 and Dminghui63 (Fig. 1(j)), microkernels were observed in both strains and the frequency was 0.05/PMC and 0.08/PMC, respectively. The frequency of microkernel per PMC in D46B was lower than that of restorers. Only a microkernel was observed in D46B in that period (Fig. 1(k)) (Tables 5 and 6).

3.1.5 Telophase II (TII)

No microkernel was observed in the diploid control in TII. But microkernels with a lower frequency than in TI were observed in all the three autotetraploid strains. The frequency of normal quartered microspore in Minghui63 was up to 98.30% but a little lower than that of D46B (98.68%). The frequencies in TP-4 and Dminghui63 were comparatively lower, which were 96.70% and 92.70%, respectively. No significant difference was observed between TP-4 and D46B, but Dminghui63 showed a significantly lower frequency than D46B (Tables 5 and 6) (Fig. 1(l), (m), (n)).

3.2 Correlation analysis among pollen fertility, seed set and meiosis in autotetraploid rice

As shown in Table 2, the pollen fertility of TP-4 and Dminghui63 was 81.78% and 80.76%, respectively; the seed set of TP-4 and Dminghui63 was 73.40% and 71.00%, respectively. However, the pollen fertility in D46B was 81.04% with seed set of 72.30%, which was higher than that of Dminghui63 but lower than that of TP-4, and similar to that of the diploid control (Table 2).

Correlation analysis was done to study the relationships among chromosome behaviors in MI, frequencies of lagging chromosomes in AI abnormal PMC in TI, abnormal quartered microspore in TII, pollen fertility and seed set. The items in meiosis referred above were negatively related to seed set or pollen fertility. Thus those items were consequently passive factors of improving seed set or pollen fertility. And only a few ($n = 4$) strains were compared, which might be the cause of being not significant. Pollen fertility was significantly related to seed set at $P < 0.01$ level. Frequency of lagging chromosomes in AI was positively and significantly related to abnormal PMC in TI. It might reveal that the microkernels formatted in TI were caused by those lagging chromosomes in AI. Abnormal PMC in TI and frequency of lagging chromosomes in AI were positively related to the frequency of abnormal quartered microspore in TII. The correlation

Table 6 *U*-test of statistical analysis of autotetraploid rice and diploid control in anaphase I (AI), telophase I (TI) and telophase II (TII)

material	anaphase I (AI)		telophase I (TI)		telophase II (TII)	
	percentage of the cell with chromosome lagging/%	*U*	percentage of abnormal cell in TI/%	*U*	frequency of abnormal cell in TII/%	*U*
TP-4 Vs	19.44	10197.00*	4.00	4899.50	3.30	13404.00
D46B	10.62		1.00		1.32	
Dminghui63	23.14	10	6.00	4853.00	7.70	11100.00**
Vs D46B	10.62	974.00**	1.00		1.32	

Note: * and ** indicate significant difference at $P < 0.05$ and $P < 0.01$ levels, respectively.

coefficient was 0.92 and 0.87, respectively, although they were not significant but seemed logically worthy of consideration yet. Univalent was significantly related to multivalent, which was consistent with the phenomenon of observing univalents and multivalents occurring in the same PMC. But the cause was not certain yet. Frequency of abnormal quartered microspore in TII was significantly related to multivalent number in PMC, which revealed multivalent might not only influence AI (0.72) and TI (0.79), but also significantly influence chromosome separation in meiosis II and consequently formation of normal quartered microspore in T II (Table 7).

4 Discussion

4.1 Relationships among meiosis, pollen fertility and seed set

Eminent agronomical traits were observed in the two restorers TP-4 and Dminghui63 and the maintainer D46B (especially in restorers). They included big panicles, huge seeds, leaf shrub, strong stems, ideal plant type, lodging, eminent adaptability, high seed set and so on. So the strains should be prospective ones of the three lines (Tu et al., 2003). It revealed that the chromosome number of the strains of autotetraploid rice in a PMC in meiosis was $2n = 48$. That was consistent with the result of mitosis observation (Fig. 1(a) and (b)). Generally speaking, PMC in meiosis showed comparatively ideal chromosome behaviors: eminence in M, and low frequency of abnormal chromosome in TI and TII. That may be a cause of the high frequency of pollen fertility in double fertilization.

Some studies with other plants have been done to ascertain the relationships between chromosome behaviors in meiosis and seed set. However, there are mutual differences among their results and generally they turn out to be two inconsistent opinions: (1) there are no relationships among abnormal meiosis and pollen fertility and seed set (Merler, 1971); (2) abnormal meiosis constrains the rise of pollen fertility and consequently seed set. Namely, the abnormal meiosis is related to seed set (Weimark, 1973; Weimark, 1975). Our experiment revealed that several items in meiosis were negatively related to pollen fertility and seed set. So they might be logically the passive factors for the rise of pollen fertility and seed set. The correlation coefficients were not up to significance yet. Maybe it is just because the number of compared strains was not big ($n = 4$). Pollen fertility was related to seed set at $P < 0.01$ level, which was consistent with the opinion authorized nowadays. The frequency of lagging chromosomes in AI was positively and significantly related to abnormal PMC in TI. It revealed that chromosome lagging in AI was the main cause of microkernel formation in TI. In a word, our results tend to support the latter opinion, namely, meiosis is related to seed set.

4.2 Cytogenetical characteristics of the two restorers and the maintainer

The three autotetraploid strains were observed having abnormal chromosome pairing and separation in meiosis that resulted in univalent, trivalent, quadrivalent and lagging. It revealed that the strains including the two restorers and the maintainer tended to produce more univalents, trivalents and other abnormal behaviors than diploid control in MI in meiosis. So autotetraploid rice would have more complex chromosome configurations and more diverse variations in meiosis, which was consistent with the result of Habib and Shahida (2004) who observed plenty of imbalance separations in *Brassica rapa* L.

Table 7 Correlation analysis of abnormal chromosome behaviors in meiosis, pollen fertility and seed set

	univalent	bivalent	trivalent	quadrivalent	multivalent	percentage of the cell with chromosome lagging in AI	percentage of abnormal cell in TI	frequency of abnormal cell per PMC in TII	pollen fertility
bivalent	0.27								
trivalent	–0.12	–0.54							
quadrivalent	0.13	–0.21	0.93						
multivalent	0.95*	0.53	–0.38	–0.09					
percentage of the cell observed chromosome lagging in AI	0.54	0.95	–0.38	–0.01	0.72				
percentage of abnormal cell in TI	0.61	0.92	–0.41	–0.03	0.79	0.995**			
frequency of abnormal cell per PMC in TII	0.82	0.75	–0.52	–0.19	0.96*	0.87	0.92		
pollen fertility	–0.62	–0.43	–0.41	–0.72	–0.56	–0.66	–0.66	–0.54	
Seed set	–0.56	–0.53	–0.35	–0.67	–0.54	–0.73	–0.72	–0.56	0.99**

Note: * and ** indicate significant difference at $P < 0.05$ and $P < 0.01$ levels, respectively.

It also revealed that the chromosome separation of quadrivalents in anaphase I tended to be II + II form. But only a few quadrivalents were observed separating in III + I or II + I + I. That might be the cause of low frequencies of trivalents lagging and microkernels in TI.

The number of bivalent and quadrivalent of the two restorers per PMC in MI was observed being significantly different from that of the maintainer. It revealed that the restorers tended to have more bivalents but the maintainer tended to have more quadrivalents. More quadrivalents usually mean better pairing capacity of chromosome in meiosis. However, the difference of univalent between the restorers and maintainer was not significant, which might confirm that the maintainer D46B tended to have more eminent genetic stability. Zhang et al. (1997) observed the lower frequency of quadrivalent in hybrid strains of *Sorghum bicolor* (L.) Moench, and stated that hybrids should show more univalent theoretically. It revealed that better chromosome pairing in meiosis could do help reduce the production of univalents. Our result was consistent with the assumption.

The frequency of lagging chromosomes in AI was positively significantly related to the abnormal PMC in TI, revealing that chromosomes lagging in AI were the main cause of microkernel formation in TI. That was consistent with the result of our study on F_1 hybrids and their parents. Kaul and Murthy (1985) stated that meiosis was a delicately precise and ordered process controlled by series of genes especially dominant genes. We deduced the chromosome separation in AI and microkernel formation in TI was controlled by a dominantly single gene or major gene in a QTL.

Normal quartered microspore frequency of D46B was not only higher than that of the two restorers but a little higher than that of the diploid control. That could confirm that D46B tended to have better genetic stability than TP-4 and Dminghui63. It may surely do good to germplasm cultivation and selection, which would be feasible to improve agronomical traits (pollen fertility, seed set, etc.).

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