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AGRONOMIC TRAITS AND CYTOGENETIC EVALUATION OF NEWLY DEVELOPED AUTOTETRAPLOID RICE LINE

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Autotetraploid rice is an important germplasm and has a great scope to increase rice yield but low seed set is a major encumbrance in its utilization. The present study was planned to observe the cytogenetic and agronomic characteristics of newly developed highly fertile autotetraploid rice line (1162). Agronomic traits, male and female gametophytes of newly developed autotetraploid rice (1162) and their hybrids showed better performance as compared to its parents and other autotetraploid rice lines. The mean of panicle length, number of grains per panicle, filled grains per panicle, 1000-grain weight, grain length, grain width, and seed set were 25.04cm, 103.38 grains, 83.56 grains, 43.44g, 9.54mm, 4.04mm and 81.58%, respectively. The meiotic analysis showed that the mean meiotic configurations of 1162 at diakinesis were (0.13±0.05) I + (4.89±0.26) II +(0.02±0.00) III +(9.45±0.13) IV. The frequency of trivalent and univalent in new line was lower than the autotetraploid lines (control) at diakinesis and metaphase I, and the configuration of chromosome in 1162 was more symmetrical, which was in favor of 2/2 segregation of homologous chromosome, although higher number of quadrivalents were found than parents. Heterosis of hybrids crossed with 1162 was not only positive but also attained highly significant levels for important quantitative traits. The results showed that high fertility can be inherited and have important applications and research value.
Keywords: Agronomic traits, autotetraploid rice, *Oryza sativa*, polyploidy, seed setting.

INTRODUCTION

Rice is one of the most important crops in China and whole world, which is the model cereal organism because of its small genome. The yield of rice is related to the food security of China and world. So, how to improve the yield and resistance of rice have attracted the attention of breeders. One of the most important aspects of breeding methods to raise rice production is the utilization of the heterosis at the diploid level. The rice yield has been obviously increased by the use of the heterosis, which improve living standards of the people. However, as the climate warms up, the environment worsens and the damage of the diseases and pests increases, the methods of rice breeding are now facing challenges such as: the productivity of rice has been stagnant and the resistance level is not increased significantly. The breeding of autotetraploid rice seems to be an approach worth exploring, because of its gigantic characteristics (Cai *et al*., 2001; Shahid *et al*., 2011 and 2013a). Autotetraploid rice is a new germplasm which is obtained by chromosome doubling through colchicine treatment. Autotetraploid rice showed higher genetic variation, greater stability across varying environments, resistant to lodging, higher hybrid vigor, greater grain length and width and resistant to insect pest and diseases than diploid rice (Song and Zhang, 1992; Luan *et al*., 2008; Tu *et al*., 2007; Shahid *et al*., 2011, 2012; Wu *et al.*, 2013). However, Low seed set is the major barrier in autotetraploid rice breeding (He *et al*., 2011a; Shahid *et al*., 2013a). There are many reasons for low seed setting of autotetraploid rice, such as pollen and embryo sac abortion (Huang *et al*., 1999; Zhang *et al*., 2003; Guo *et al*., 2006; Hu *et al*., 2010; Shahid *et al*., 2010), abnormal chromosome behavior and microtubule distribution pattern, and differentially expressed genes and miRNAs (He *et al*, 2011a,b; Wu *et al.*, 2014, 2015; Li *et al*., 2016). *Indica-japonica* autotetraploid rice hybrids have shown strong yield potential, which would provide a new way to the rice breeding (Shahid *et al*., 2012). But the poor fertility or low seed set is the inherent problem which has limited the yield advantages of autotetraploid hybrid rice breeding. In order to overcome this problem, through continuous hard work for 20 years, we have developed a large number of autotetraploid rice lines after chromosome doubling with varying seed set. Some new autotetraploid rice lines with high fertility were developed in our lab from 2007. Therefore, the present study was planned to 1) investigate the cytogenetic and agronomic characteristics of newly developed high fertility autotetraploid rice line, 2) and to observe the hybrid vigor by crossing with other 42 autotetraploid rice lines. These lines could provide the important germplasm for autotetraploid rice breeding, and practical and theoretical basis for heterosis studies, and for commercial utilization of autotetraploid rice.

MATERIALS AND METHODS

Plant materials: High fertility autotetraploid rice line "1162" was developed in our lab in 2010 by crossing autotetraploid Jackson-4x and 96025. Jackson-4x, which was kindly donated by Prof. YQ Li, South China Botanical Garden, Chinese academy of sciences (SCBU-CAS), was obtained by chromosome doubling through colchicine treatment from an American rice variety, Jackson-2x. 96025 is an autotetraploid rice line, and kindly provided by Prof. YH Zhang, Chinese Academy of Agricultural Sciences. 1162 and its parents were used for investigating chromosome behavior during meiosis in the present study. Forty two hybrids were developed by crossing 1162 with 42 autotetraploid rice lines to investigate mid-parent heterosis and better-parent heterosis. Pollen and embryo sac fertility of 1162, its parents, and 1162×Jingxian 89-4x, 1162×4001-1-4x 1162×Yuhei1-4x and 1162×IR36-4x were observed. All these materials were planted at the farm of South China Agricultural University.

***Chromosome behavior observation*:** Inflorescences were collected from shoots of rice plants with 1-4 cm between their flag leaf cushion and second-to-last leaf cushion, and fixed in Carnoy solution (ethanol : acetic acid=3:1) for at least 24 h. The samples were then stored in 70% ethanol at 4˚C. Anthers were separated from the floret using forceps and dissecting needle and placed in a small drop of 1% carmine acetate. After 2-3 min, glass slide was covered with a slide cover and examined under microscope (Olympus CX31). The numbers of the quadrivalent, bivalent, trivalent and univalent were also observed according to He *et al*. (2011b).

***Investigation of pollen and embryo sac fertility and seed setting percentage*:** About five mature spikelets were collected from various panicles of the plant to investigate the pollen fertility, and then fixed in Carnoy solution for 24 h. Potassium Iodide solution (I_2-KI, 1%) was used to stain the spikelets and observed under microscope. Pollen fertility was divided into four categories i.e., normal pollens, stained abortive pollens, spherical abortive pollens and typical abortive pollens (Shahid *et al*., 2013b).

Whole-mount eosin B-staining confocal laser scanning microscopy (WE-CLSM) was used to observe embryo-sac structure. Embryo sac fertility was investigated according to Shahid *et al*. (2010) and seed setting was counted according to the method of Shahid *et al*. (2013b).

The amount of heterosis or hybrid vigor (H) was evaluated using two measurements: (1) mid-parent heterosis (H_{PM}) was calculated using the equation: $H=(F_1-(P_1+P_2)/2)/\ (P_1+P_2)/2$ (where P_1 and P_2 are the means of the corresponding parents), and (2) better-parent heterosis using the following equation (H_{PB}): $H=\ (F_1-P_b)/\ P_b$ (where P_b represents the value of the better-parent).

RESULTS

***Performance of main agronomic traits in 1162*:** The means of ten agronomic traits of 1162 and its parents were assessed by analysis of variance (Table 1). The results indicated that six traits of 1162, including plant height, the number of filled grains per panicle, seed setting, 1000-grain weight, grain width and grain length-width ratio, were significantly (p<0.01) higher than parents. The number of filled grains per panicle, seed setting and 1000-grain weight were closely related with yield. Number of grains per panicle and grain length of 1162 significantly increased as compared to its parents, while there was non-significant difference in the

Table 1. Analysis of variance for agronomic characters of 1162 and its parents.

Agronomic characters	96025	Jackson-4x	1162	*F* value
PH/cm	84.86±1.63c	93.32±0.81b	120.78±2.78a	95.67**
NP	4.00±0.32c	3.00±0.32c	4.00±0.45c	2.50
PL/cm	26.64±1.02c	25.86±0.71c	25.34±0.66c	0.65
NGP	91.56±3.98b	114.94±6.54a	106.98±6.05ab	4.45*
NFGP	49.44±4.01b	32.14±1.90c	89.80±6.33a	43.99**
SS (%)	53.82±2.89b	28.03±1.15c	83.74±2.28a	156.83**
TGW/g	43.18±0.83b	25.18±0.87c	43.37±0.75b	162.58**
GL/mm	9.24±0.14c	10.52±0.24b	9.46±0.14c	14.54*
GW/mm	3.34±0.09b	2.96±0.068c	3.96±0.14a	24.04**
L/W	2.78±0.11b	3.56±0.07a	2.40±0.11c	38.34**

PH=Plant Height, NP=Number of Panicles, PL=Panicle Length, NGP= Number of Grains per Plant, NFGP= Number of Filled Grains per Panicle, SS=Seed Setting, TGW=1000-Grain Weight, GL=Grain Length, GW=Grain Width, L/W=Grain Length-Width Ratio.

*, ** Significantly different from zero at p<0.05 and p<0.01, respectively number of panicles and panicle length between 1162 and parents. 1162 also showed higher seed setting (83.74%) than its parents, which reached at the level of diploid rice (Fig. 1).

Figure 1. Panicles of 1162 and its parents.

***Chromosome configuration during pollen mother cell meiosis in high fertile autotetraploid rice*:** The high fertile autotetraploid rice and its parents were used to observe pollen mother cell (PMC) meiosis and chromosome behavior. The results showed that meiosis division of high fertility autotetraploid rice line and its parents were consistent with diploid rice. Meiosis process include: prophase I, metaphase I, anaphase I, telophase I, prophase II, metaphase II, anaphase II and telophase II. Prophase I could be subdivided into leptotene (Plate I-1, II-1), zygotene (Plate I-2, II-2), pachytene (Plate I- 3), diplotene (Plate I-4, II-3) and diakinesis (Plate I-5, II-4). Diakinesis is the best stage to identify the configuration and number of chromosomes. There were highly significant differences between newly developed autotetraploid rice line and their parents for chromosome configuration at diakinesis and following results were obtained.

96025: 2n=48= (0.27±0.08) I + (6.26±0.38) II + (0.03±0.02) III + (8.59±0.20) IV

Jackson-4x: 2n=48= (0.39±0.08) I + (8.93±0.41) II + (0.12±0.04) III + (7.02±0.20) IV

1162: 2n=48= (0.13±0.05) I + (4.89±0.26) II + (0.02±0.00) III + (9.45±0.13) IV

There were highly significant differences between parents and 1162 for the number of quadrivalents. The numbers of quadrivalents per cell ranged from 3-12, the average numbers of quadrivalents per cell were over 7. New line 1162 had the highest number of quadrivalents per cell (9.45), whereas female parent (Jackson-4x) had the lowest number of quadrivalents per cell (7.02). The main types of quadrivalents were: ring-shape (Plate II-4), double ring-shape (Plate II-4), chain-shape (Plate I-5), frying pan-shape, Y shape, OK shape (Plate I-5), X shape and -O- shape. Ring and double ring shapes were the most frequent configurations of quadrivalents. 1162 and Jackson-4x had the highest (4.28) and the lowest (2.91) number of double rings quadrivalents, respectively (Table 2).

There were different number of bivalents (ranging from 5 to 9), trivalents, univalents and multivalents in 1162 and its parents (Table 2). Jackson-4x had the highest number of bivalents per cell (8.93), while 1162 had the lowest number of bivalents per cell (4.89). The types of bivalents in autotetraploid rice were rod and ring shape as in diploid rice. There were more ring bivalents than rod bivalents in autotetraploid rice, and a few quadrivalents changed into trivalents and univalents. The main types of trivalents were chain and frying pan shape. Average frequency of trivalents was less than 1 in all autotetraploid rice lines. Jackson-4x with the lowest seed setting had the highest number of univalents and multivalents, while the new line has significantly lower number of univalents and multivalents than Jackson-4x and 96025.

Metaphase I: Most of the chromosomes arranged at the equator of the spindle (Plate I -6, II-5). Chromosome straggling (Plate III-1) and abnormal spindle (Plate III-2) were also observed during this stage. The maximum percentage of PMCs with chromosome straggling was 33.08% in Jackson-4x, while the minimum percentage of chromosome straggling (18.40%) was found in 1162.

Anaphase I: Homologous chromosomes were separated towards opposite poles of the cell (Plate I-7, II-6). The main types of abnormalities include: chromosome straggling (Plate III-3), chromosome bridge (Plate III-4), abnormal spindle and asymmetric division (Plate III-5).

Table 2. Chromosome configurations at diakinesis in autotetraploid rice lines.

Materials	N	Quadrivalents per cell							Trivalents per cell			Bivalents per cell			Uni-valent per cell	Multi-valents per cell
		Ring	Chain	F.pan	Y	D rings	Others	Total	Chain	F.pan	Total	Rod	Ring	Total		
96025	93	3.42 ±0.18	0.91 ±0.09	0.37 ±0.08	0.01 ±0.01	3.62 ±0.23	0.42 ±0.08	8.59 ±0.20	0.03 ±0.02	0.00	0.03 ±0.02	1.79 ±0.17	4.71 ±0.34	6.26 ±0.38	0.27 ±0.08	0.00
Jackson-4x	85	2.99 ±0.15	0.66 ±0.09	0.39 ±0.07	0.02 ±0.02	2.91 ±0.16	0.26 ±0.05	7.02 ±0.20	0.02 ±0.02	0.09 ±0.03	0.12 ±0.04	0.64 ±0.09	8.31 ±0.39	8.93 ±0.41	0.39 ±0.08	0.16 ±0.05
1162	105	3.87 ±0.17	0.86 ±0.09	0.19 ±0.05	0.04 ±0.02	4.28 ±0.19	0.27 ±0.05	9.45 ±0.13	0.02 ±0.01	0.00	0.02 ±0.00	0.58 ±0.09	4.30 ±0.24	4.89 ±0.26	0.13 ±0.05	0.00

N=No. of cells; Ring=Ring shape; Chain=Chain shape; F.pan=Frying pan shape; Y=Y shape; D rings=Double rings shape.

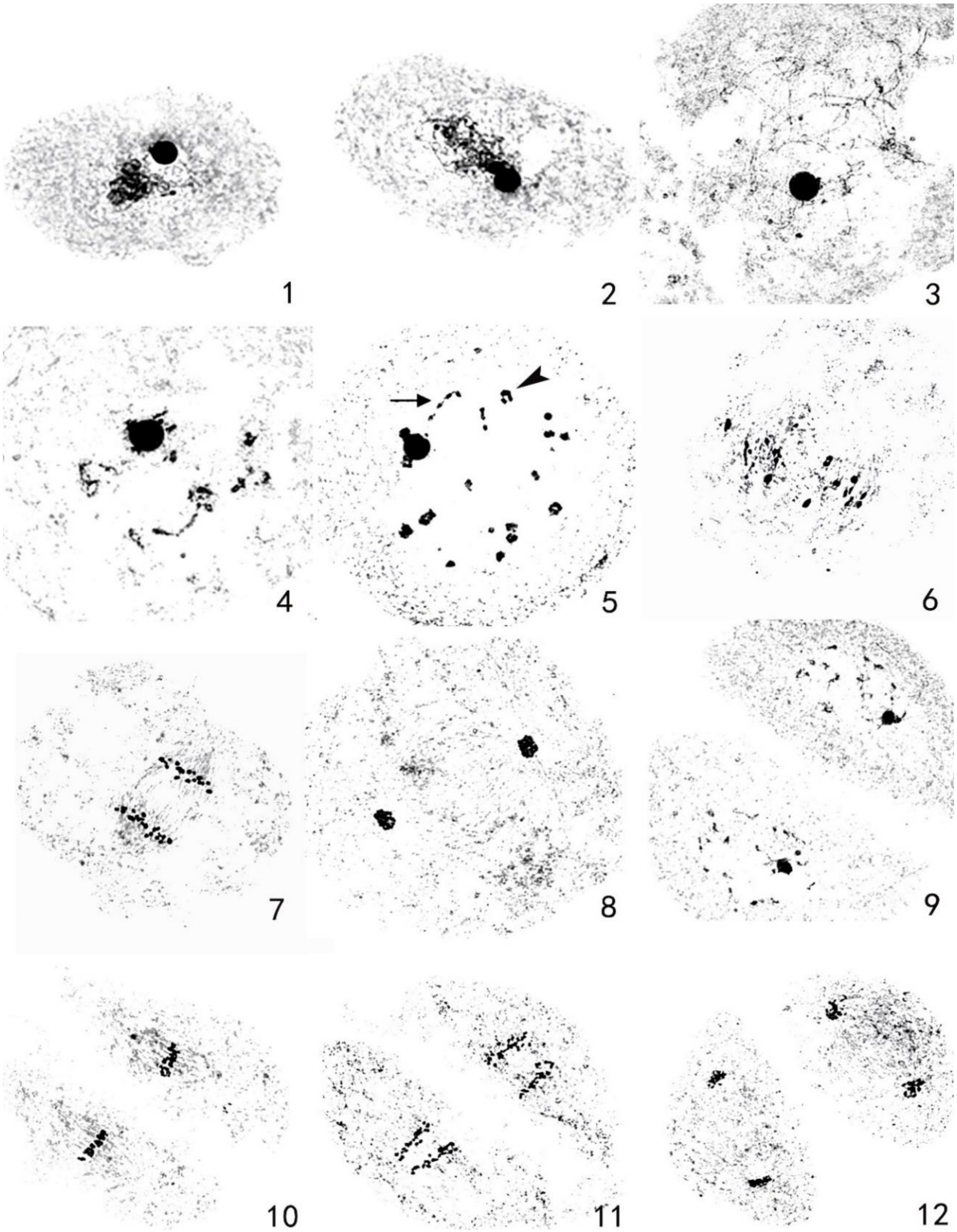

Plate I. Chromosome behavior of PMCs in Jackson-4x, ×1000.

1. Leptotene; 2. Zygotene; 3. Pachytene; 4. Diplotene; 5. Diakinesis, VII +7 IV +6 II + I, chain-shape quadrivalent (arrow head), OK shape quadrivalent (arrow); 6. Metaphase I, 7 IV +10 II; 7. Anaphase I; 8. Telophase I; 9. Prophase II; 10. Metaphase II; 11. Anaphase II; 12. Telophase II

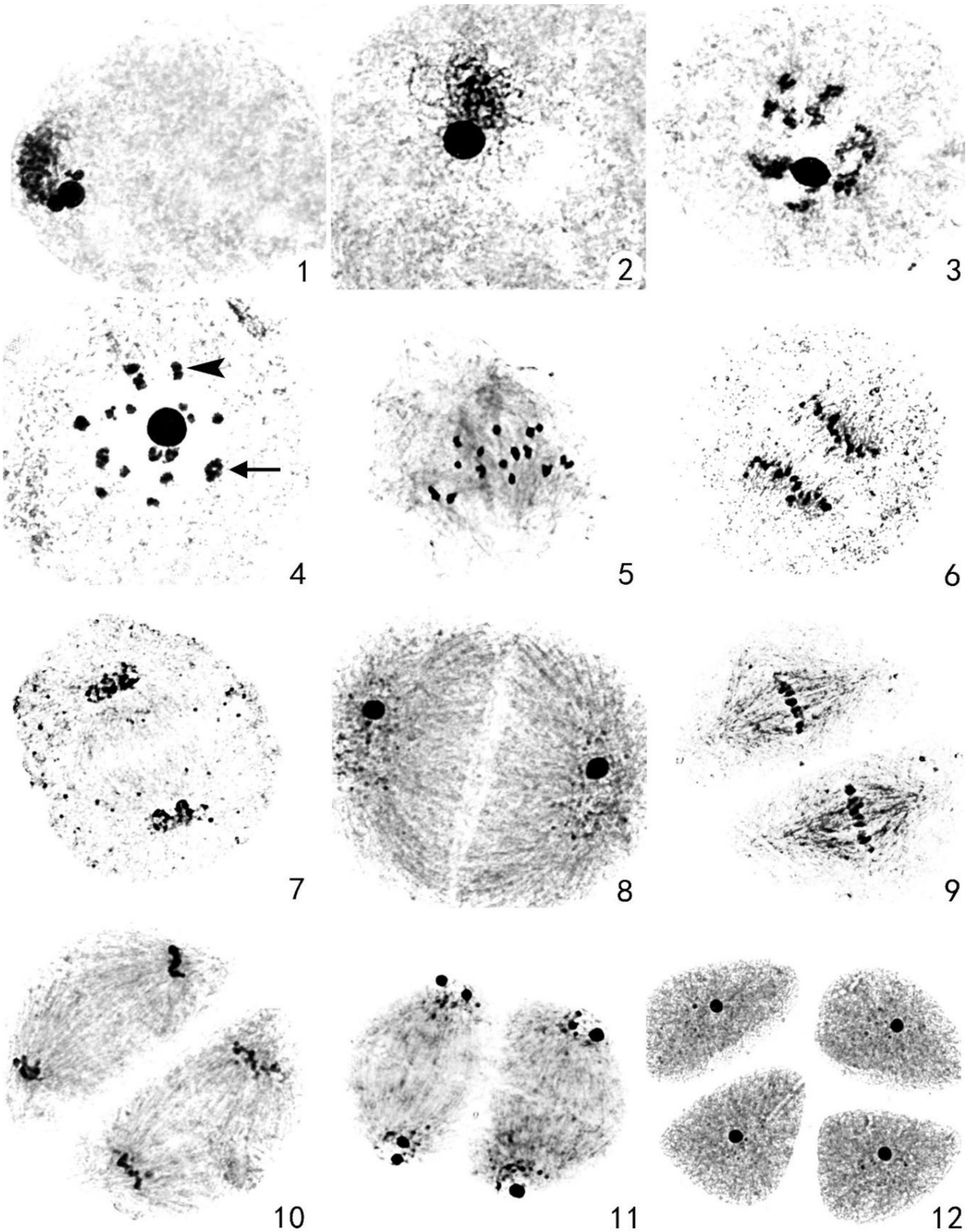

Plate II. Chromosome behavior of PMCs in 1162, ×1600.
1. Leptotene; 2. Zygotene; 3. Diplotene; 4.Diakinesis, 8 IV+7 II+2 I, ring-shape quadrivalent (arrow head), double ring-shape quadrivalent (arrow); 5. Metaphase I, 11 IV+2 II; 6. Anaphase I; 7. Telophase I; 8. Prophase II; 9. Metaphase II; 10. Anaphase II; 11. Telophase II; 12. Tetrads

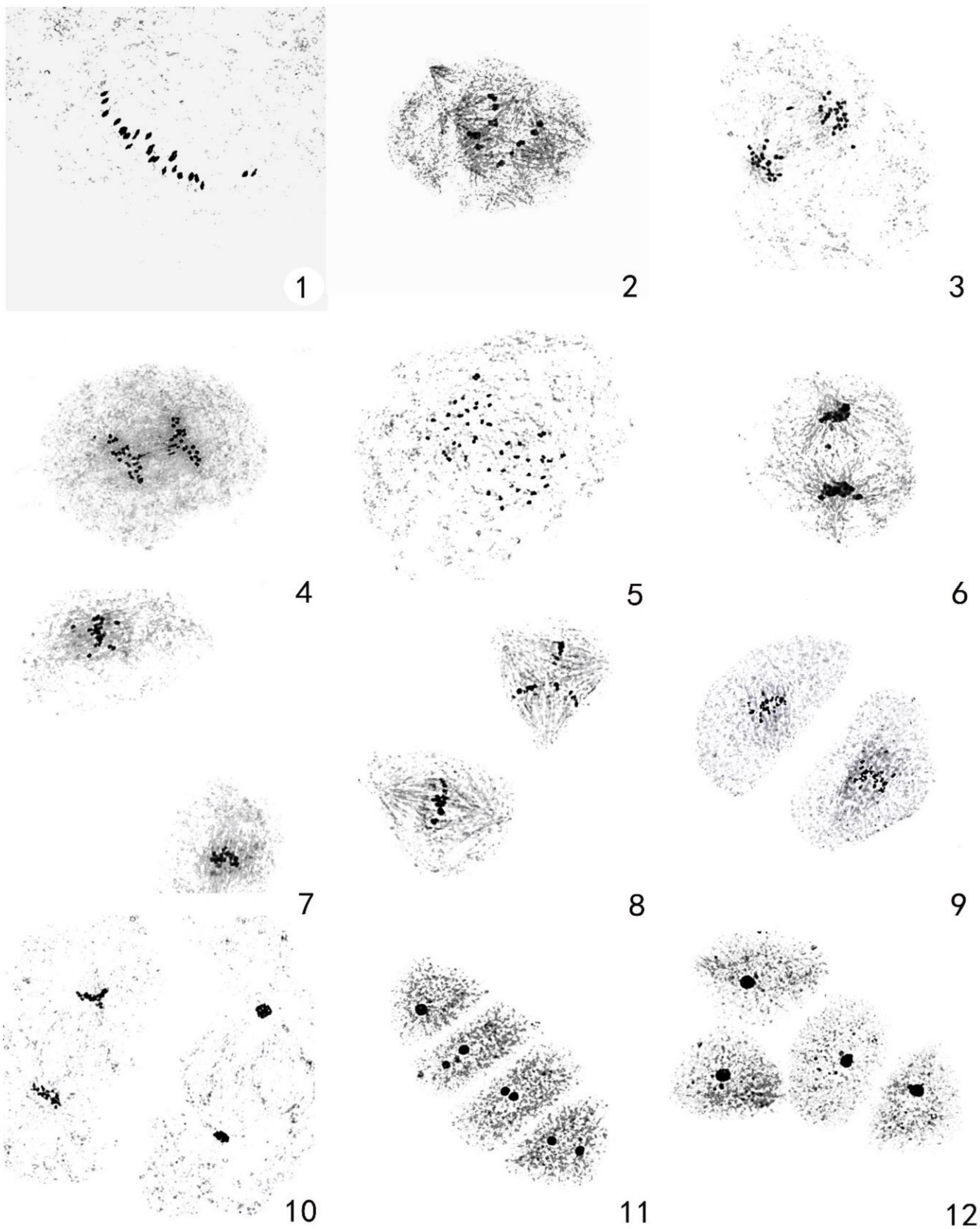

Plate III. Abnormal chromosome behavior of PMCs in autotetraploid rice, ×1000.
1. Metaphase I, straggling (Jackson-4x); 2. Metaphase I, multipolar spindle (96025); 3. Anaphase I, Straggling (Jackson-4x); 4. Anaphase I, chromosome bridge (96025) 5. Anaphase I (Jackson-4x); 6. Telophase I, Straggling (96025); 7. Metaphase II, straggling (Jackson-4x): 8. Metaphase II, triad (96025) 9. Metaphase II, T spindle (Jackson-4x); 10. asynchronous division, (Jackson-4x); 11. Telophase II, linear tetrad (96025); 12. Telophase II, T tetrad (96025)

Table 3. Frequency of abnormal chromosome behaviors during meiosis I of autotetraploid rice lines.

Name	Metaphase I				Anaphase I				Telophase I				
	N	Normal (%)	Lag (%)	Multipolar spindle (%)	N	Normal (%)	Strag (%)	Bridge (%)	N	Normal (%)	Strag (%)	Triad (%)	Micronucleus (%)
96025	203	77.83	15.76	6.40	75	81.33	17.33	1.33	101	90.10	9.90	0.00	0.00
Jackson-4x	130	66.92	33.08	0.00	82	86.59	13.41	0.00	118	80.51	16.10	0.00	3.39
1162	163	71.78	18.40	9.82	162	87.65	12.35	0.00	118	91.53	5.08	1.69	1.69

N= Number of cells observed; Lag=Lagging; Strag=Straggling

Table 4. Frequency of abnormal chromosome behaviors at metaphase II of autotetraploid rice lines.

Name	N	Normal (%)	Lag (%)	Tripolar spindle (%)	V spindle (%)	T spindle (%)	I spindle (%)	Chaos (%)	Out of step (%)
96025	74	60.81	2.70	9.46	10.81	8.11	2.70	5.41	0.00
Jackson-4x	150	55.33	24.67	0.00	8.00	2.00	0.00	0.00	10.00
1162	124	62.10	15.32	8.87	0.00	8.06	0.00	5.65	0.00

N= Number of cells observed; Lag=Lagging

Table 5. Frequency of abnormal chromosome behaviors at anaphase II of autotetraploid rice lines.

Name	N	Normal (%)	Strag (%)	Triad (%)	Chaos (%)	Out of step (%)
96025	27	40.74	7.41	0.00	3.70	48.15
Jackson-4x	87	37.93	12.64	0.00	0.00	49.42
1162	57	54.39	1.75	7.02	0.00	36.85

N= Number of cells observed; Strag=Straggling

Table 6. Frequency of abnormal chromosome behaviors at telophase II of autotetraploid rice lines.

Name	N	Normal/%	Strag/%	Abnormal tetrad/%	Pentads/%	Out of step/%	Polyad/%	Micronucleus/%
96025	152	84.87	1.32	13.16	0.66	0.00	0.00	0.00
Jackson-4x	84	73.81	8.33	0.00	0.00	4.76	0.00	13.10
1162	121	86.78	0.00	3.31	3.31	2.48	4.13	0.00

N= Number of cells observed; Strag=Straggling

The frequency of chromosome straggling was ranged from 12.35 to 17.33%. The frequency of normal cell in new line was significantly higher than parents (Table 3). Straggled chromosomes were also observed at Telophase I, but their frequency was less in new line than its parents. Prophase II, fission begins with division of nucleus, followed by cytoplasm division, and then a dyad was formed (Plate I-9, II-8). This stage was rather short, and no chromosomal aberration was observed.

Metaphase II: In most of the cells, chromosome arranged precisely on the equatorial plate and two spindles were parallel to each other (Plate I-10, II-9). However, a number of anomalies, including chromosome straggling (Plate III-7), tripolar spindle (Plate III-8), V spindle, T spindle (Plate III-9) and I spindle, were observed. Autotetraploid line, 96025, showed the highest frequency of abnormal spindles (31.08%) during Metaphase II (Table 4). In addition, around 10.00% asynchronous division of the dyad cell was found. The frequency of abnormal chromosome behavior of newly developed line was significantly lower than its parents.

Anaphase II: The chromatids separated and move towards opposite poles (Plate I-11, II-10). The chromosome straggling was relatively low, while high frequency of asynchronous division (Plate III-10) was found in this stage. Autotetraploid rice line 96025 showed the highest number of asynchronous divisions (Table 5).

Telophase II: The chromosomes of the dyad cell reached at the two poles of each cell and condensed to reform nuclear membrane and nucleolus (Plate I-12, II-11), then the isobilateral tetrads were formed. In addition to the normal tetrads (Plate II-12), some abnormalities, linear tetrad (Plate III-11), T tetrad (Plate III-12), polyad and micronucleus cell, were found at telophase II (Table 6).

***Pollen and embryo sac fertility of new line 1162 and its hybrids*:** Pollen fertility of new line 1162 and its hybrids was generally higher than the parents or other autotetraploid lines (Table 7). Pollen fertility of IR36-4x was 45.79%, lower than other materials (65%). While pollen fertility of F_1 hybrid (IR36-4x × 1162) was improved to 76.45% (Table 7). Stained abortive pollens and small pollens were the main types of abortive pollens in parents and F_1, respectively.

The new line 1162 and its hybrids mostly produced normal embryo sacs and fertility of embryo sac was over 80% (Table 8). Normal embryo sac contains one egg cell, two synergids at the micropylar end, two polar nuclei above the egg apparatus and a group of antipodal cells at chalazal end.

Table 7. Pollen fertility of autotetraploid rice and hybrids.

Name	N	Normal/%	Stained abortive pollen (%)			Round abortive pollen (%)	Typical abortive pollen (%)
			Medium pollen	Large pollen	Small pollen		
1162	192	91.03±3.56	0	0	1.40±2.19	0.63±1.40	2.82±4.12
1162×Jingxian 89-4x	219	68.28±7.13	10.19±1.57	0	15.18±2.83	0	0.40±0.89
1162×4001-1-4x	338	83.39±3.96	0	0	0.82±0.77	0.31±0.70	0.31±0.70
1162×Yuhei 1-4x	268	80.81±4.19	3.77±0.91	0	2.97±1.69	0.60±1.19	1.78±1.30
1162×IR36-4x	387	76.30±6.25	2.15±2.55	0	3.67±0.82	1.67±0.54	4.68±2.74
96025	148	65.27±16.77	21.38±9.04	0.74±1.47	6.34±7.62	5.14±1.61	2.58±3.33
Jackson-4x	167	70.29±7.60	4.88±4.22	0.76±1.31	2.72±2.97	0.49±0.85	2.12±2.29
Jingxian 89-4x	154	70.61±6.34	17.89±5.11	1.09±1.26	0	0.57±1.14	3.98±3.46
Yuhei 1-4x	78	74.80±5.56	13.14±2.55	0	1.75±3.04	1.08±1.86	1.19±2.06
IR36-4x	204	45.79±11.23	31.11±6.39	3.20±1.95	6.05±3.49	4.00±0.95	1.12±0.97

N=No. of pollen observed.

Table 8. Fertility of embryo sacs in autotetraploid rice.

Name	N	Normal (%)	Polar nuclei at abnormal position (%)	Embryo sac Degeneration (%)	Female germ unit degeneration (%)	Synergid Degeneration (%)	'Double set' of embryo sac (%)	No egg apparatus (%)	No polar nuclei (%)	Small Embryo sac (%)
1162	11	90.91	9.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1162×Jingxian 89-4x	66	81.82	4.55	3.03	3.03	0.00	1.52	1.52	1.52	3.03
1162×4001-1-4x	64	90.63	0.00	6.25	0.00	0.00	0.00	1.56	0.00	1.56
1162×Yuhei1-4x	44	88.64	2.27	2.27	0.00	2.27	0.00	0.00	0.00	4.55
1162×IR36-4x	58	94.83	1.72	0.00	0.00	0.00	0.00	0.00	0.00	3.45

N=No. of embryo sacs observed.

Table 9. Fertility and seed setting in autotetraploid rice.

Name	SS (%)	Pollen fertility (%)	Name	SS (%)	Pollen fertility (%)	Embryo sac fertility/%
96025	38.10±16.61	55.72±9.67	1162	81.58±1.90	91.02±3.56	90.91
Jackson-4x	33.73±11.70	72.71±9.65	1162×Jingxian89-4x	47.03±5.28	68.28±.13	81.82
Jingxian89-4x	25.68±12.38	70.27±7.00	1162×T416	48.62±12.53	80.81±4.19	88.64
Yuhei1-4x	10.71±6.16	71.06±8.46	1162×Yuhei1-4x	91.88±0.68	83.3±3.96	90.63
IR36-4x	27.93±14.67	45.59±11.48	1162×IR36-4x	64.36±1.65	76.30±.25	94.83

SS= Seed setting.

Low percentage of abnormal embryo sacs found in all autotetraploid rice lines, which included embryo sac degeneration, embryo sac without female germ unit, egg apparatus degeneration, embryo sac without polar nuclei, embryo sac with polar nuclei at abnormal position and small embryo sac.

Pollen fertility and seed setting of autotetraploid rice hybrids were significantly higher than parents (Table 9). Seed setting of hybrids increased significantly as compared to other autotetraploid rice parents with low seed setting. The results suggested that the high fertility of 1162 is heritable.

***Analysis of main agronomic characteristics and heterosis of new line 1162*:** Data regarding performance of 1162 evaluated during late season 2010 and early season 2011, and results are presented in Table 10. The mean of panicle length, number of grains per panicle, filled grains per panicle, 1000-grain weight, grain length, grain width, grain length-width ratio were 25.04cm, 103.38 grains, 83.56 grains, 43.44g, 9.54mm, 4.04mm and 2.37, respectively. The plant height of 1162 was high (124.13cm), while the number of panicles per plant was less and only 4 panicles per plant were found. Seed setting was ranged from 70%~89.22%, and the average seed set (81.58%) of newly developed line was in accordance with the seed set of diploid rice. Variation coefficient of most traits was less than 10% except for the number of panicles per plant, number of grains per panicle and filled grains per panicle. It showed that all the individuals of new line have almost similar morphological characteristics.

The results of heterosis of 13 agronomic characters of 1162 crossed with other 42 autotetraploid lines are listed in Table 11. Obvious heterosis was observed in most of the quantitative characters of F_1 hybrids, especially the characters related to the yield. The heterosis in 10 quantitative characters was not only positive but also attained highly significant

Table 10. Performance of main agronomic traits of the new line 1162.

Agronomic traits	Max	Min	Variation amplitude	Mean±SD	CV(%)
PH/cm	135.00	110.00	25.00	124.13±2.04	5.19
NP	5.00	2.00	3.00	4.00±0.37	28.87
PL/cm	27.85	22.93	4.92	25.04±0.57	7.17
NGP	145.00	70.20	74.80	103.38±8.41	25.71
NFGP	106.70	59.00	47.70	83.56±5.91	22.35
SS (%)	89.22	70.00	19.22	81.58±1.90	7.38
TGW/g	45.71	41.03	4.68	43.44±0.51	3.70
GL/mm	9.70	8.90	0.80	9.54±0.07	2.48
GW/mm	4.30	3.60	0.70	4.04±0.07	5.86
L/W	2.64	2.06	0.58	2.37±0.05	7.27

CV=Coefficient of Variation; See Table 1 for traits abbreviations

Table 11. Mean heterosis and transgressive heterosis of main quantitative traits.

Traits	(H_{PM}) Mid-parent heterosis			(H_{PB}) Better-parent heterosis		
	Mean	H>0 Combination	H<0 Combination	Mean	H>0 Combination	H<0 Combination
PH	0.309**	41(37)	1(0)	0.244	41(19)	1(0)
NP	-0.026	20(6)	22(7)	-0.260*	16(1)	26(19)
PL	0.151**	36(23)	6(0)	0.111	33(17)	9(9)
NGP	0.473**	40(28)	2(0)	0.376	38(11)	4(4)
NFGP	0.834**	41(29)	1(0)	0.513	37(12)	5(5)
SS	0.429**	39(33)	3(0)	0.001	26(6)	16(13)
TGW	-0.032	18(10)	24(12)	-0.218**	0	42(34)
SWP	0.690**	40(22)	2(0)	0.119	21(2)	21(16)
GWP	0.797**	39(28)	3(0)	0.724	38(22)	4(4)
GWP/SWP	0.155**	27(12)	15(8)	-0.408**	7(0)	35(28)
GL	0.062**	35(24)	7(2)	-0.020**	21(8)	21(16)
GW	0.103**	40(24)	2(0)	0.038	31(2)	11(6)
GL/GW	-0.048**	10(5)	32(8)	-0.146**	4(0)	38(31)

PH=Plant Height, NP=Number of Panicles, PL=Panicle Length, NGP= Number of Grains per Plant, NFGP= Number of Filled Grains per Panicle, SS=Seed Setting, TGW=1000-Grains Weight, SWP=Straw Weight per Plant, GWP=Grain Weight per Plant, GWP/SWP= Ratio of GWP to SWP, GL=Grain Length, GW=Grain Width, GL/GW=Grain Length-Width Ratio

*, ** Significantly different from zero at p<0.05 and p<0.01, respectively. The values in parenthesis represent the number of combinations with significantly different from zero at p<0.05

level. The mid-parent heterosis of these 10 quantitative characters was in sequence of number of filled grains per panicle>grain weight per plant>straw weight per plant>number of grains per plant>seed setting>plant height>grain-straw ratio>panicle length>grain width>grain length. The mid-parent heterosis of other remaining three quantitative characters was negative. The population mid-parent heterosis for number of filled grains per panicle in 41 cross sets was significant, and 29 cross sets showed positive heterosis, while one cross set showed negative heterosis. A higher mid-parent heterosis value was also found for grain weight per plant, straw weight per plant, number of grains per plant, seed setting and plant height. In total, more than 38 cross sets showed positive heterosis, and 20 cross sets attained significant level. Heterosis for seed setting in 39 cross sets was positive, 33 cross sets showed significant positive heterosis, while three cross sets showed negative heterosis. The results showed that the mid-parent heterosis for most of the quantitative characters of 1162 was significant as compared to other autotetraploid lines.

Better-parent heterosis values of 8 quantitative characters, including plant height, panicle length, number of grains per panicle, number of filled grains per panicle, seed setting, straw weight, grains weight per plant and grain width, was positive, but didn't reach the remarkable level. Grain weight per plant showed the maximum better-parent heterosis value (0.724) and number of filled grains per plant ranked second (0.513). A total of 38 and 37 cross sets expressed positive heterosis for grain weight and number of filled grains per plant, respectively. Better-parent heterosis value of seed setting was 0.001, positive heterosis of 6 cross sets was significant. These results indicate that mid-parent heterosis and better-parent heterosis for most of the quantitative characters of the hybrids crossed with 1162 have the significant population mid-parent heterosis and better-parent heterosis, and have a wide heterosis, especially for grain weight per plant, filled grains per plant and seed setting. Most of the cross sets for these characters showed the positive heterosis. In addition, it is worthwhile to mention that heterosis for seed setting was significant among most of the cross sets.

DISCUSSION

***Low trivalent and univalent might be the main reason for high fertility in newly developed autotetraploid rice*:** Abnormal meiosis chromosome behavior, such as multivalents and univalents, happened frequently in autotetraploid plants, and was considered important reasons for low seed setting of the autotetraploids (Luan *et al*., 2007; Long *et al*., 2007; He *et al*., 2011a,b). Multivalents of chromosome included trivalents, quadrivalents, pentavalents, and so on, in autotetraploid plants. There are two viewpoints about the influence of quadrivalent on seed setting of autotetraploid plants. The first one pointed out that the more number of quadrivalents cause reduction in seed setting of autotetraploid plants (Sebastiampillai *et al*., 1977). Another study on chromosome configuration of autotetraploid rice and its hybrids, revealed that ring quadrivalents and number of quadrivalents had negative correlation with pollen fertility and seed setting, respectively (He *et al*., 2011b). He *et al.* (2011a) reported higher number of multivalent, trivalent and univalent in autotetraploid rice that lead to low seed in autotetraploid rice hybrids. The second viewpoint considered that the frequency of quadrivalents was not the crucial factor that influences the seed setting. The numbers of quadrivalents were approximately similar among the hybrids and their parents with different pollen fertility (Zhao *et al*., 2006). Luan *et al*. (2007) studied the cytology of the autotetraploid restorer lines and found that univalents, trivalents and multivalents result in low seed setting, while the quadrivalent had non-significant correlation with seed setting. They considered that the number of quadrivalents within a range may not exert great effect on pollen fertility or seed setting. Long *et al*. (2007) considered that the autotetraploid rice maintainer lines with more number of quadrivalents exhibited more stable inheritance than the autotetraploid rice restorer lines, and believed that the increase in quadrivalents results in good chromosome pairing of maintainer lines. High percentage of trivalents and univalent were generally resulted in low seed setting of autotetraploid plants (Cai *et al*., 2007; Luan *et al*., 2007; He *et al*., 2011a,b; Wu *et al*., 2014).

In our study, more number of quadrivalents were found in newly developed autotetraploid rice 1162 than that in its parents with low seed setting, while the frequency of trivalent and univalent was lower in the new line comparing with its parents at diakinesis and metaphase I. Moreover, the configuration of chromosome in 1162 was symmetrical, which may be in favor of 2/2 separation of homologous chromosomes. These results suggested that high quadrivalents and low trivalent and univalent improve the hereditary stability of the new line 1162 than its parents, and it might be the important factor for high fertility of new autotetraploid line.

***Newly developed autotetraploid line 1162 is a new germplasm that could be used for the breeding of autotetraploid rice*:** High fertility autotetraploid rice is a key germplasm for autotetraploid rice breeding. As a result, rice breeders kept on developing high fertility autotetraploid rice lines for more than 60 years. Till to nineties in twentieth century, some autotetraploid rice hybrids with high seed setting were developed. Two autotetraploid rice lines, PMeS-1 and PMeS-2, with more than 65% seed setting were produced (Cai *et al*., 2007). Luan *et al*. (2008) reported two autotetraploid rice restorers with high seed set. These studies give a hope to autotetraploid rice breeders for commercial production. Here, we developed a new autotetraploid rice line with high fertility that could be used to generate autotetraploid hybrids with high fertility.

***Conclusion*:** In present study, we reported a newly developed line (1162) that showed very high pollen and embryo sac fertility, and high seed setting (>80%). Further, newly developed line showed high hybrid vigor and better agronomic traits than parents. Substantial evidences proved that the new line had the potential to improve the seed set of autotetraploid lines and had great potential to increase the yield of autotetraploid rice because of its gigantic features, such as long and wide grains, long panicle and high seed setting. Therefore, further studies on the new line have significant implications for genetic improvement of autotetraploid rice. This research may promote autotetraploid rice breeding to a new level.

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