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Cytogenetics of Ditelosomic Alien Addition Lines in rice (*Oryza sativa* L.) Each Carrying an Extra Pair of Telocentric Chromosomes of *O. punctata* Kotschy

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Ditelosomic alien addition lines (DtAALs: 2n=2x+2t) of rice each carrying a pair of telocentric chromosomes of *Oryza punctata* were isolated at low frequencies (2.5–11.1%) from the progenies of respective monotelosomic alien addition lines (MtAALs: 2n=2x+1t) 7, 11 and one unidentified MtAAL. During the meiosis, the alien telocentric chromosomes of three DtAALs completely paired at the pachytene and usually separated to each daughter cell at anaphase I, giving rise to viable gametes with an alien telocentric chromosome at high frequencies. These DtAALs were characterized by stable transmission of the alien telocentric chromosome in the progenies. The transmission rates of the alien chromosome were considerably high in the DtAALs, and most plants of their self-pollinated progeny carried at least one alien telocentric chromosome. The pollen and seed fertility were different among three DtAALs. DtAAL 11 carrying a short telocentric chromosome of *O. punctata* showed high pollen and seed fertility similar to the disomics. The DtAALs showed relatively stable transmission of alien telocentric chromosome(s), where gametes with an extra telocentric chromosome are functional in both female and male germ cells. These suggest that small chromosome fragment with functional centromere, that is a telocentric chromosome, can be transmitted to the progenies and be stable in the next generation. High transmission rates of the alien telocentric chromosome in these DtAALs assure the efficient changes in genetic background of any recipient cultivars by backcrossing.

INTRODUCTION

Disomic alien addition lines (DAALs: 2n=2x+2) and ditelosomic alien addition lines (DtAALs: 2n=2x+2t) can be used to introduce alien traits because a pair of alien chromosomes normally form a bivalent at meiosis and at least a copy of that alien chromosome is transmitted to the whole progeny of the next generation. The meiotic chromosome behavior and the transmission of the alien chromosome in the offsprings of D(t)AALs have been observed in several species such as wheat (Riley and Chapman 1958) and beet (Lange *et al.*, 1993). These D(t)AALs in polyploid species could tolerate the condition harboring an extra pair of alien chromosomes in addition to the normal complement and produced viable progenies. In diploid species, several tetrasomics can often tolerate the presence of an extra pair of chromosomal fragments in addition to the diploid complement and produce viable progenies as in barley (Fedak and Helgason 1970, Tsuchiya 1969, Wiebe 1975) and rice (Yasui *et al.*, 1995).

In this study, rice plants each carrying an extra pair of alien telocentric chromosomes in addition to 24 chromosomes (DtAALs) were obtained at low frequencies in the self-pollinated progenies of three MtAALs and analyzed for the genetic basis by observing morphology, cytology, seed and pollen fertility and alien chromosome transmission.

MATERIALS AND METHODS

Plant materials

Three DtAALs (2n=2x+2t) of rice each carrying an extra pair of telocentric chromosomes of *Oryza punctata* Kotschy were isolated in the self-pollinated progenies of the three MtAALs X, 7 and 11. MtAAL 7 and MtAAL 11 had been isolated from the progenies of monosomic alien addition line (MAAL) 7 (Type I) and MAAL 11, respectively (Yasui and Iwata, 1998). MtAAL X had been isolated from the progenies of an unidentified MAAL (MAAL X) with green leafhopper resistance. In the self-pollinated progenies of the three MtAALs, the morphological features of the segregants were investigated at maturity. The plants showing deviated morphological features were isolated on the basis of morphological difference from those of disomics and parental MtAALs. These isolated DtAALs were studied to identify the extra pair of alien chromosomes and characterize their morphological and reproductive features.

Cytological analysis

Chromosome number of the aberrants were determined from root tip tissues. The plants discriminated by morphological deviation from disomics were used for mitotic chromosome analysis. The plants presumed as DtAALs were subsequently investigated at the diakinesis and metaphase I of PMCs by the acetocarmin squash methods. The telocentric chromosome was designated as small letter "t" in the meiotic chromosome configuration such as 12II+1tII or 12II+2tI.

Characterization of DtAALs

Pollen and seed fertility of the DtAALs were investigated at heading stage and after harvesting, respectively. The transmission rates of extra alien chromosomes were examined from the rates of segregating MtAALs and DtAALs characterized by their respective morphological features such as grain shape.

RESULTS

Isolation and identification

The three MtAALs were unstable and gave rise to the respective DtAALs at a range

Table 1. Frequency of DtAALs in the self-pollinated progenies of the three MtAALs each carrying an *O. punctata* teloentric chromosomes.

Parental MtAAL	The number of plants observed	The number of plants segregated in the selfed progenies			% of DtAAL
		Normal	MtAAL	DtAAL	
MtAAL X	126	72	49	5	4.0
MtAAL 7	120	88	29	3	2.5
MtAAL 11	9	5	3	1	11.1

from 2.5% to 11.1% (Table 1). The three DtAALs were putatively designated as DtAAL X, DtAAL 7 and DtAAL 11. The morphological features of these DtAALs deviated from those of disomics and MtAALs. The extra pair of telocentric chromosomes incorporated in the diploid complement were identified by karyotype analysis. DtAAL X carried an additional pair of telocentric chromosomes with a medium length, while DtAALs 7 (Fig. 1) and 11 each had an additional pair of telocentric chromosomes with a short length.

Fig. 1. Somatic metaphase chromosomes of a monotelosomic and a ditelosomic alien addition lines in rice carrying chromosome(s) of *O. punctata* Kotschy (Genome: 2n=2x=BB). (a) 2n=24+1 telocentric 7B chromosome. (b) 2n=24+2 telocentric 7B chromosomes. Arrows show telocentric 7B chromosome(s). A bar=5μm.

Meiotic chromosome behavior

The meiotic chromosome behavior were observed in DtAALs X, 7 and 11. The typical results are shown in Figures 2, 3 and 4. A telocentric bivalent with short length in addition to 12 bivalents with relatively long lengths were observed at the pachytene in all DtAALs (Figs. 2a, 3a and 4a, b). As shown in Table 2, more than 90% of the PMCs in the DtAALs formed 12II+1tII (Figs. 2b, c, 3b, d and 4c, d) at the diakinesis and metaphase I

Fig. 2. Meiosis in ditelosomic alien addition line X. (a) A pachytene stage showing 12II+1tII chromosome association; (b) An early diakinesis showing 12II+1tII chromosome association including one telosomic bivalent; (c) A metaphase stage showing 12II+1tII chromosome association; (d) A metaphase stage showing 12II+2tI chromosome association; (e) An anaphase stage showing 13:13 segregation. Arrows and arrowheads show telocentric *O. punctata* chromosome(s). A bar shows 5 μm.

Fig. 3. Meiosis in ditelosomic alien addition line 7. (a) A pachytene stage showing 12II−1tII chromosome association; (b) An early diakinesis showing 12II+1tII chromosome association including one telosomic bivalent; (c) An early diakinesis showing 12II+1tII chromosome association; (d) A metaphase stage showing 12II+1tII chromosome association; (e) A metaphase stage showing 12II+2tII chromosome association; Anaphase stages showing 13:13 segregation (f) and with lagging chromosomes(g). Arrows and arrowheads show telocentric 7B chromosome(s). A bar shows 5 μm.

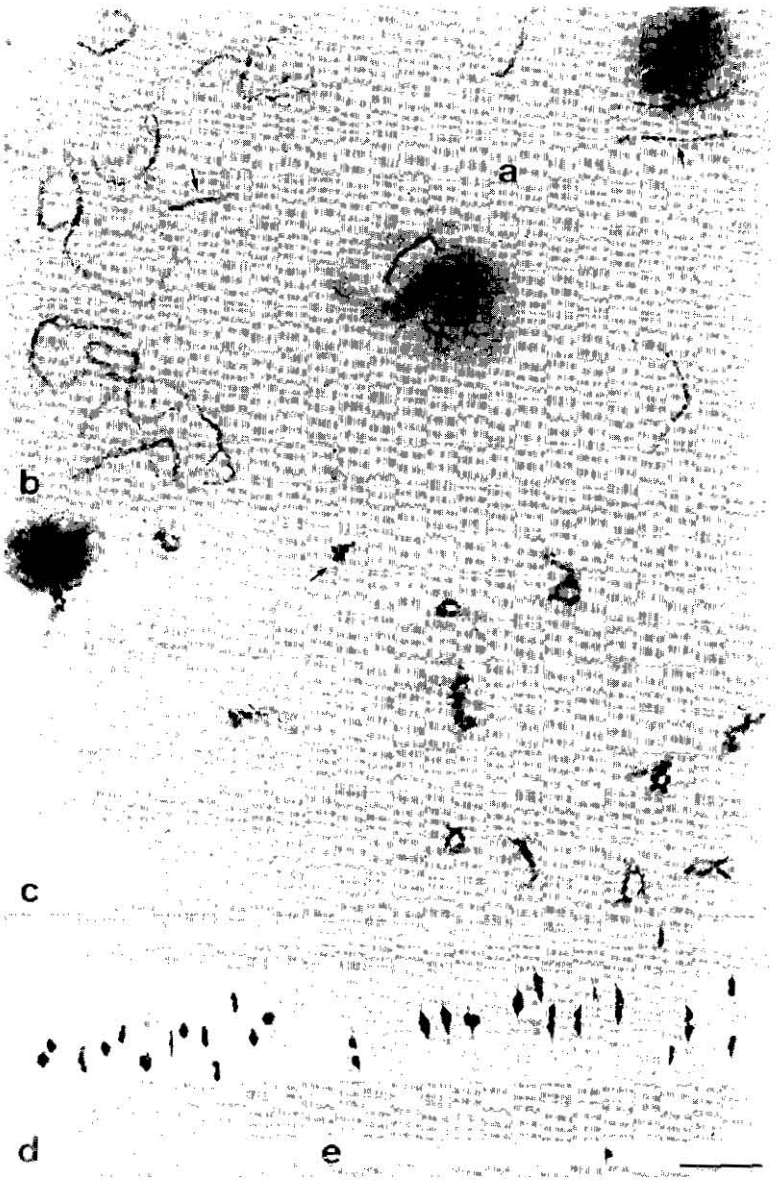

Fig. 4. Meiosis in ditelosomic alien addition line 11. (a, b) A pachytene stage showing 12II+1tII chromosome association; (c) A diakinesis showing 12II+1tII chromosome association including one telosomic bivalent; (d) A metaphase stage showing 12II+1tII chromosome association; (e) A metaphase stage showing precocious segregation. Arrows show telocentric 11B chromosome(s). A bar shows 5μm.

while the rest being 12II+2tI (Figs. 2d, 3c, e and 4e) showing frequently precocious separation. Most of the PMCs showed 13:13 segregation at anaphase I (Figs. 2e and 3f). Lagging chromosomes sometimes occurred at anaphase I (Fig. 2g).

Table 2. Meiotic chromosome behavior of three DtAALs each carrying a pair of telocentric chromosomes of *O. punctata* Kotschy.

Ditelosomic alien addition lone	Stage[a]	Number of PMCs				% of 12II +1tII
		12II +1tII	12II +2tI	Others[b]	Total	
DtAAL X	DK	82	1	0	83	98.8
	MI	101	9	2	112	90.2
DtAAL 7	DK	111	2	0	113	98.2
	MI	79	4	2	85	92.9
DtAAL 11	DK	35	0	0	35	100.0
	MI	61	1	2	64	95.3

a) Dk; diakinesis, MI; metaphase I.
b) Two PMCs of each ditelosomic alien addition line showed precocious separation at MI.

Morphological features and pollen and seed fertility

The diagnostic characteristics as well as pollen and seed fertility of the DtAALs X, 7 and 11 are shown in Table 3. The degrees of morphological deviation of the three DtAALs from disomic plants were more intensified than those of the parental MtAALs. DtAAL X showed more slender kernels than the parental MtAAL. DtAAL 7 was characterized by

Table 3. Comparison of morphological and reproductive features of MtAALs and DtAALs each carrying single or a pair of chromosome(s) of *O. punctata* Kotschy.

Line	Diagnostic morphological features	Pollen fertility (%)	Seed fertility (%)
MtAAL X	Semi-dwarf, semi-lax panicles, semi-rolled leaves, narrow grains with tiny awn	91.2	85.5
DtAAL X	Dwarf, lax panicles, rolled leaves, narrow grains with tiny awn	66.0	14.2
MtAAL 7	Semi-short panicles, semi-round grains, slightly brown pericarp	89.7	93.3
DtAAL 7	Short panicles, round grains, darkish brown pericarp	82.4	78.6
MtAAL 11	Pseudo-normal, slightly compact panicles, grains with pointed apiculi	>90.0*	95.6
DtAAL 11	Compact panicles, slender grains with pointed apiculi	>90.0*	91.9
Disomics	Normal	>90.0*	93.8

*Average of two sets of data taken at different years.

more round kernels and more brownish pericarp than the parental MtAAL due to gene(s) located on chromosome 7 of *O. punctata*. DtAAL 11, on the other hand, has slender kernels than the parental MtAAL. In addition, the panicles of DtAAL 11 were more incompletely exerted compared with the parental MtAAL. The pollen fertility of the three DtAALs were considerably high especially DtAAL 11 which was almost the same as the disomics. The seed fertility of DtAALs 7 and 11 can be classified high (78.6% and 91.1%, respectively), while that of DtAAL X as low (14.2%).

Alien chromosome transmission

Alien chromosome transmission of the three DtAALs were shown in Table 4. All of the three DtAALs were recovered in their self-pollinated progenies and more than 95% of the self-pollinated progenies each had at least one alien chromosome. DtAAL X showed complete transmission of the alien chromosome in the self-pollinated progeny.

Table 4. Chromosome stability in the self-pollinated progenies of DtAALs.

Parental DtAAL	Number of plants identified	Number of plants			% of MtAAL +DtAAL
		Disomics (2n=24)	MtAAL (2n=25)	DtAAL (2n=26)	
DtAAL X	28	0	3	25	100.0
DtAAL 7	41	2	11	28	95.1
DtAAL 11	29	1	2	26	96.6

DISCUSSION

Three DtAALs of rice each carrying an extra pair of telocentric chromosomes of *Oryza punctata* occurred at low frequencies (2.5–11.1%) from the respective MtAALs X, 7 and 11. The aberrant plant types with the expression of chromosome-specific effects must have originated through the fertilization of male and female gametes each carrying a telocentric chromosome of *O. punctata*. At meiosis, the alien telocentric chromosomes of the three DtAALs fully paired at pachytene and usually separated to each daughter cell at anaphase I, giving rise to the viable gametes with the alien telocentric chromosome at high frequencies. The transmission rates of the alien chromosome were considerably high in the DtAALs, while most plants in their self-pollinated progenies carrying at least one alien telocentric chromosome. Pollen and seed fertility were different among DtAALs. DtAAL 11, carrying a short telocentric chromosome of *O. punctata*, showed high pollen and seed fertility similar to the disomics.

The three DtAALs of rice isolated in the present study can be used in transferring alien traits from *O. punctata* to *O. sativa*. These DtAALs showed relatively stable transmission of alien telocentric chromosome(s), where gametes with an extra telocentric chromosome are functional both in female and male gametes. These facts suggest that small chromosome fragments with functional centromere, that is a telocentric

chromosome, can be transmitted to the progenies with certain stability. In addition, pollen and seed fertility were considerably restored in the DtAALs carrying small fragments such as telocentric chromosomes 7 and 11 of *O. punctata*. The following procedures to develop materials for breeding program are, therefore, proposed: (1) The screening of MtAALs derived from spontaneous misdivision of the additional alien univalents in MAALs, and (2) the selection of fertile DtAAL stocks for the respective 24 chromosome arms of *O. punctata*.

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