Theor Appl Genet (2000) 101:564–568

Z.K. Cheng · H.X. Yu · H.H. Yan · M.H. Gu
L.H. Zhu

B chromosome in a rice aneuploid variation

Received: 8 June 1999 / Accepted: 30 December 1999

Abstract An awned rice plant was discovered among the progeny of the triploid Zhongxian 3037. Cytological investigation showed that this awned plant was a variation with extra chromosomes. Based on the properties of the extra chromosomes during both meiosis and mitosis – e.g., short stature, darkly staining, instability in chromosome number, and lack of synapsis with A chromosomes – they could be considered to be B chromosomes in rice. The B chromosome(s) in both asexual and sexual progenies exhibited a unique segregation that is different from that of telotrisomics and other aneuploids. Moreover molecular marker analysis detected no dosage effects between the B-chromosome plant and the normal diploid of Zhongxian 3037, indicating that the B chromosomes might not be directly derived from any A-chromosome fragments in rice.

Key words Rice · B chromosome

Introduction

B chromosomes are one kind of numerical variation of chromosomes in higher living species. Unlike A chromosomes, they are actually dispensable to each organism and do not pair with A chromosomes (Jones and Rees 1982). It is generally thought that B chromosomes have no major genes with phenotypic effects on organism development.

Since the first discovery of B chromosomes in maize (Longley 1927), they have been found in more than 1,000 living species, including human, animals, plants, and other types of organisms (Beukeboom 1994). Among cereal crops, in addition to maize, only other two organisms, rye and sorghum, have been reported to have B chromosomes (Porter and Rayburn 1990; Wu 1980, 1984, 1992; Wilkes et al. 1995). So far, a large amount of cytogenetic and molecular knowledge has been accumulated on B chromosomes in rye and maize, but there has been no report of B chromosomes in rice.

Here we report the presence of B chromosomes in rice. These were observed in a variation of rice aneuploids derived from the progeny of the triploid of an *indica* rice variety, Zhongxian 3037 during the isolation of rice trisomics (Cheng et al. 1996).

Materials and methods

Plant materials

Test-tube plants of the triploid of Zhongxian 3037 were grown in the field and pollinated with pollen of the diploid of the same variety, by which a population showing variation in chromosome number was constructed. All variants morphologically different from the original diploid and trisomic sibs were selected for further cytological identification. In addition, a whole set of rice telotrisomics with the same genetic background as that of Zhongxian 3037 were also used to monitor the molecular marker dosage effects on different chromosome arms.

Chromosome preparation

The young panicles of the rice variants at meiosis stage were harvested and fixed in 3:1 Carnoy's solution supplemented with 0.5% $FeCl_3$. Squashes were prepared in an acetic-carmine solution according to Wu's procedure (1967).

Roots of the rice variants were harvested from the plants growing in the field, pretreated in 0.002 *M* 8-hydroxyquinoline at 20°C for 2 h to accumulate prometaphase cells, fixed in methanol-acetic acid (3:1), and stored at –20°C. Root tips were macerated in 2.5% cellulose at 37°C for 1.5 h. Squashes were made in the fixative on a glass slide and flame-dried. The chromosomes were then stained with 2% Giemsa solution.

Southern analysis for dosage effects of RFLP markers

Genomic DNAs of the diploid, the variants, and the telotrisomics were extracted from young leaves according to McCouch et al.

Communicated by K. Oono

Z.K. Cheng · H.H. Yan · L.H. Zhu (✉)
Institute of Genetics, Chinese Academy of Sciences,
Beijing 100101, P.R. China
e-mail: Lhzhu@ss10.igtp.ac.cn

Z.K. Cheng · H.X. Yu · M.H. Gu
Yangzhou University, Yangzhou 225009, P.R. China

(1988). DNA digestion, electrophoresis, Southern blotting, and hybridization were also carried out according to their procedures. The DNA in each lane was adjusted to equal amounts using a specific control. Briefly, DNA concentrations of the different aneuploids were determined and adjusted to equal amounts as follows. All DNAs were digested with *Dra*I. For each plant, serial volumes of digested DNA were run on a 0.8% agarose gel and stained with ethidium bromide. The DNA images were captured with a digital camera and analyzed with molecular analysis software. Subsequently, the nearly equal aliquots of DNAs of each sample could be loaded for electrophoresis on one agarose gel and then transferred to a Hybond-N+ membrane (Amersham) using an alkali transfer method. Probes were labeled with [^{32}P] by the random hexamer priming method and then hybridized to membranes overnight at 65°C. The membranes were washed sequentially in 2×, 1×, and 0.5×SSC plus 0.1% SDS for 20 min each at 65°C and then exposed at –70°C to intensifying screens for 3–7 days.

Restriction fragment length polymorphic (RFLP) markers with prefixes RG, RZ, and CDO were kindly provided by Dr. S.D. Tanksley and Dr. S.R. McCouch at Cornell University, USA, while clones with the prefixes G, L, and C were obtained from the MAFF DNA Bank at NIAR, Japan.

Results

Origin and cytological identification of the variant with extra chromosomes

An awned plant was discovered among the progeny of a rice triploid plant backcrossed to its parental diploid plants of Zhongxian 3037. In the selfing progeny of this awned variant two types of rice plants, with or without awns, were observed (Fig. 1a, b).

In order to know the chromosomal variation of the variant, we conducted a cytological investigation during both meiosis and mitosis. At pachytene stage in meiosis, most PMCs of the awned plant had an extra chromosome that was darkly stained, short in length, and drifting in the nucleus as a univalent (Fig. 1c). There were also a few PMCs with two extra chromosomes (Fig. 1d) that neither paired with each other nor with the A chromosomes. Among the 89 PMCs at diakinesis, 68 had one extra chromosome, with a synaptic configuration of 12II+I; the other 21 PMCs had no extra chromosomes, showing the synaptic configuration of 12II. At metaphase, the extra chromosomes could all be seen as univalents. Two types of abnormal PMCs were detected at metaphase I – one with the univalent outside the plate, the other with the univalent precociously separated into two chromosomes (Fig. 1e). These phenomena were also detected in the PMCs at anaphase I (Fig. 1f).

During mitosis most of the prometaphase cells from the variant plant had an extra chromosome. Among the 92 cells observed at the prometaphase stage there was an instability in chromosome number; 66 had one extra chromosome (Fig. 1g), 1 had five extra chromosomes (Fig. 1h), and the other 25 cells had no extra chromosomes. As the extra chromosomes were short in length – no more than half the length of the shortest chromosome in the rice chromosome complement – darkly stained, and telocentric, they could be easily distinguished from the A chromosomes in a prometaphase cell. Based on these properties we ascribed the extra chromosomes to be B-chromosome material in rice.

Transmission of the B chromosomes during somatic propagation and in the selfing generation

To investigate the transmission of the B chromosome during somatic propagation, the nodes with auxiliary buds of the plants with B chromosomes were excited, sterilized, and cultivated on an inducing medium to develop adventitious buds. After propagation of several generations, the test-tube plants were transplanted to the field, and their roots were harvested for further cytological identification. It was noted that, at the flowering stage, two types of tillers, awned and awnless, were generated by the individual plants. B chromosomes were detectable only in the awned tillers and never in the awnless ones. The number of the two types of tillers was almost equal.

The selfing progenies of the plants with B chromosomes, including a total of 172 awnless and 88 awned individuals, were also examined for their prometaphase chromosomes at flowering stage, the B chromosomes could only be detected in the awned plants.

This feature of extra chromosomes in both asexual and sexual progenies that is clearly distinguishable from those of telotrisomics and other aneuploids in rice is quite unusual. Therefore, we believe that the initial variant with extra chromosomes must have been a B-chromosome variation in rice.

Morphological characters of the B-chromosome plants

To detect the effects of the B chromosomes on plant development, we analyzed the characters of the B-chromosome plants and the diploids of Zhonyxian 3037. By comparing morphological data between the two types of plants (Table 1), we found slightly positive effects in the plants with B chromosomes, at a highly significant level, on characters such as plant height, length of panicle, length of grain, and weight of grain and slightly negative effects, at a highly significant level, on the number of tillers and width of grain. In general, the B chromosomes had a slightly positive effect on plant development.

Molecular marker analysis of the B chromosomes

If our assumption of the presence of B chromosomes in rice is valid, where did they come from in this particular aneuploid? To answer this question, we first supposed that the B chromosomes were derived from a piece of a chromosome arm.

To find any clues concerning the origin of the B chromosomes, we performed Southern blot hybridization on membranes containing equal amount of *Dra*I-digested DNAs from the B-chromosome plant and the diploid of

Fig. 1a–h Morphology and cytological identification of the variant. **a** Plants of the diploid (*left*) and the variant (*right*), **b** panicles of the diploid (*left*) and the variant (*right*), **c** pachytene chromosomes of the variant; *arrow* showing the extra chromosome, **d** pachytene chromosomes of the variant; *arrows* showing the two extra chromosomes, **e** chromosomes at metaphase I of the variant; *arrow* showing the extra chromosome, **f** chromosomes at anaphase I of the variant; *arrow* showing the extra chromosome, **g** prometaphase chromosomes of the variant; *arrow* showing the extra chromosome, **h** prometaphase chromosomes of the variant; *arrows* showing the five extra chromosomes

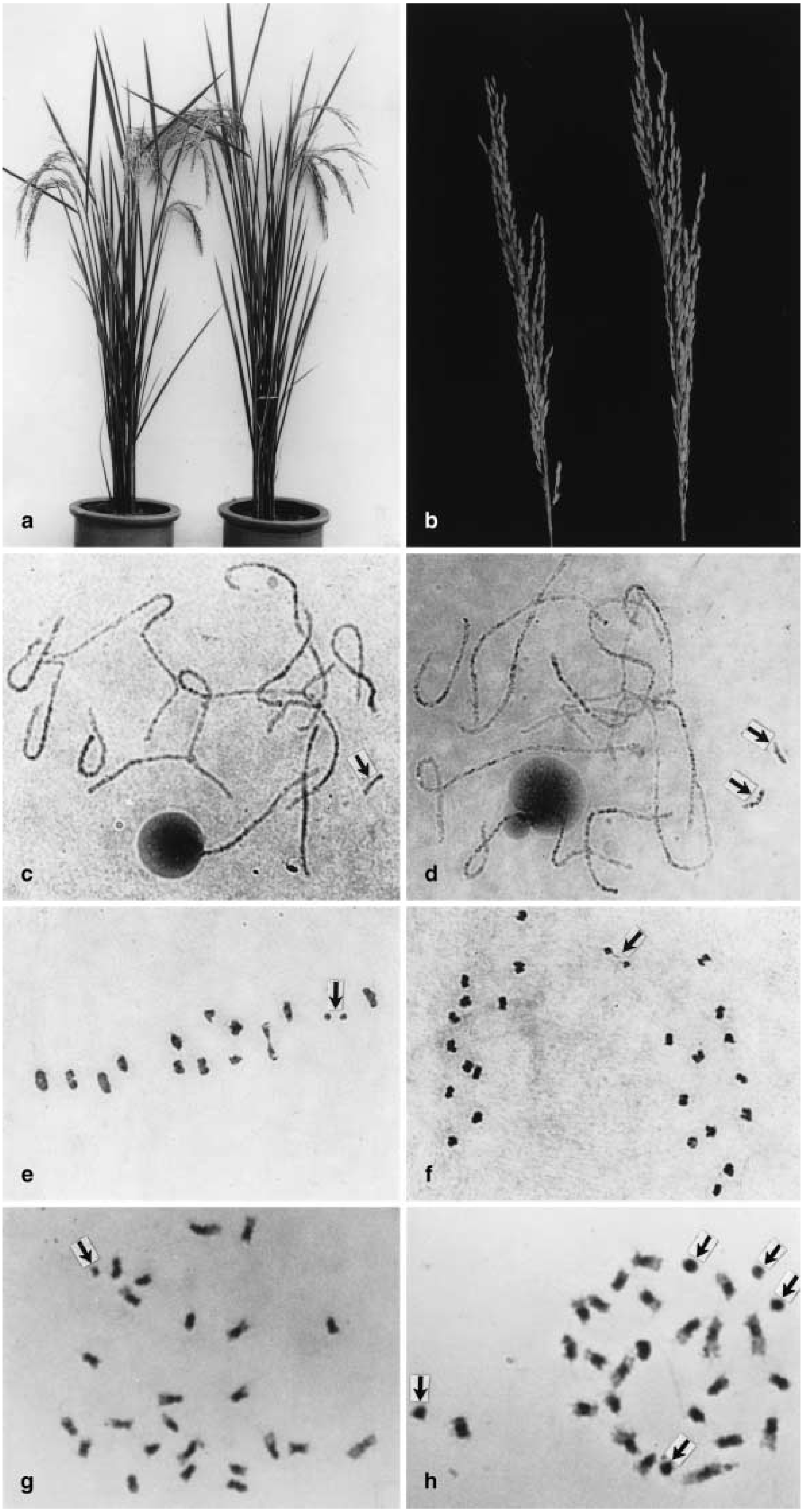

Table 1 Comparison of characters between B-chromosome plants and diploids of Zhongxian 3037

Factors	Diploid	B chromosome Plant	*t*-test
Plant height (cm)	103.5±2.40	108.6±3.10	**
No. of tillers	9.8±0.80	8.7±0.50	
Length of panicle (cm)	26.68±0.40	30.60±0.39	**
No. of spikelet	229.78±8.35	241.70±7.46	**
Length of grain (mm)	9.24±0.06	9.35±0.09	**
Width of grain (mm)	2.68±0.02	2.57±0.05	**
Weight of 1000 grains (g)	21.35±0.51	23.67±0.42	**
Seed setting (%)	83.89±3.16	84.30±1.77	

** 1% significant level; *n*=10

Table 2 Markers tested for dosage effects on different chromosome arms

Chromosome arms	Tested markers	Chromosome arms	Tested markers
1S	C749, C146, RG811	7S	RZ488, RG128, C1057
1L	RG350, RZ276, G370	7L	G20, C213, RG351
2S	G365, RG509, G357	8S	RG20, C400, G278
2L	RG322, G275, G45	8L	G1073, RG1, RG136
3S	RG409, RZ891, RG450	9S	RG757, G95, C711
3L	RG558, RG910, RZ328	9L	CDO590, G385, RG662
4S	CDO456, C708 , RG190	10S	RZ892, C701, L169
4L	RG449, G271, RG620	10L	G291, C16, RG561
5S	RG556, G396, RG360	11S	RG304, RG118, G320
5L	RG13, G81, C246	11L	L190, RG2, G257
6S	RZ450, RG213, RZ2	12S	RG574, RZ816, RZ397
6L	RG716, RZ405, CDO218	12L	RG241, RG190, RG181

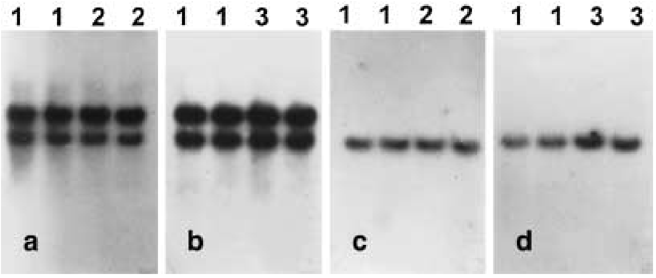

Fig. 2a–d Dosage analysis of different RFLP markers. *Lane 1* DNA from the diploid, *lane 2* DNA from the B-chromosome plant, *lane 3* DNA from 2n+•6S. **a, b** Probed with G275 in 2L, **c, d** probed with RG213 in 6S

Zhongxian 3037. As a control, *Dra*I-digested DNAs from the diploid and one of the telotrisomics were also blotted onto a membrane to monitor the dosage effects of the molecular markers on different chromosome arms. For example, when the membrane with DNAs from the diploid and the B-chromosome plant and the membrane with DNAs from the diploid and the telotrisomic 2n+•6S were both probed with RFLP marker G275 on the long arm of chromosome 2, the bands in all the lanes on the membranes were similar in intensity, indicating that the DNAs mounted on the different lanes were almost equal (Fig. 2a, b). When they were probed with RG213 on the short arm of chromosome 6, the bands for the diploid and for the B-chromosome plant showed a similar intensity (Fig. 2c), indicating equal DNA dosage of this marker in the two rice plants, whereas, as expected from the dosage effect, the bands for 2n+•6S were significantly stronger than those in the diploid (Fig. 2d). The other 2 markers on the arm of 6 S, i.e., RZ2 and RZ450, also gave similar hybridization patterns. It seems that the B chromosome may not derive directly from 6S. For further surveying the possible origin of the B chromosomes, we used the other 69 markers on the remaining 23 chromosome arms, 3 for each arm, as probes (Table 2), but no dosage effects could be detected (data not shown). As all the markers employed in the present study belong to single-copy sequences, we can not exclude the possibility that the B chromosomes are homologous to A chromosomes in some repetitive sequences and the most of the other single-copy sequences have not yet been probed. However, it seems impossible that the first B chromosome was directly derived from an A-chromosome fragment.

Discussion

B chromosomes, which are supernumerary to the A chromosome set, have been known to exist in about 15% of living species. As B chromosomes differ from A chromosomes in morphology, many efforts have been made to characterize B chromosomes in a variety of organisms. In general, B chromosomes have the following characteristics: (1) small in size and darkly stained; (2) variable in chromosome number among different individuals in the same population and even among different cells from a same individual; (3) not homologous to and therefore incapable of pairing with A chromosomes; (4) inherited in a non-Mendelian fashion; (5) little effects on organ development; (6) lack of genes with major effects and nucleolus organizers (Beukeboom 1994). The extra chromosomes we found in the aneuploid rice have most of the above-mentioned features of B chromosomes, and they are obviously different from any extra chromosome arms in telotrisomics and other aneuploids regarding their mitotic and meiotic behavior, transmission modes in both asexual and sexual progenies, and dosage effects of molecular markers. All these characters indicate that the extra chromosome is a true B chromosome in rice.

As our knowledge of B chromosomes increases several of their previously defined features such as their lack of homology with A chromosomes and their absence of nucleolus organizers, have become redundant. Consequently, the First B Chromosome Conference defined B chromosomes simply as ”a dispensable supernumerary chromosome that does not recombine with the A chromosomes and follows its own evolutionary pathway” (Beukeboom 1994). In the present study, suggestive gene functions of the B chromosomes in rice were detected. For example, the presence and absence of awn in rice is a complex trait controlled by one major gene, or by some mini-effect genes. The parental variety Zhongxian 3037 is an awnless *indica* rice variety, but its triploid and triplo-6 both have awns (Cheng et al. 1996). This phenomenon might be caused by the dosage effects of the awn-related mini-effect genes situated on chromosome 6. As all the B-chromosome plants investigated in the present study also have awns, we assume that there might be some genes on the B chromosome which are related to rice awn expression. In addition, some morphological differences were also found between the diploids and the B-chromosome plants, indicating that the B chromosomes in rice have other effects on plant development. In this respect the modern definition of B chromosomes is certainly more comprehensive than the earlier one.

Even though much has been learnt about the characteristics and behaviors of B chromosomes, their origin is still a matter of speculation. It has been assumed that B chromosomes originated from A complements in corresponding genomes. As the previously reported B chromosomes were all detected in natural varieties and existing populations, the researchers could not study where B chromosomes had actually come from. These kind of obstacles has limited the formulation of hypotheses regarding the origin of B chromosomes. In the present study, B chromosomes were discovered in an aneuploid that was derived from the triploid of Zhongxian 3037, but they were not observed in the triploid as well as Zhongxian 3037, the parent itself. Therefore, it is not unreasonable to assume that the B chromosome may have come from a variation in the progeny of the triploid. The variation might somehow bear resemblance to the occurrence of telocentric chromosomes, isochromosomes, or translated chromosomes in the aneuploids, which were also derived from the triploid, e.g., telotrisomics, secondary trisomics, or tertiary trisomics. However, unlike all these chromosomes, the B chromosome, due to its distinctive origin, can not pair with A chromosomes and misdivides in both mitosis and meiosis. Therefore, continued study of the DNA constitution of the B chromosomes presented in this paper will facilitate our understanding of the origin of B chromosomes.

References

Beukeboom LW (1994) Bewildering Bs: an impression of the 1st B-chromosome conference. Heredity 73:328–336

Cheng ZK, Yu HX, Li X, Gu MH (1996) A new set of primary trisomics in indica rice, its breeding and cytological investigation. Acta Genet Sin 23:263–272

Jones RN, Rees H (1982) B chromosomes. Academic Press, London

Longley AE (1927) Supernumerary chromosomes in *Zea mays*. J Agric Res 35:769–784

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Porter HL, Rayburn AL (1990) B-chromosome and C-band heterochromatin variation in Arizona maize populations adapted to different altitudes. Genome 33:659–662

Wilkes TM, Francki MG, Langridge P, Karp A, Jones RN, Forster JW (1995) Analysis of rye B-chromosome structure using fluorescence in situ hybridization (FISH). Chromosome Res 8:466–472

Wu HK (1967) Note on preparing of pachytene chromosomes by double mordant. Sci Agric 15:40–44

Wu TP (1980) Cytogenetic studis of the B chromosomes in *Sorghum nitidum*. Proc Natl Sci Coun ROC 4:297–306

Wu TP (1984) B chromosomes in *Sorghum purpureosericeum*. Proc Natl Sci Coun ROC 8:198–209

Wu TP (1992) B-chromosomes in Sorghum stipoideum. Heredity 68:457–463