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ORIGINAL ARTICLE

The *r-X1* deletion induces terminal deficiencies in the maize B chromosome

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Abstract In addition to causing the nondisjunction of maize B and normal A chromosomes at the second megaspore division during embryo sac development, the *r-X1* deletion results in terminal deficiencies (TDs) in various A chromosomal arms, but whether the *r-X1* deletion also induces TDs of the maize B chromosome remains unknown. To answer this question, the chromosomal composition in the *r-X1*-containing progeny of *r-X1/R-r* female parents carrying two standard B chromosomes was determined. Nine of 104 (8.7%) examined kernels contained a smaller telocentric B chromosome, and one of these (designated Bdef-1) was further identified as a TD with a breakpoint in the third distal heterochromatic region of the B chromosome. Thus, the results indicated that the *r-X1* deletion could also induce TDs of the maize B chromosome during megaspore divisions. The Bdef-1 chromosome lacked nondisjunctional behavior, and this behavior was restored by the presence of the B chromosome in the cell. A transmission analysis of the Bdef-1 chromosome revealed that loss of the distal portion of the B chromosome reduced female but not male transmission of the B chromosome. Furthermore, the Bdef-1 chromosome was used to more finely map B-derived miRNA genes on the B chromosome. Our results indicate that the *r-X1* deletion results in TDs of the B chromosome in maize, and the *r-X1* deletion system can thus be used to generate a series of terminally truncated B chromosomes that may be used to map features of the B chromosome, including genes and properties related to B chromosome functions.

Keywords *r-X1* deletion · B chromosome · Terminal deficiency · Meiotic loss · Mapping · Maize

Abbreviations

CK	Centromeric knob
DAPI	4',6-Diamidino-2-phenylindole
DE	Distal euchromatin
DH	Distal heterochromatin
FISH	Fluorescence in situ hybridization
RFLP	Restriction fragment length polymorphism
PE	Proximal euchromatin

TD Terminal deficiencyIntroduction

The *r-X1* deletion is a small intercalary deletion located within the long arm of maize chromosome 10 and was originally produced by L.J. Stadler via X-ray induction. This deletion, which is only transmitted

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through the female gamete (Weber 1983), induces nondisjunction of normal A chromosomes at the second megaspore division during embryo sac development (Lin and Coe 1986; Simcox et al. 1987) and generates monosomes and trisomes of various A chromosomes (Weber 1983). Monosomes generated with the *r-X1* deletion system have been employed in a variety of applications, including the study of gene dosage effects, the analysis of univalent chromosomal behavior, the characterization of monosomic syndromes, and the mapping of genes to chromosomes (Weber 1983). In addition to the production of aneuploid gametes, the *r-X1* deletion can induce breaks in A chromosomes to generate terminal deficiencies (TDs) in maternal plants (Lin 1987; Lin et al. 1990), and these TDs have been used to physically map restriction fragment length polymorphism (RFLP) markers on specific chromosomal arms (Lin et al. 1997).

Our previous study showed that the *r-X1* deletion can also induce nondisjunction of the maize B chromosome during the second megaspore division and the first microspore division, but whether the *r-X1* deletion causes TDs of the B chromosome was not analyzed (Tseng et al. 2018). The maize B chromosome is an additional nonessential chromosome that has no phenotypic effect on plants (Jones et al. 2008) and is mitotically telocentric (Randolph 1941). However, the occurrence of spontaneous B chromosomal variations is rare, and few cases have been reported to date (Randolph 1941; Cheng et al. 2016). At least three mechanisms have evolved for ensuring B chromosome survival: nondisjunction during the second microspore division (Longley 1927; Roman 1947), preferential fertilization of the egg by sperm containing B chromosomes (Roman 1948), and prevention of univalent meiotic loss (Carlson 1986). The nondisjunction mechanism requires *trans*-acting elements located in the proximal and distal euchromatic regions of the B chromosomal long arm (Ward 1973; Lin 1978), and at least one proximal region and one distal region of the B chromosome suppress the meiotic loss of the univalent B chromosome (Carlson and Roseman 1992). The maize B chromosome has been widely used for the deletion mapping of B- or A-chromosome-located genes and molecular markers via experiments involving B-A translocations (Roman and Ullstrup 1951; Beckett 1978; Alfenito and Birchler 1993; Cheng and Lin 2003; Peng et al. 2005; Lamb et al. 2007; Peng and Cheng 2011; Chien et al. 2014; Lin et al. 2014; Kao et al. 2015; Hong et al. 2020; Huang et al. 2020).

The purpose of the current study was to determine whether the *r-X1* deletion also induces TDs of the maize B chromosome during megaspore divisions. We analyzed the chromosomal composition of the *r-X1*-containing progeny of maternal *r-X1* plants carrying two standard B chromosomes. The results indicate that the *r-X1* deletion also induces breaks in the B chromosome during megaspore divisions. Subsequently, we cytologically characterized one B chromosomal TD and found that the studied TD resulted from a break in the third distal heterochromatic region of the B chromosome. The truncated chromosome lost its nondisjunction property, and a transmission analysis suggests that loss of the distal portion of the B chromosome reduces female but not male transmission of the B chromosome. Additionally, the physical locations of three B-derived miRNA genes were determined relative to the truncation breakpoint.

Materials and methods

Plant materials

r-X1/R-r, *r/r*, and *R-r/R-r* stocks of inbred W22 maize carrying the typical B chromosomes have been propagated in our laboratory for decades (Tseng et al. 2018). From the *r-X1/R-r* (W22) x *R-r/R-r* (W22) + 2B cross, three *r-X1* plants with two standard B chromosomes (*r-X1/R-r* + 2B) were identified by chromosomal observation and used as female plants for crossing with *r/r* (W22) plants to identify B chromosomal deficiencies. The chromosomal composition of the resulting F1 sibling *r-X1/r* (colorless) kernels was analyzed, and plants carrying B chromosomal deficiencies were crossed as male parents with *r/r* (W22) females for propagation.

Cytological procedures

The chromosomal composition in Feulgen-stained root tip cells was determined as described by Lin (1977). Chromosomes at the pachytene stage were prepared from pollen mother cells following the standard protocol (Cheng and Lin 2003). Fluorescence in situ hybridization (FISH) was performed

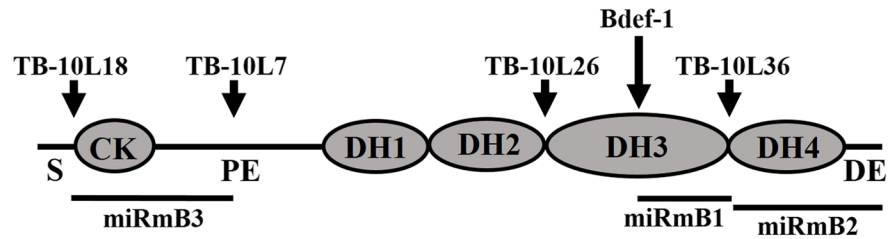

Fig. 1 Map positions of three B-derived miRNA genes (*black lines*) in relation to breakpoints (*arrows*) of four B-10L translocations and the Bdef-1 chromosome. *S* short arm, *CK* centromeric knob, *PE* proximal euchromatin, *DH1-DH4* distal heterochromatin 1–4, *DE* distal euchromatin

according to Cheng (2010). A total of six B chromosome sequences, including the B-repeat (Alfenito and Birchler 1993; Cheng 2010), Stark repeat (Stark et al. 1996; Lamb et al. 2007), CL-repeat (Cheng and Lin 2004), CentC repeat (Ananiev et al. 1998; Peng and Cheng 2011), telomere repeat (Wang and Chen 2003), and 180-bp knob repeat (Peacock et al. 1981; Cheng and Lin 2003), were used as FISH probes. The resulting fluorescence signals were captured using a cooled charge-coupled device camera (DP72, Olympus Corp.) on an Olympus BX51 fluorescence microscope and were processed using Photoshop software (Adobe, San Jose, CA, USA).

B-derived miRNA analysis

Primer pairs targeting three B-derived miRNAs, namely, miRmB1, miRmB2 and miRmB3, were used in the stem-loop RT-PCR procedure as previously described by Huang et al. (2020). The mapping of the positions of the three B-derived miRNA genes on the B chromosome using four B-10L translocations (TB-10L7, TB-10L18, TB-10L26, and TB-10L36) was reported by Huang et al. (2020) and is summarized in Fig. 1 along with the position of the deletion breakpoint of the B chromosomal deficiency determined in this study.

Results

The *r-X1* deletion induces deficiencies in the maize B chromosome

To determine whether the *r-X1* deletion could induce deficiencies in the maize B chromosome, *r-X1*/*R-r* + 2B (W22) plants, which served as the female parents, were crossed with *r/r* (W22) males, and the chromosomal constitution of F_1 sibling *r-X1*/*r* (colorless) kernels from three ears was analyzed (Table 1). Six (5.8%) of 104 analyzed kernels were aneuploid; among these six kernels, five were monosomes, and the remaining kernel was a trisome. In addition, 72.1% (75/104) of the *r-X1*/*r* kernels carried 1B, and 15.4% (16/104) and 3.8% (4/104) carried 0B and 2B, respectively. This result was consistent with previously reported findings that the *r-X1* deletion can induce B chromosome nondisjunction during megaspore divisions (Tseng et al. 2018). Moreover, nine (8.7%) kernels contained 20 A chromosomes and one

Table 1 Cytological classification of colorless kernels produced by the *r-X1*/*R-r* + 2B (W22) x *r/r* (W22) crosses

		Chromosomal composition						
Ear no	Total no	20 + 1B	20 + 0B	20 + 2B	19 + 1B	19 + 0B	21	20 + 1Bdef[b]
1	31	22 (70.1) [a]	4 (12.9)	2 (6.5)	0	1 (3.2)	0	2 (6.5)
2	40	27 (67.5)	6 (15.0)	1 (2.5)	1 (2.5)	1 (2.5)	1 (2.5)	3 (7.5)
3	33	23 (69.7)	3 (9.1)	1 (3.0)	2 (6.1)	0	0	4 (12.1)
Total	104	72 (69.2)	13 (12.5)	4 (3.9)	3 (2.9)	2 (1.9)	1 (1.0)	9 (8.7)

[a] The numbers in parentheses are percentages

[b] Bdef B chromosomal deficiency with a size smaller than that of the standard B chromosome

smaller telocentric chromosome. These additional chromosomes were clearly smaller than the standard B chromosome, and their sizes were visually different from each other (Fig. 2), which indicated the presence of TDs in the B chromosome induced by the *r-X1* deletion.

Cytological analysis of B chromosomal deficiency

Among the nine possible B chromosomal deficiencies, one (designated Bdef-1) was successfully propagated and used for further analysis. At mitotic metaphase, the morphology of the Bdef-1 chromosome was similar to that of the B chromosome but had a smaller size (Fig. 3a). FISH signals were observed on the Bdef-1 chromosome using probes corresponding to the Stark repeat sequence specific to the third distal heterochromatic region of the B chromosome (Lamb et al. 2007) and the B chromosome centromere-specific sequence B-repeat (Alfenito and Birchler 1993), which confirmed that the Bdef-1 chromosome was derived from the B chromosome (Fig. 3b). Although a strong signal from the B-repeat probe was observed on the Bdef-1 and intact B chromosomes, a markedly weaker signal from the Stark repeat probe was obtained with the Bdef-1 chromosome than with an intact B chromosome, and this result is consistent with a truncation breakpoint in the long arm of the B chromosome in the region enriched for the Stark repeat.

To determine the detailed structure of the Bdef-1 chromosome, bivalent chromosomes were observed at the pachytene stage. The pachytene B chromosome consists of a short arm, a centromeric knob (CK), a proximal euchromatin (PE), four distal heterochromatins (DH1-DH4), and a distal euchromatin (DE) (Fig. 3c). The pachytene Bdef-1 chromosome was similar to the B chromosome but lost DE, DH4, and the distal half of DH3 (Fig. 3d). A heteromorphic pair of the B and Bdef-1 chromosomes was very clear at the pachytene stage; at this stage, the homologous pairing of the short arm, CK, PE, DH1, DH2 and a portion of DH3 (DH3') from the Bdef-1 chromosome was complete, and the hemizygous portion contained DH4, DE, and the distal half of DH3 (Fig. 3e).

To further investigate the organization of the Bdef-1 chromosome, five repetitive elements in maize that have been mapped to various regions of the B chromosome, including the B-repeat, 180-kb knob repeat, telomere repeat, CL-repeat, CentC repeat, and Stark repeat, were used as FISH probes for the hybridization of pachytene bivalents of the B or Bdef-1 chromosome (Fig. 4). B-repeat signals occupied the distal half of CK on the B and Bdef-1 chromosomes, and telomere signals were observed at both ends of the two chromosomes (Fig. 4a, b). Signals of the 180-bp knob repeat were detected in the proximal half of CK on the B and Bdef-1 chromosomes (Fig. 4c, d), and the CL-repeat probe hybridized to the first three heterochromatic regions of both chromosomes (Fig. 4d, f). CentC signals were observed in multiple regions along the length of the B chromosome, as described by Lamb et al. (2005), and these regions included a CK region that colocalized with a region of intense B-repeat signaling and the four heterochromatic regions (Fig. 4g). The distribution of CentC signals on the Bdef-1 chromosome was identical to that on the B chromosome with the exception that the signal at DH4 was lost on the Bdef-1 chromosome (Fig. 4h). The Stark repeat was detected specifically at DH3 of the B chromosome and DH3' of the Bdef-1 chromosome (Fig. 4i, j), but the Stark repeat signals at DH3' were markedly weaker.

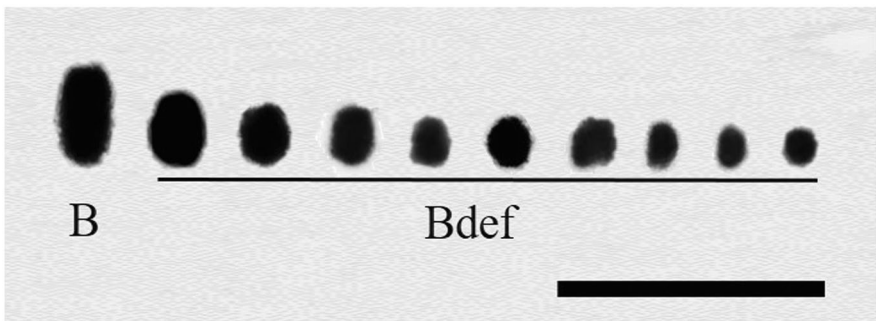

Fig. 2 Mitotic metaphase B chromosome and B chromosomal deficiencies. All B chromosomal deficiencies are telocentric and smaller than the standard B chromosome. *B* B chromosome, *Bdef* B chromosomal deficiency, *Scale bar* 10 μm

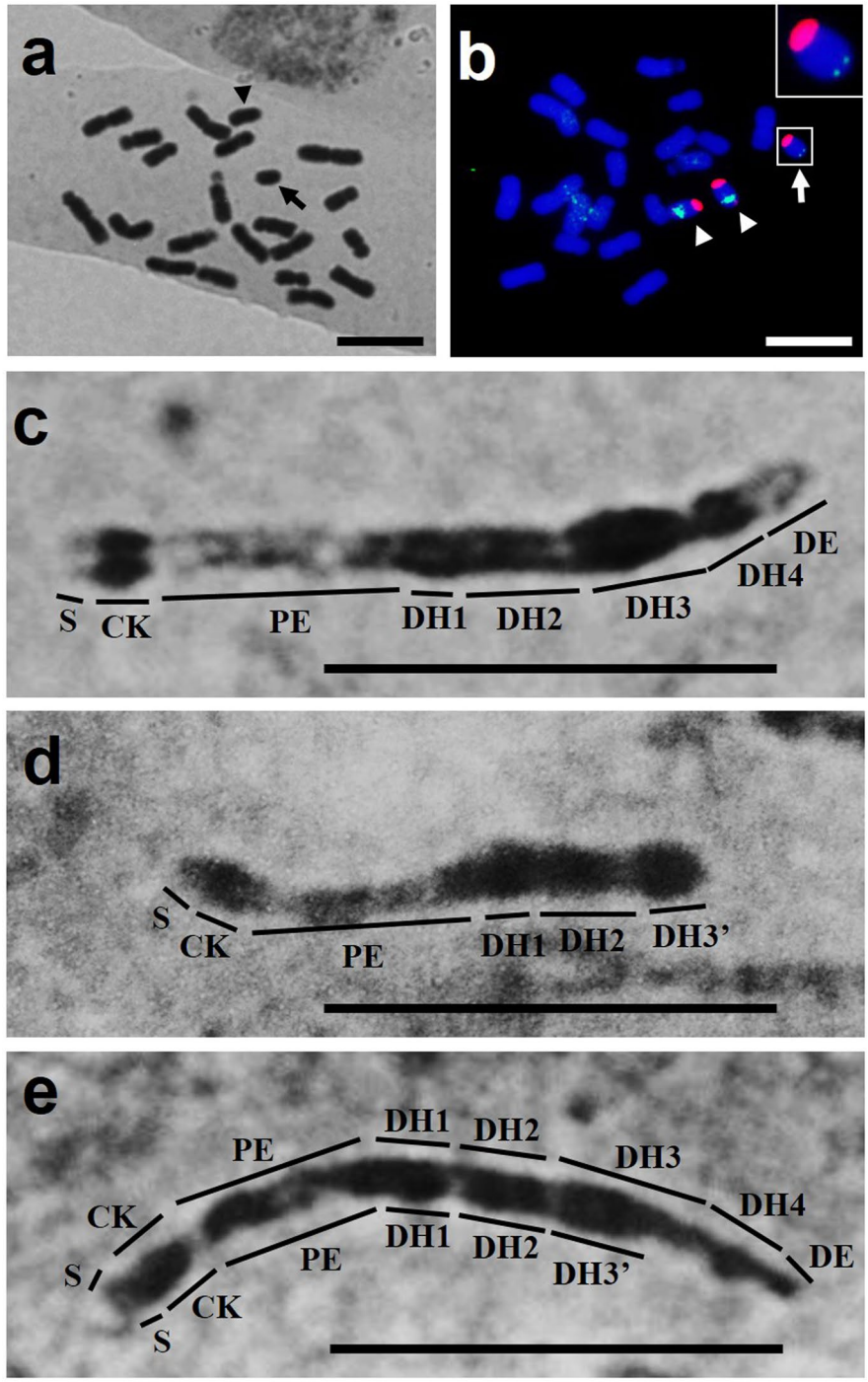

Fig. 3 Chromosomal structure of the B and Bdef-1 chromosomes. **a**, **b** A mitotic metaphase cell containing the typical B (*arrowhead*) and Bdef-1 (*arrow*) chromosomes. Maize B-specific B-repeat (*red*) and Stark repeat (*green*) probes were used to detect the B and Bdef-1 chromosomes. The chromosomes were stained with DAPI (*blue*). **c** The pachytene B chromosome consists of a short arm (*S*), a centromeric knob (*CK*), proximal euchromatin (*PE*), four distal heterochromatins (*DH1-DH4*), and distal euchromatin (*DE*). **d** The pachytene Bdef-1 chromosome is similar to the B chromosome but does not have DE, DH4, and a portion of DH3. **e** Heteromorphic pairing of the B and Bdef-1 chromosomes at the pachytene stage. *DH3'* indicates the residue of DH3 on the Bdef-1 chromosome. All the *scale bars* are equal to 10 μm

Transmission of the Bdef-1 chromosome

The transmission frequencies from the male and female parents recorded in W22 plants carrying a univalent B or Bdef-1 chromosome were 43.1% and 29.4% for the B chromosome, respectively, and 47.5% and 12.4% for the Bdef-1 chromosome, respectively (Table 2). The transmission frequencies of the univalent Bdef-1 and B chromosomes from male parents were similar, but the transmission

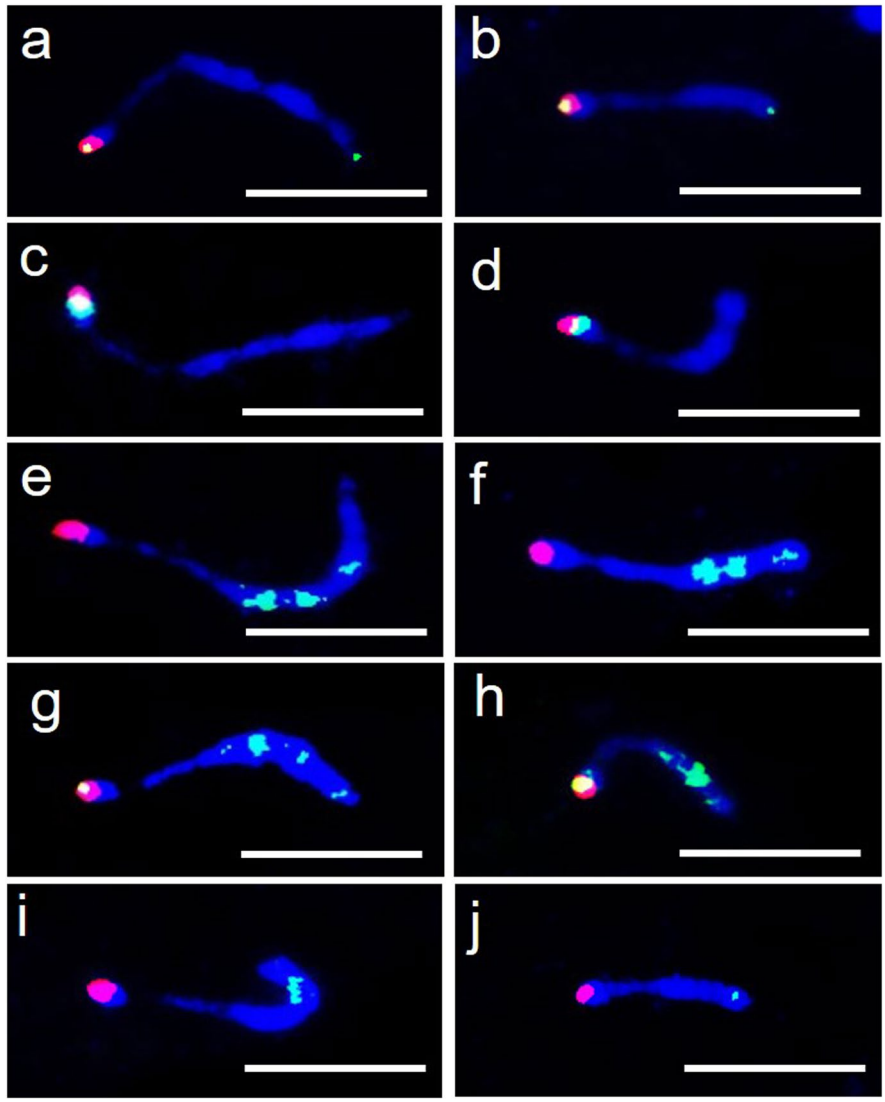

Fig. 4 FISH analysis of the pachytene Bdef-1 chromosome. Pachytene bivalents of the B (**a**, **c**, **e**, **g**, **i**) and Bdef-1 (**b**, **d**, **f**, **h**, **j**) chromosomes were hybridized with the B-repeat probe (*red*) and various maize repetitive elements (*green*), including a telomere repeat (**a**, **b**), 180-bp knob repeat (**c**, **d**), CL-repeat (**e**, **f**), CentC repeat (**g**, **h**), and Stark repeat (**i**, **j**). The chromosomes were stained with DAPI (*blue*). All the *scale bars* are equal to 10 μm

frequency of the Bdef-1 chromosome from the female parent was significantly lower than that of the B chromosome. Nondisjunction of the B chromosome was observed from the male parent, but the Bdef-1 chromosome lacked the ability to undergo nondisjunction. However, the coexistence of the Bdef-1 chromosome with the normal B chromosome restored its nondisjunction ability (Table 3). This result was reasonable because the Bdef-1 chromosome lacks the DE region of the B chromosome, which is essential for nondisjunction (Ward 1973).

Physical mapping of three B-derived miRNA genes on the B chromosome using the Bdef-1 chromosome

Primer pairs that amplify three B-derived miRNAs (miRmB1, miRmB2, and miRmB3) whose genes have been mapped to definitive B chromosomal regions were used for stem-loop RT-PCR analysis of the total RNAs of W22 + 0B, W22 + 1B, and W22 + 1Bdef-1. As shown in Fig. 1, the miRmB3 gene was located in a region that includes CK and the proximal half of PE, and the miRmB1 gene was located in DH3. The miRmB2 gene was mapped

Table 2 Transmission of univalent B and Bdef-1 chromosomes

Female parent	Male parent	Ear no	B or Bdef-1 frequency in progeny			Total (γ)	Transmission frequency (%)[a]
			0	1 (α)	2 (β)		
W22	W22 + 1B	1	31	1	8	40	42.5
		2	30	0	7	37	37.8
		3	35	0	11	46	47.8
		Total	96	1	26	123	43.1
W22 + 1B	W22	1	34	15	0	49	30.6
		2	26	14	0	40	35.0
		3	29	8	0	37	21.6
		Total	89	37	0	126	29.4
W22	W22 + 1Bdef-1	1	41	41	0	82	50.0
		2	19	20	0	39	51.3
		3	24	15	0	39	38.5
		Total	84	76	0	160	47.5
W22 + 1Bdef-1	W22	1	73	11	0	84	13.1
		2	36	4	0	40	10.0
		3	32	5	0	37	13.5
		Total	141	20	0	161	12.4*

[a] Transmission frequency = $(\alpha + 2\beta)/\gamma$ X 100 (%)

*The transmission frequency of the Bdef-1 chromosome from the female parent was significantly different from that of the B chromosome based on Student's *t* test ($p < 0.01$)

to a region including DH4 and DE of the B chromosome (Huang et al. 2020). The B-specific PCR products of miRmB1 and miRmB2 were present in W22 + 1B RNA but absent in W22 + 1Bdef-1 RNA (Fig. 5), which suggests that the positions of both miRNA genes are distal to the breakpoint of the Bdef-1 chromosome. The mapped position of the miRmB3 gene was proximal to the breakpoint of the Bdef-1 chromosome because it could be amplified from both W22 + 1B and W22 + 1Bdef-1 RNAs (Fig. 5). According to the results, the position of the miRmB1 gene can be narrowed down to the distal half of DH3 between the breakpoints of TB-10L36 and the Bdef-1 chromosome. The mapped positions of the three B-derived miRNA genes in relation to the breakpoints of the four B-10L translocations and the Bdef-1 chromosome are summarized in Fig. 1.

Discussion

Previously reported data show that the *r-X1* deletion in maize induces the nondisjunction of chromosomes A and B during the second megaspore division (Lin and Coe 1986; Tseng et al. 2018) and causes breaks to generate TDs on various A chromosomal arms (Lin 1987; Lin et al. 1997). The frequency of TDs varies among different A chromosomes and ranges from 0% to approximately 1.2% (Lin et al. 1990). However, whether the *r-X1* deletion also induces TDs on the maize B chromosome is unclear. If this is true, B chromosome deletions would be observed in *r-X1*-containing kernels generated from *r-X1/R-r* + 2B female parents, and the deleted region would be sufficiently large to be distinguished by light microscopy. Among the examined *r-X1*-containing kernels, 8.7% contained various B chromosomal deficiencies with sizes smaller than that of the standard B chromosome

Table 3 Nondisjunction of the Bdef-1 chromosome in the presence of the B chromosome

Female parent	Male parent	B and Bdef-1 frequency in progeny						Total
		0	2B	2B + 2Bdef-1	4B	1Bdef-1	2Bdef-1	
W22	W22 + 2B + 1Bdef-1	16 (41%)	12 (30.8%)	3 (7.7%)	2 (5.1%)	1 (2.6%)	5 (12.8%)	39

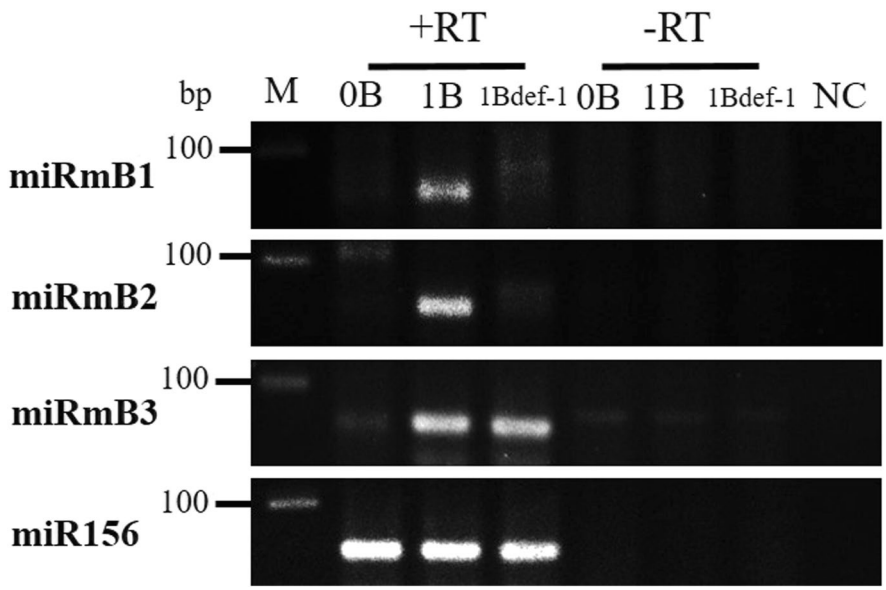

Fig. 5 Mapping of B-derived miRNA genes with the Bdef-1 chromosome. Stem-loop RT-PCR primers of three B-derived miRNAs, namely, miRmB1, miRmB2, and miRmB3, were used to analyze total RNAs from W22 + 0B, W22 + 1B, and W22 + 1Bdef-1. *miR156* was used to confirm equal amounts of RNA. *M* 100-bp marker, *NC* negative control (water)

at mitotic metaphase (Table 1, Fig. 2). The frequency of TDs in the B chromosome substantially exceeds that of A chromosomes, and this finding is rational because the B chromosome is nonessential and exerts no phenotypic effect on plants (Jones et al. 2008). Thus, the *r-X1* deletion can induce breaks in the B chromosome during megaspore divisions.

One of these B chromosomal deficiencies, Bdef-1, was further proven to be a TD with a breakpoint in the middle of DH3 (Fig. 3d, e). A FISH analysis of various B chromosome repetitive elements showed an identical signal distribution between the pachytene B and Bdef-1 chromosomes (Fig. 4c–j), which indicated that no obvious chromosomal rearrangements occurred during the formation of the Bdef-1 chromosome. Moreover, FISH signals of telomere repeats were present at both ends of the Bdef-1 chromosome (Fig. 4b), which suggested that de novo telomere formation occurred at the breakpoint of the Bdef-1 chromosome, as described by Santos-Serejo and Aguiar-Perecin (2016). The Bdef-1 chromosome lost its nondisjunction ability due to the absence of DE, which contains a *trans*-acting element essential for B chromosome nondisjunction (Ward 1973). However, the presence of a standard B chromosome restored the nondisjunction of the Bdef-1 chromosome (Table 3), which was similar to the behavior of mini B chromosomes generated by other means (Han et al. 2007; Cheng et al. 2016) because DE was again present in the cell.

The meiotic loss of the univalent B-9 chromosome of TB-9Sb in females has been reported previously (Carlson 1986), and the results show that at least one proximal region and one distal region (although without DE) on the B chromosome suppress meiotic loss (Carlson and Roseman 1992). In our study, the transmission frequency of the univalent B chromosome from the female parent was only 29.4%, which indicated a high rate of meiotic loss (Table 2). However, the transmission frequency (12.4%) of the univalent Bdef-1 chromosome from the female parent was significantly lower than that of the univalent B chromosome (Table 2). This result indicated that DH4 and the distal half of DH3 may be needed for the suppression of meiotic loss. In contrast, the transmission frequencies of both the univalent Bdef-1 and B chromosomes from the male parents were close to the theoretical 50% frequency of a univalent chromosome (Table 2). This result indicated that the meiotic loss of a univalent chromosome might not occur in the univalent B chromosome from the male parent or that the B chromosomal regions proximal to the Bdef-1 breakpoint strongly suppress meiotic loss through the male parent.

The breakpoints of A chromosomal TDs induced by the *r-X1* deletion have been determined to be random (Lin et al. 1990) and have been used for the physical mapping of RFLP markers on four A chromosomal arms (Lin et al. 1997). However, the utilization of A chromosomal TDs for long-term physical mapping in maize is not possible because these TDs

cannot be propagated. Due to the lack of phenotypic effects of the B chromosome on maize plants (Jones et al. 2008), it is possible to produce and propagate a large collection of B chromosomal TDs mediated by the *r-X1* deletion, which may offer a new system for assigning B-specific molecular markers or genes to particular B chromosomal regions by deletion mapping. Using the Bdef-1 chromosome identified in this study, we further physically mapped the B-derived miRmB1 gene to the distal half of DH3 (Fig. 1), but whether the *r-X1* deletion acts randomly on the B chromosome needs to be further determined.

In this study, we confirmed that *r-X1* deletion could induce TDs in the maize B chromosome of maternal plants. One B chromosomal TD with a breakpoint in the middle of DH3 of the B chromosome was identified, and this TD causes loss of nondisjunction ability at the second pollen mitosis and reduces the female but not male transmission of the B chromosome. Moreover, the TD was used to map B-derived miRNA genes. Accordingly, the *r-X1* deletion system will be a valuable resource for generating B chromosomal TDs with various breakpoints along the entire B chromosome.

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