New Phytologist

Unravelling the unusual: chromosome elimination, nondisjunction and extra pollen mitosis characterize the B chromosome in wild sorghum

Miroslava Karafiátová[1], Tereza Bojdová[1,2], Magdaléna Stejskalová[1], Natália Harnádková[1], Vinod Kumar[3], Andreas Houben[4], Jianyong Chen[4], Alžběta Doležalová[1], David Honys[3] and Jan Bartoš[1]

[1]Institute of Experimental Botany of the Czech Academy of Sciences, Centre of Plant Structural and Functional Genomics, Šlechtitelů 31, 779 00, Olomouc, Czech Republic; [2]Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Šlechtitelů 27, 779 00, Olomouc, Czech Republic; [3]Laboratory of Pollen Biology, Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 02, Prague 6 – Lysolaje, Czech Republic; [4]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466, Seeland OT Gatersleben, Germany

Author for correspondence:
Miroslava Karafiátová
Email: karafiatova@ueb.cas.cz

Received: *5 March 2024*
Accepted: *17 June 2024*

New Phytologist (2024) **243:** 1840–1854
doi: 10.1111/nph.19954

Key words: accumulation mechanism, B chromosome, chromosome drive, chromosome elimination, extra pollen mitosis, nondisjunction, pollen mitosis, polymitosis.

Summary

- The B chromosomes exhibit diverse behaviour compared with conventional genetic models. The capacity of the B chromosome either to accumulate or to be eliminated in a tissue-specific manner is dependent on biological processes related to aberrant cell division(s), but here yet remains compatible with normal development.
- We studied B chromosome elimination in *Sorghum purpureosericeum* embryos through cryo-sections and demonstrated the B chromosome instability during plant growth using flow cytometry, molecular markers and fluorescent *in situ* hybridization techniques. Consequently, using B chromosome-specific probes we revealed the non-Mendelian inheritance of B chromosomes in developing pollen.
- We disclosed that the occurrence of the B chromosome is specific to certain tissues or organs. The distribution pattern is mainly caused by an extensive elimination that functions primarily during embryo development and persists throughout plant development. Furthermore, we described that B chromosome accumulation can occur either by nondisjunction at first pollen mitosis (PMI) or the initiation of extra nuclear division(s) during pollen development.
- Our study demonstrates the existence of a not-yet-fully described B chromosome drive process, which is likely under the control of the B chromosome.

Introduction

Historically, the simplistic depiction that individuals of the same species have the identical chromosome number, which is stable in all the cells of a given individual, has been widely accepted (Zufall *et al.*, 2005). However, findings from the past decades prove that this assumption is not absolute. Recent studies described the genomes as plastic and dynamic structures generating perpetually significant intraspecific genome variability (Becher *et al.*, 2021; Cuevas *et al.*, 2021) and even differences in genetic content within an organism (Wang & Davis, 2015; Biederman *et al.*, 2018). In some animals, the elimination of particular DNA sequences and chromosomes is part of their developmental programme associated with germ-soma differentiation or sex determination (Goday & Esteban, 2001; Sánchez, 2014).

Another possible cause of genetic makeup differences is the presence of extra genetic elements, such as B chromosomes (Jones *et al.*, 2008; Borodin *et al.*, 2022). B chromosomes are often considered as a 'selfish' component that carries no genes essential for the host, but their presence is tolerated to some extent by the carrier. Their presence becomes burdensome with increasing numbers, but in lower copy numbers, they are usually phenotypically neutral. Several studies even show the positive or adaptive effect of B chromosome on the host. Interestingly, the possessing of the B chromosomes was found advantageous, especially in species that do not have a drive mechanism (Häkansson, 1954; MsLeisch, 1954; Fröst, 1957, 1969). Alternatively, the beneficial effects of the presence of the B chromosome can be manifested only in stressful conditions (Plowman & Bougourd, 1994; Pereira *et al.*, 2017). B chromosome may offer better survival rates in the way of increased germination rate in chives or heat/drought stress mitigate in rye (Plowman & Bougourd, 1994; Pereira *et al.*, 2017). In crop plants, the idea has been to use them as a platform to develop engineered minichromosomes to introduce

the valuable traits. A number of applications of B-derived synthetic chromosomes have been described, but so far this strategy has not been adopted by breeders as an efficient approach (Birchler, 2015).

B chromosomes are typically detected only in some populations and vary in number among their members (Rees, 1974; Chen *et al.*, 2022). The copy number of B chromosomes is generally constant in all tissues, but exceptions to this distribution pattern were observed in some organisms (Jones & Rees, 1982). Despite the fact that most of the known 2951 B chromosome-carrying species are plants (*c.* 74%), the phenomenon of numerical B chromosome instability among the tissues is relatively unexplored in plants (Gutiérrez *et al.*, 2023). In animal species, the level of intraindividual genome variability due to the B chromosomes is better studied (Stevens & Bougourd, 1994; Schmid *et al.*, 2002; Bernardino *et al.*, 2017). In plants, if the B chromosome is eliminated during development, it tends to be maintained in reproductive tissues (Jones & Rees, 1982; Karafiátová *et al.*, 2021).

To date, the only comprehensive study about the tissues-specific B chromosome elimination process was conducted in the goatgrass *Aegilops speltoides* (Ruban *et al.*, 2020), where the B chromosome is missing in roots. This atypical pattern of B chromosome distribution was discovered to be established yet in developing embryo of young seed, where the B chromosome elimination from proto-root cells was remarkable already in very early embryo development. The B chromosome elimination was accompanied by the presence of B-positive micronuclei as a result of lagging B chromosomes during the anaphase (Ruban *et al.*, 2020). Besides the *Aegilops*, other plant species exist with B chromosomes depleted from primary roots (Nygren, 1957; Östergren & Frost, 1962), adventitious roots (Baenziger, 1962), leaves (Müntzing & Nygren, 1955) and sterile florets (Darlington *et al.*, 1941). Nevertheless, in those studies the knowledge is limited only to austere reports pending further investigation.

The tissue-wide distribution of the B chromosome in wild sorghum *Sorghum purpureosericeum* is more restricted. This species tolerates up to six B chromosomes (Janaki-Ammal, 1940). In wild sorghum, the B chromosome is absent in roots, leaves and sterile inflorescence, whereas the B chromosome has been shown to be stable in male meiocytes of fertile florets and possibly in young shoots (Darlington *et al.*, 1941; Wu, 1984). Additionally, mitotic irregularities, such as lagging chromosomes and micronuclei formation, were noticed in somatic tissues of floral parts, suggesting the presence of the B chromosomes is cell type-specific (Darlington *et al.*, 1941).

In order to ensure its transmission into the progeny, B chromosomes had to evolve the mechanisms of accumulation that are collectively referred to as a drive (Houben, 2017). Based on progeny analysis, it was concluded that the B chromosome drive of wild sorghum occurs at first pollen mitosis (Darlington *et al.*, 1941). Interestingly, an extraordinary nuclear division in pollen carrying the B chromosome was noted (Darlington *et al.*, 1941). This extra pollen mitosis takes place before the final division of the generative nucleus, where the two sperm arise. In order to name this phenomenon, the authors introduced the term 'polymitosis' (Darlington *et al.*, 1941).

A recent analysis characterized the repeat composition of *S. purpureosericeum* and revealed B chromosome- and centromere-specific repeats which were used to design polymerase chain reaction and cytogenetic markers (Karafiátová *et al.*, 2021). Here, we characterized the main plant tissues and organs of this species regarding the presence of the B chromosome and traced the dynamics of the B chromosome from microsporogenesis to mature pollen grains. In addition, we studied the postmeiotic drive and phenomenon of extra pollen mitosis and suggest on the master role of the B chromosome in altering pollen development.

Materials and Methods

Plant material and cultivation

The progeny of wild sorghum *Sorghum purpureosericeum* (Hochst. ex A.Rich.) Schweinf. & Asch. plants carrying B chromosomes (+B plants) was used in all experiments. The plants with one (1B), two (2B), three (3B) and four (4B) copies of B chromosome were grown for individual set of experiments (specified in particular sections). All plants represented progeny of a single 1B plant; hence, the B chromosomes were identical in all individuals. Plants were cultivated under short-day conditions of 10 h of daylight and a temperature regime of 29°C : 25°C, day : night. At the stage of the first spike emergence, the B-presence of the plants was evaluated on floral tissue using flow cytometry according to the protocol developed by Karafiátová *et al.* (2021).

Detection of the B chromosome in plant tissues and organs

Flow cytometric evaluation of specific plant tissues Twenty different tissues and organs (stem, root, leaf meristem, leaf sheath, leaf, side shoot (without apical meristem (SAM−)), last node, shoot apical meristem (SAM), side shoot (including SAM (SAM+)), sessile floret, pedicellate floret, stigmas, ovaries, anther, protective floral tissues (palea, lemma and glume), peduncle, rachis, awns, embryo and endosperm) of tillering 2B plants (possessing two copies of B chromosome) were selected to determine the B chromosome presence (Table 1). The B chromosome was detected in the tissues based on the methodology developed by Bednářová *et al.* (2021). Nuclei isolation was done according to Doležel *et al.* (1989), and samples were analysed using a FACSAria SORP (BD Biosciences, San Jose, CA, USA). The B chromosome distribution profile was explored in identical tissues of three independent 2B plants.

PCR-based evaluation B-specific polymerase chain reaction marker 'Spu_B1' was derived from a sequence identified in wild sorghum +B plant (GeneBank, accession no.: PP319393; Supporting Information Methods S1) and used to identify +B tissues and organs. DNA from each tissue of 2B plants (Table 1) was isolated using Monarch Genomic DNA Purification Kit (cat. no.: T3010S; NEB, Ipswitch, MA, USA) and the DNA concentration

Table 1 B chromosome profile in *Sorghum purpureosericeum* tissues and organs evaluated by independent approaches.

		B chromosome status				
Tissue/organ		Flow cytometric	Polymerase chain reaction -based	Cytological	B-positive nuclei	Micronuclei
Figs S3(a–g), S4(a,b)	Stem	B0	B0	B0	No	No
	Root	B0	B0	B0	No	No
	Leaf meristem	B0	B0	B0	No	No
	Leaf sheath	B0	B0	B0	No	No
	Leaf	B0	B0	B0	No	No
	Side shoot (SAM−)	B0	B0	B0	No	No
	Last node	B0	B+	B0	No	No
	SAM	B+	B+	B+	Yes	Yes
	Side shoot (SAM+)	B+	B+	B+	Yes	Yes
Figs S4(c–i), S5	Sessile floret	B+	B+	B+	Yes	Yes
	Pedicelate floret	B+	B+	B+	Yes	Yes
	Stigmas	B+	B+	B+	Yes	Yes
	Ovary	B+	B+	B+	Yes	Yes
	Anther	B+	B+	B+	Yes	Yes
	Palea, lemma, glume	B+	B+	B+	Yes	Yes
	Peduncle	B+	B+	B+	Yes	Yes
	Rachis	B+	B+	B+	Yes	No
	Awns	B+	B+	B+	Yes	No
	Embryo	B+	B+	B+	Yes	Yes
	Endosperm	B+	B+	B+	Yes	No

was measured using Nanodrop ND-1000 (Saveen Werner, Malmo, Sweden). The presence of the B chromosome was assessed using polymerase chain reaction (Methods S1). Genomic DNA isolated from plants with (+B) and without B (0B) chromosome were used as positive and negative controls, respectively. Polymerase chain reaction detection of B-specific amplicon was performed on set of tissues in three replicates isolated from individual plants.

FISH-based tissue characterization B-status of tissues was determined by fluorescent *in situ* hybridization (FISH). The tissue samples (Table 1) were prepared independently from three 2B plants according to Kato *et al.* (2004) with minor modifications (for details see Methods S1). Postfixation of slides was carried out according to Said *et al.* (2018).

The B-specific repeat CL135 (Karafiátová *et al.*, 2021), with a unit size of 1132 bp, was labelled by nick translation (see Methods S1). The specificity of the CL135 repeat for the B chromosome was verified on meiocytes isolated from 0B and 2B plant. In 0B plant, the signal was not observed on any of the five bivalents. In 2B plant, the probe hybridized in two loci in one bivalent and two strong signals were regularly detected (Fig. S1). The centromeric probe CL29 (Karafiátová *et al.*, 2021) was used as a positive control. FISH was performed as published previously by Karafiátová *et al.* (2021). The signals were observed using epifluorescent microscope Zeiss Axio Imager Z.2 (Carl Zeiss GmBH, Jena, Germany) and adjusted in PHOTOSHOP CS.5.

Embryo cryo-sectioning Embryos (25 d after pollination (DAP)) were collected from 2B plants. The embryos were fixed in 4% formaldehyde (Sigma-Aldrich) in 1× phosphate-buffered saline (PBS) for 6 h at 4°C. Fixed embryos were washed 10 min in 1× PBS and transferred into the 5% sucrose for overnight incubation. Subsequently, the embryos underwent cryo-protective treatment in a sucrose gradient 10%, 15%, 20% and 30%, each for 2 h at room temperature. The sectioning was done using the cryostat Leica CM1950 (Leica Biosystems, Nussloch, Germany), which was cooled down to −20°C. Fixed and cry-protected embryos were embedded into the drop of Surgipath cryo-gel FSC22 blue (cat. no.: 3801481; Leica, Buffalo Grove, IL, USA) on a precooled disc in a longitudinal orientation. Ten- to fifteen-micrometre embryo sections were collected onto the Silane-prep microscopic slides (cat. no.: S4651-72FA; Sigma-Aldrich).

FISH on embryo sections First, the embryo sections were immobilized onto slides in a thin layer of polyacrylamide gel, according to Němečková *et al.* (2020), with minor modifications. Concisely, the slides with sections were rinsed in 1× PBS in horizontal position to remove the cryo-gel. Next, 60 µl of polyacrylamide mixture (PAA mix and 1× MBA buffer 1 : 2) was added to the dried sections and covered with 22 × 40 mm coverslips (Němečková *et al.*, 2020). The slides were incubated for at least 1 h at 37°C and high humidity for polymerization. Coverslips were removed, and the slides were stored in 1× PBS at 4°C till use. Before hybridization, the slides were taken out of PBS buffer and decanted. Forty microlitres of hybridization mixture containing 28 µl of hybridization buffer HB 50 (50% formamide, 2× SSC) and 250 ng of B-repeat CL135 was added onto each slide, and sections were denatured for 5 min at 99°C. FISH was performed at 37°C in humid conditions overnight. After, sections were washed twice 2× SSC/15 min (20× SSC: 77.4 g

$HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$, 175.3 g NaCl, ddH_2O up to 1 l, pH 7.0), twice 1× SSC/15 min and 4× SSC + 0.2% Tween 20/15 min. DNA of embryo sections was counterstained by adding 15 µl of Vectashield mounting medium with DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride) (2 mg ml^{-1}; Vector Laboratories, Newark, CA, USA). The distribution pattern of the B chromosome was evaluated on 10 embryo section. Signals were observed using the Leica TCS SP8 STED 3X confocal microscope (Leica Microsystems, Wetzlar, Germany), equipped with an HC PL APO CS2 ×100/1.44 Oil objective, Hybrid detectors (HyD) and the Leica Application Suite X (LAS-X) software v.3.5.5 with the Leica Lightning module (Leica).

Transmission of the B chromosome through the male microsporogenesis

FISH of meiocytes 1B, 2B and 3B plants were used to collect the anthers for meiotic analysis. Anthers were fixed 10 min in 90% acetic acid. The slides were prepared and probe hybridization was performed according to Karafiátová *et al.* (2013; for details, see Methods S1).

Suspension FISH on pollen grains Anthers at the appropriate stage of pollen development were isolated from panicles of 2B plant. For each developmental stage of +B pollen, four fractions were collected from independent 2B plants representing four biological replicates. Ten-microlitre aliquots of pollen suspension (fixed 10 min in 90% acetic acid) were placed into a 0.5-ml tube and pulse-centrifuged. The supernatant was removed, and the hybridization mixture containing B-specific CL135 and centromeric CL29 oligo probes was added to the pollen pellet (for details, see Methods S1). FISH was performed as described by Ebrahimzadegan *et al.* (2023). For the analysis of aborted pollen architecture, five independent fractions of mature pollen were collected and the number of nuclei was examined.

Pollen viability and germination analysis

The viability of the pollen grains was assessed using the Alexander staining method in pollen collected from 0B, 1B, 2B, 3B and 4B plants (Alexander, 1969). To monitor the tube growth, pollen of 2B plant was germinated in 1X pollen germination media (10% sucrose, 0.005% H_3BO_3, 10 mM $CaCl_2$, 0.05 mM KH_2PO_4, 6% PEG4000 and 0.3% (w/v) agar (Molecular biology grade; AppliChem, Darmstadt, Germany)). Therefore, pollen from anthers at the dehiscence stage was spread onto a thin layer of media on microscopic slides. Slides were enclosed in a humid chamber and incubated at 29°C for different time intervals ranging from 5 min to 2 h. DAPI staining was used to visualize the nuclei of the germinated pollen. To compare the germination efficiency, pollen grains containing three/four nuclei were scored in parallel on each microscopic slide. Pollen was considered germinated when a bud appeared at the germ pore. Pollen was collected from three independent plants, and at least two technical replicates for the individual plant were set for microscopy image analyses. Images of the germinated and nongerminated pollen were acquired by Olympus BX60 microscope. IMAGEJ software was employed to quantify the germinated and nongerminated pollen. Experimental values were evaluated using a *t*-test to confirm statistical differences between the groups.

Results

The B chromosome of wild sorghum undergoes extensive elimination during embryo development except in undifferentiated meristematic cells

To assess the cellular distribution of the B chromosome at the initial phase of plant development, we performed *in situ* localization of the B-specific repeat CL135 on longitudinal cryo-sections of differentiated *S. purpureosericeum* embryos of 25 DAP (Fig. 1e). At this stage of development, embryonic ground and aerial organs are established and individual organs of embryo axis are noticeable in tissue sections (Hariprasanna & Patil, 2015; Fig. 1a). The ground part comprised of embryonic root (radicle) with a differentiated root cap (calyptra) at its end and perceptible procambial cells of central cylinder. The whole proto-root is surrounded by coleorhiza – scutellar cells protecting the radicle. The embryo axis above the radicle bears a sheath-like layer of scutellum termed as coleoptile, which encloses three foliage leaves, plumule (shoot apex) and bud in the axil of the coleoptile. Besides, the vascularization of the coleoptile via procambial strands is apparent in the mesocotyl (Fahn, 1990; Hariprasanna & Patil, 2015).

The radicle cells are free from B chromosomes (Fig. 1b,c). Similarly, the absence of B-specific FISH signals was noticed in the cells of organs of the upper part of the embryo axis. The most evident regions lacking the B chromosomes were the mesocotyl and foliage leaves. Contrastingly, a remarkable population of nuclei carrying B chromosomes were detected in the most inner part of the coleoptile corresponding to the plumule and in an unspecified cell lineage bordering the marginal layer of the youngest foliage leaf (Fig. 1b–d). Furthermore, B-specific signals were spotted in the axillary bud and in scutellar cells with a distinct cell layer at the embryo/endosperm interface (scutellar epidermis). Thus, already during embryo development, extensive B chromosome elimination occurs. Only the zone of undifferentiated meristematic cells, which will develop into future aerial organs, possess B chromosomes.

Tissue atlas demonstrates the B chromosome elimination from most of the plant organs

To study the presence of the B chromosome in adult plants, we applied flow cytometry, polymerase chain reaction and FISH (Fig. 2). In addition to the organs (roots, leaves and anthers), where the B-status is known (Janaki-Ammal, 1940; Wu, 1984; Karafiátová *et al.*, 2021), we investigated 17 additional tissue and organ types with a focus on reproductive organs (Table 1; Fig. S2).

First, we employed flow cytometry to get an overview of the presence of the B chromosome in different tissues (Fig. 2a,b). Expectedly, no B chromosome was found in the root, stem, last

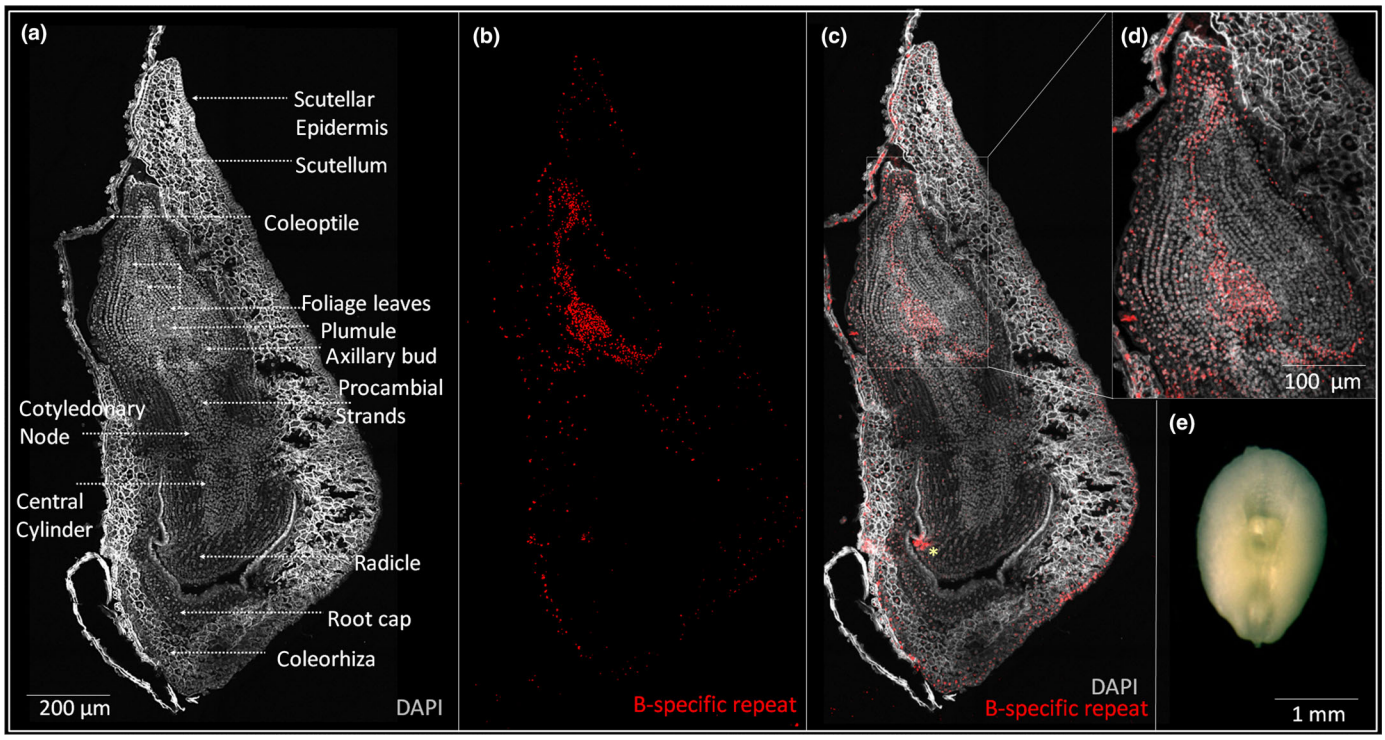

Fig. 1 Tissue-specific distribution of the B chromosome in the developing embryo of *Sorghum purpureosericeum*. Cryo-section of an embryo *c*. 25 d after pollination (DAP) reveals embryonic tissues and organs (a). B chromosome detection using fluorescent *in situ* hybridization (FISH) with the B-specific repeat CL135 (in red) (b), merged picture (c); a detail of future aerial organs harbouring a large number of +B nuclei (d), isolated 25 DAP embryo – ventral side (e). Section was counterstained with the DNA-specific dye DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride) (in grey). The strong red signal in the distal region of the radicle tip (indicated with an asterisk *) represents an unspecific signal.

node and leafy tissues (leaf, leaf sheath, leaf meristem and side shoot (SAM^-)) (Table 1; Figs 2a, S3). Nuclei carrying the B chromosome were detected in 13 tissue types (rachis, peduncle, protective floral tissue, anthers, ovaries, stigmas, side shoot (SAM+), sessile and pedicellate spikelets, awns, SAM, embryo and endosperm; Table 1; Figs 2b, S4). Among them, nine tissues were found contributing to the formation of the reproductive organ – panicle, and mature embryos together with endosperm were determined as +B. The B-positive profile of the side shoot and the SAM is likely based on their undifferentiated cells. This presumption was confirmed by flow cytometry, where SAM and leafy parts of the side shoot were separately analysed (Figs S3f, S4a,b).

In +B tissues, the population of +B nuclei was of variable size, reflected by the +B peak high in the histograms (Table S1). Among all, the female gametophytic tissues, ovaries and stigmas exhibited the most abundant population of +B nuclei, where the frequency of +B nuclei exceeded those without B chromosomes (Fig. S4h,i). In the remaining tissues, B chromosomes were only found in a minority of nuclei. The +B population was the smallest in samples of the side shoot, SAM, rachis, peduncle and embryo, where it exists only in a residual population (Fig. S4a–d).

Interestingly, in all analysed tissues and organs, with the exception of the endosperm, a mixture of the +B and 0B nuclei exists in variable proportions. In +B seed, the uniformity of endosperm nuclei was observed. No peak splitting was noted, and the endosperm-typical 3C peak position was shifted (Fig. S5a,b). Endosperm nuclei in +B seed uniformly showed higher DNA content, implying the presence of the B chromosome in the entire endosperm (Fig. S5b).

The B chromosome presence was further verified using polymerase chain reaction and FISH (Figs 2c–e, S6,S7). Selected tissues were scored with a marker (Table 1; Methods S1), and the presence of the B-specific polymerase chain reaction product in individual tissues was assessed. FISH confirmed the mosaic profile of +B tissues comprising nuclei with and without B chromosomes (Fig. S7m–o). Only the results for the last node were not consistent. Constantly, we found no significant +B population in the histogram and no B chromosome-specific FISH signal despite a weak polymerase chain reaction product being detected in this tissue. Thus, the B-status of the last node remains elusive.

The FISH analysis revealed the presence of +B micronuclei in tissues evaluated as B-containing (Fig. S7m–o). The constant detection of micronuclei in most B-positive organs suggests that B chromosome elimination is a process accompanying cell differentiation during plant growth.

Meiotic B chromosome transmission followed the typical mode of segregation of A chromosomes

Meiotic transmission was studied on squashed pollen mother cells isolated from plants possessing 1B, 2B and 3B chromosomes.

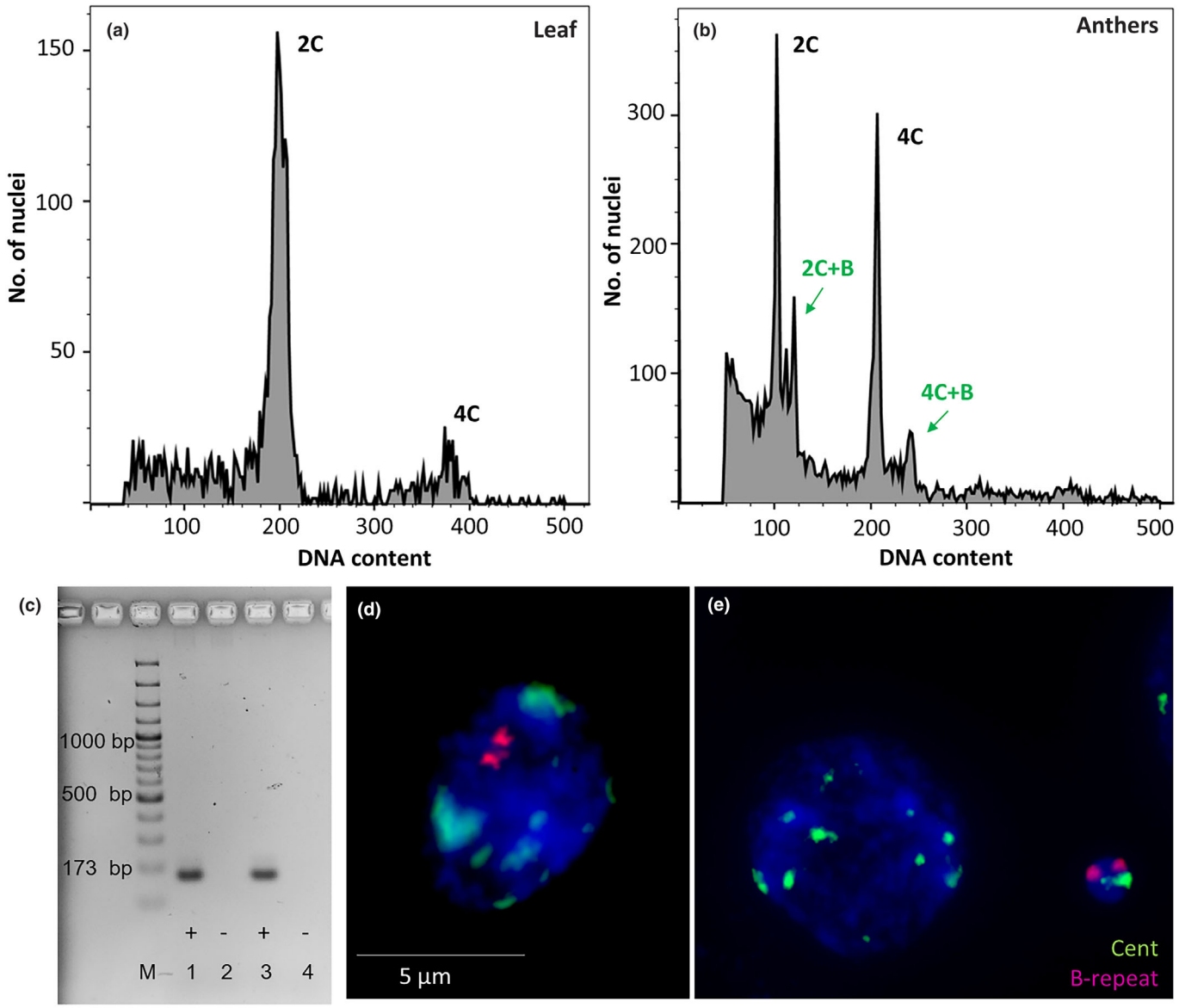

Fig. 2 Combined approach for evaluation of B chromosome presence in selected tissues/organs of *Sorghum purpureosericeum* plant (with leaf and anther as examples). (a, b) Flow cytometric strategy of B chromosome detection relied on detection of extra peak(s) representing +B nuclei (green-labelled) (b) that were not present in 0B samples (a); (c) B-status evaluated based on polymerase chain reaction using B-specific marker (lines: 1 – positive control; 2 – negative control; 3 – DNA from anthers; 4 – DNA from leaf). (d) presence of nuclei (4′,6-Diamidino-2-phenylindole dihydrochloride-blue) carrying B chromosome in anthers visualized using fluorescent *in situ* hybridization (FISH) with B-specific repeat CL135 (magenta) and centromeric probe CL29 (green); (e) B chromosome undergoing elimination in +B tissue of anther and forming micronucleus.

B chromosome was localized using FISH with B-specific repeat CL135 on 885 meiocytes in total (Fig. 3). The B chromosome mirrored the behaviour of A chromosome and displayed no significant irregularities. In 1B plants, the B univalent (Fig. 3a) was preferably (in 78% of 245 cells) passed undivided towards one of the poles of the mitotic spindle at anaphase I (Fig. 3b). In the remaining pollen mother cells (PMCs) (17%), the sister chromatids of the B chromosome univalent separated precociously and moved towards the opposite poles (Fig. S8a). In 5% of the cells, the B univalent lagged in the central zone and formed a micronucleus (Fig. S8b). Consequently, 78% of the tetrads (of 194 cells) showed two adjacent microspores free of B chromosomes and two carrying one B chromosome each (0 + 0 : 1 + 1) (Fig. 3c). In tetrads arising from diads containing precociously separated B chromatids, each of the chromatids was moved to one of the poles and formed two alternate microspores with B chromatids and two without (1 + 0 : 1 + 0; 15%, 30/194) (Fig. S8c). Micronucleation of B univalents was detected in only 7% of the tetrads (Fig. S8d).

Meiotic segregation of 2B chromosomes followed the standard rules of inheritance. The two B chromosomes in the majority of

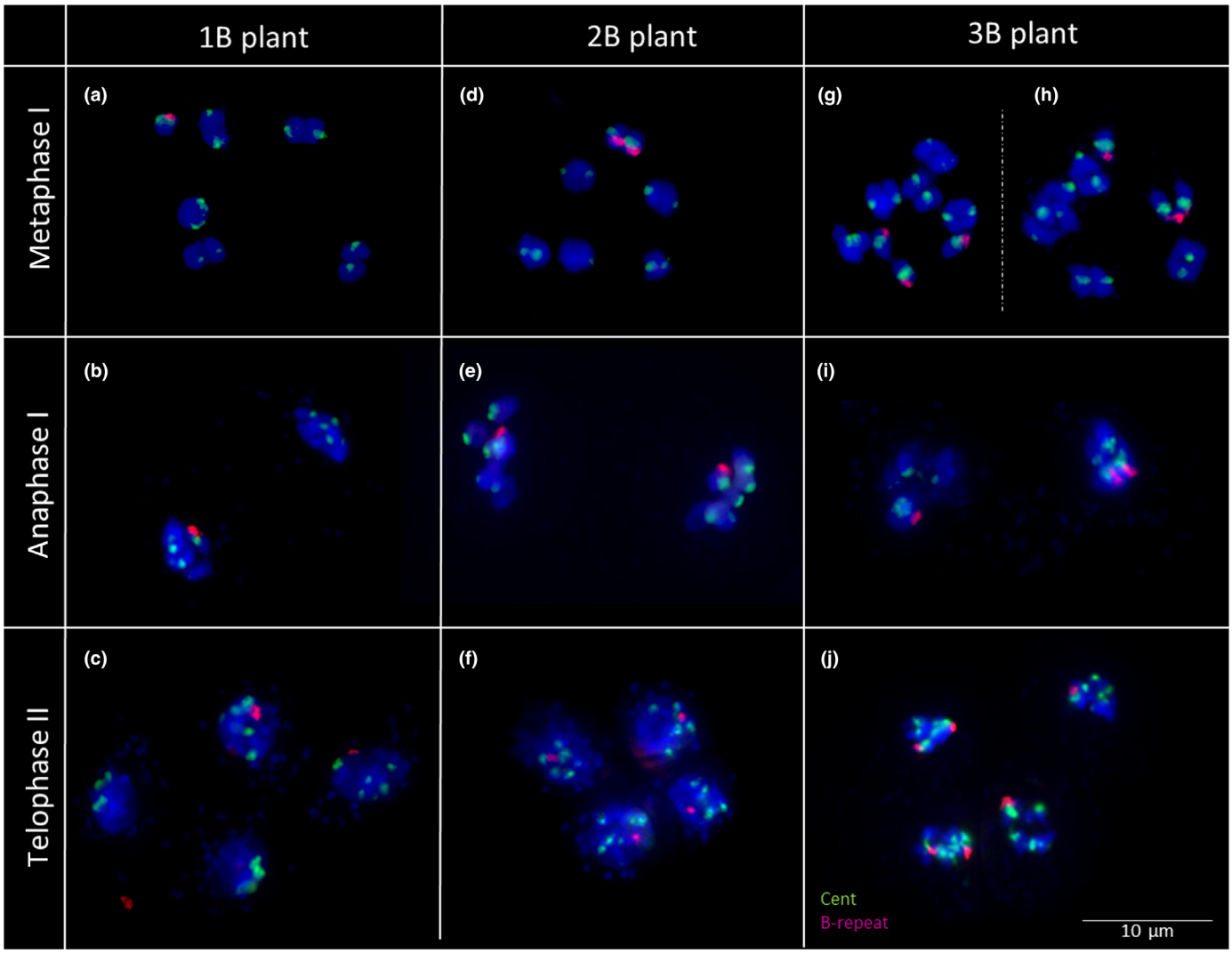

Fig. 3 Meiotic transmission of the B chromosome in male gametophytes of *Sorghum purpureosericeum*. The prevalent segregation patterns are shown for 1B, 2B and 3B plants at metaphase I (a, d, g, h), anaphase/telophase I (b, e, i) and telophase II (c, f, j). Fluorescent *in situ* hybridization (FISH) was performed with the B-specific repeat CL135 (magenta) and the centromere repeat CL29 (green). Chromosomes were counterstained with 4′,6-Diamidino-2-phenylindole dihydrochloride.

the PMCs paired at metaphase I (Fig. 3d), and the B bivalents divided normally producing two daughter nuclei harbouring 1B chromosome each at telophase I (97%; 88/91 cells, Fig. 3e). In 3% of 91 cells, a precocious separation of B sister chromatids occurred during anaphase I. Nevertheless, chromatids segregated normally – two chromatids to each daughter cell (Fig. S8e). Only sporadically, B bivalents did not divide and were pulled to one of the poles (Fig. S8f). The second meiotic division produced 97% microspores (of 66 cells) carrying one B chromosome as a consequence of the standard division of 1 : 1 diads (Fig. 3f). Besides, 0B and 2B microspores were formed as a result of a segregation failure in 3% of cells (Fig. S8g). Micronuclei were not detected in any of the PMCs in 2B plant.

The pattern of B chromosome behaviour in 3B plants exhibits the combination of the features observed in both 1B and 2B plants. One percent of 186 metaphase I cells revealed B trivalents (Fig. S8h), while the rest formed bivalents and univalents (Fig. 3g, h). Pairing of the B chromosomes resulted in the formation of either rod (Fig. 3g) or ring bivalents (Fig. 2h). B bivalent divided into a regular manner, and the sister chromatids of the B univalent mostly remained cohesed and were pulled towards the same pole. In this case, the diads (2 : 1) occurred the most frequently (92%, 166/186; Fig. 3e). Exceptionally, two different pathways were observed. Either both univalent and bivalent migrated to the same pole forming a diad 3:0 (1%; 2/186; Fig. S8i) or the B bivalent segregated regularly, but the sister chromatids of univalent split and were positioned in opposite poles (diads 1.5 : 1.5) (7%; 13/186; Fig. S8j). In the second meiotic division, the most abundant diads produced microspores with expected B chromosome numbers – 2 : 2 + 1 : 1 (97%; 98/103; Fig. 3j). The division of the abnormal diads resulted in microspores with 0B to 3B chromosomes, respectively (3%; 5/103; Fig. S8k,l).

Conclusively, the tracking of B chromosome revealed no accumulation during meiosis. Our analysis proved B chromosome

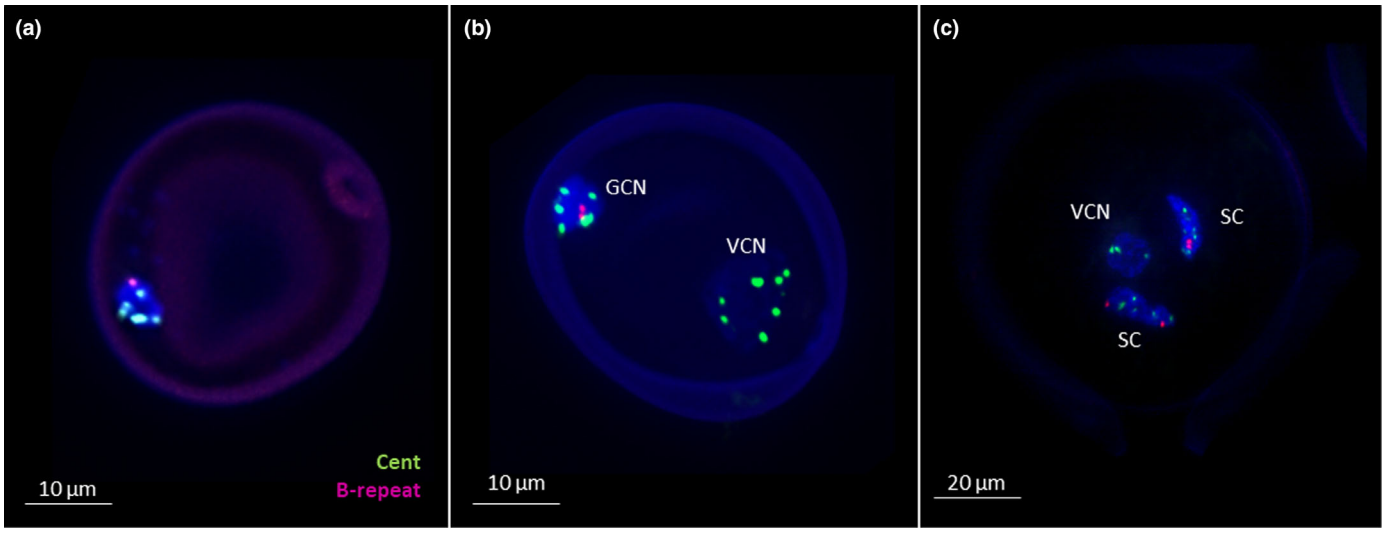

Fig. 4 Drive of the wild sorghum *Sorghum purpureosericeum* B chromosome occurs during the first pollen grain mitosis. (a) Unicellular pollen with 1B chromosome of 2B plant, (b) Bicellular pollen grain after B chromosome nondisjunction at the first pollen mitosis; two B chromosomes are accumulated in generative nucleus (GCN) and vegetative nucleus (VCN) remains free of B chromosome, (c) Tricellular mature pollen of wild sorghum with two identical sperm cells (SCs). Suspension fluorescent *in situ* hybridization (FISH) was performed with the B-specific repeat CL135 (magenta) and the centromere repeat CL29 (green). Chromosomes were counterstained with 4′,6-Diamidino-2-phenylindole dihydrochloride.

number being stable in pollen mother cells and no numerical instability was detected.

The B chromosome of wild sorghum accumulates via nondisjunction at the first pollen division

To decipher the process of B chromosome drive, we traced the B chromosome transmission at the first and second pollen mitosis using the B-specific probe CL135 for FISH in 1B microspores derived from 2B mother plant. The behaviour of the B chromosome was studied on 1094 pollen grains in total. *In situ* hybridization was performed on pollen in suspension to ensure the intactness and completeness of pollen nuclei.

Unicellular pollen harboured 1B chromosome in 96% (382/398) of the pollen analysed (Fig. 4a). Remaining 4% of the pollen lacked the B chromosome as a result of irregular transmission during meiosis. In 146 (out of 331) pollen grains in bicellular stage, B sister chromatids accumulation was detected. The B chromosome accumulated in the generative nucleus (GCN) and the vegetative nucleus (VCN) remained without a B chromosome (Fig. 4b). However, among four studied individuals, variable accumulation frequencies were observed ranging from 3% to 95% (Table S2). The further development of pollen where B chromosome accumulated in GCN was regular. The GCN divided, giving rise to two identical sperm cells (SCs) with the same number of B chromosomes (Fig. 4c). Such B chromosome distribution was documented in 192 of 365 examined mature pollen with incidence varying from 24% to 98% among four individual replicates. The generative nucleus-specific accumulation is likely a product of nondisjunction of B chromatids as reported for the B chromosomes of rye, *Ae. speltodies* and *Festuca pratensis* (Wu *et al.*, 2019; Houben *et al.*, 2021; Ebrahimzadegan *et al.*, 2023).

The B chromosome may trigger an extra mitotic division of the pollen cell in the early stage of pollen development

Unexpectedly, a fraction of +B pollen grains exhibited a different development. In 185 bicellular pollen grains (out of 331), B chromatids segregated during the first pollen mitosis and the B chromosome was found in both nuclei (Fig. 5c). The subsequent pollen development did not proceed via the typical division of GCN. Instead, another cycle of cell division occurred and extracellular pollen grains were produced (Fig. 5). It appears plausible that the additional division might have been triggered by the B chromosome, which failed to accumulate during the first division of pollen nuclei (Fig. 5a–c). In this state, one of the daughter nuclei underwent the additional mitotic division, most likely in favour of successful B chromosomes accumulation (Fig. 5d–f). The final division of GCN then followed the standard pollen development. GCN replicated, multiplied B chromosomes once again (Fig. 5g) and divided, producing two SCs – both harbouring two B chromosomes (Fig. 5h). The extra nucleus remained intact, attached to the pollen wall opposite to the germination pore and showed two signals of the B-specific probe (Fig. 5h). Such architecture was documented in 173 out of 365 mature pollen scored. Again, the frequency of alternative pathway of development was individual-dependent ranging from 2% to 76%.

If accumulation does not occur within the first extra division, the division is likely repeated cyclically until nondisjunction of the B chromosome occurs. Nevertheless, there was a certain upper limit of polymitotic cycles that were tolerated by the pollen to retain their viability. In 96% of 173 mature pollen grains, the B chromosome nondisjoined during the first extra division. Consequently, pollen grains with one additional nucleus were produced (Fig. 5h). Two rounds of additional mitosis were noticed in six pollen grains (3.5%). A maximum number of three extra

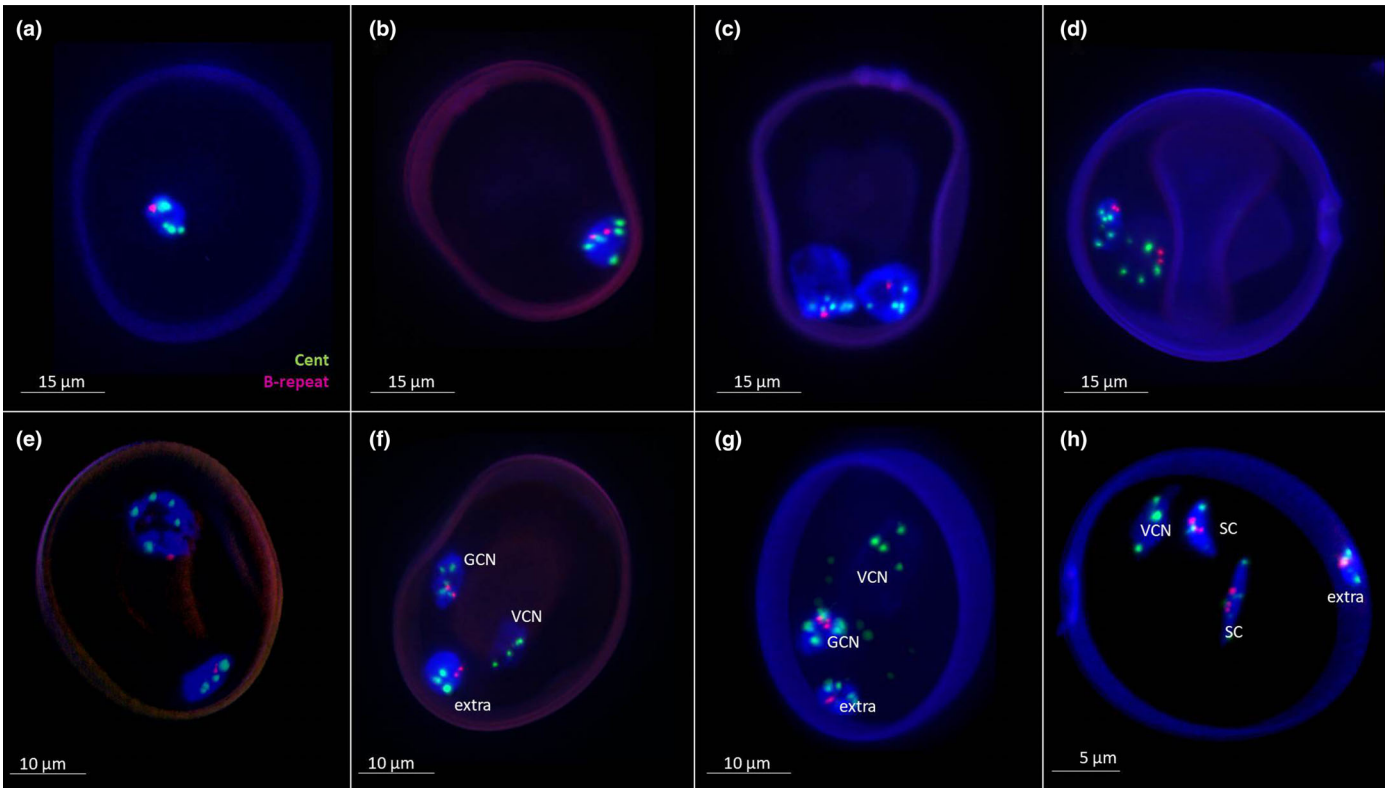

Fig. 5 B chromosome triggers an extra mitotic division during pollen development in *Sorghum purpureosericeum* 2B plant. (a) 1B unicellular pollen before and (b) after DNA replication; (c) absence of nondisjunction results in bicellular pollen with B chromosomes in both nuclei; (d, e) bicellular pollen with replicated B chromosome prior extra mitosis; (f) abnormal pollen grain after B chromosome nondisjunction at extra division; (g) GCN replication prior final division; (h) nuclei formation in abnormal mature pollen grain. B chromosomes are detected by B-repeat CL135 (magenta), centromeres CL29 are shown in green. DNA is counterstained with 4′,6-Diamidino-2-phenylindole dihydrochloride.

nuclei was observed. Nevertheless, such formation was rare and was detected in only single mature pollen grain (0.6%). If the B chromosome was not excluded from one of the daughter nuclei in those three cycles of the extra mitosis, the nuclei probably kept dividing, resulting in a defective development (Fig. 7).

The analysis of mature pollen grains supported the proposed model of the remarkable behaviour of the pollen nuclei. None of the 365 pollen collected from 2B mother plants with B-containing sperms showed B chromosomes in VCN. This is in contrast with the B distribution noticed in bicellular pollen, where B chromosomes were frequently (185 out of 331) detected in both nuclei. Additionally, the aborted pollen, where the B chromosome was found in all nuclei, was detected along with the well-developed pollen grains. Aborted pollen frequently (125 of 370 nonvital pollen scored) possessed more extra nuclei, lacked sperms and differed in size and shape from viable pollen.

Pollen with extra nuclei was only produced from microspores harbouring B chromosomes. To confirm the key role of the B chromosome in triggering an alternative pathway of pollen development, we analysed mature pollen of five independent 0B plants. In total, nearly 600 pollen grains from five 0B plants were scored, and no extra division was detected. Similarly, no extra cell division was observed in the 0B pollen developed from 0B microspores of a 1B plants. These observations suggest that the B chromosome of wild sorghum controls the process of additional pollen mitosis.

However, it cannot be unequivocally concluded if any of the pathways is dominant over another one. In order to evaluate the frequency of both mechanisms we scored additional 1364 of mature pollen from 17 independent plants. Collectively, the incidence of both pathways was rather individual-dependent and likely to be influenced by the physiological condition of the plant (Table S3). Interestingly, both pathways led to the formation of different pollen types (tricellular or extracellular), but carrying SCs whose are identical concerning the B chromosome copy number (Figs 4h, 5c). In both cases, the resulting sperms carry more B chromosomes than the original microspores, and thus, both pathways can be considered as processes of B chromosome accumulation.

The pollen development is influenced by the presence of the B chromosome

B chromosome decreases the pollen viability The pollen viability was assessed by Alexander staining in pollen fractions collected from 0B and +B plants carrying up to 4B chromosomes. An increase in aborted pollen grains was noticed and correlated with the number of B chromosomes present in the parental plant.

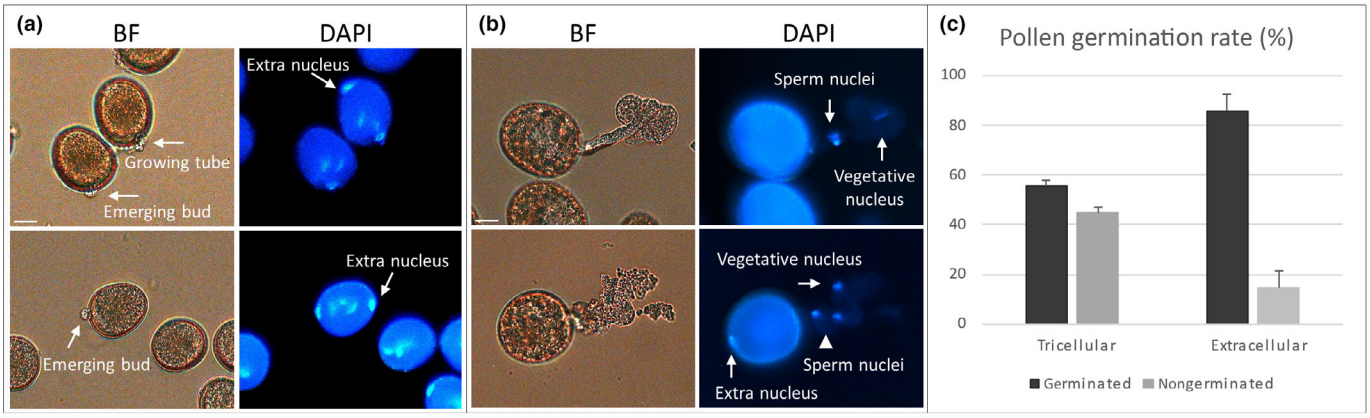

Fig. 6 Early germination of the extracellular pollen grain of 2B plant and pollen tube germination *in vitro* in *Sorghum purpureosericeum*. (a) Detail of pollen grains incubated on germinating medium in one fraction. Emerging bud of pollen tube is visible in a bright field (pointed by arrowhead), nuclei are detected after DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride) staining, extra nucleus marked with arrowhead. (b) Germinating tricellular pollen and germinating extracellular pollen grain. Pollen tube progression and nuclei migration is documented in bright field and after DAPI staining. Bar, 10 μm. (c) Evaluation of extra nucleus effect on pollen germination. Germinated multicellular pollen showed a significantly increased early germination rate difference (*t*-test, $P < 0.05$) compared with non-germinated pollen. Error bars represent SD.

The most viable pollen grains were observed in 0B plants (95%, of 928 pollen). The viability of pollen dropped down to 92% (of 944 pollen) in 1B, 91% (of 1054 pollen) in 2B and 87% of 909 pollen in 3B plants. A dramatic decrease was detected in 4B plants (maximum B chromosome number found in our seed stock), where 38% of 435 grains were scored as nonviable (Fig. S9a; Table S4). The impact of B chromosome copy number on reduced fertility was statistically proved ($P < 0.05$), except the difference between 1B and 2B samples ($P > 0.2$; Fig. S9b). Hence, a high number of B chromosomes reduce the viability of pollen.

The extra nuclei do not contribute to the fertilization in wild sorghum To apprehend the biological role of the B chromosome in pollen tube growth, we performed an *in vitro* pollen germination assay to test the standard and abnormal pollen carrying an extra nucleus. Our observation uncovered that only three nuclei (VCN and two SCs) migrated into germinating pollen tubes. Interestingly, extra nuclei remained on the peripheral part of pollen grain and did not move during pollen tube growth progression for up to 2 h in none out of 95 pollen observed (Fig. 6a, b). Thus, extra nuclei are most likely not involved in the process of fertilization.

Next, we explored whether the presence of extra nucleus affects the pollen germination. We performed a pollen germination assay to monitor the initiation of germination in 133 pollen grains of 1B pollen released from 2B plants. We scored the pollen that started to germinate within 5 min of cultivation (emerging pollen tube visible after 5 min (Fig. 6a)) and assign each pollen grain either as extracellular or tricellular +B pollen. The higher fraction of extracellular pollen (84.8%, 50 out of 59) maintained early germination compared with tricellular +B pollen grains where early germination rate was detected in only 55.4% (40 out of 74; Fig. 6c; Table S5).

Discussion

Elimination of the B chromosome starts in developing embryos and continuous throughout plant development

B chromosome mosaicism observed in several species raises the question of when and how exactly the B chromosome is depleted from specific parts of the plant. To address this notion, we scrutinized the occurrence of the B chromosome in tissues of differentiated embryos of *S. purpureosericeum* (Fig. 1). The absence of the B chromosome in specific organs indicates that the process of B chromosome elimination is not random and likely under the control of the B chromosome itself. Besides the *Ae. speltoides* (Ruban *et al.*, 2020), this would be the next example of programmed B chromosome elimination in plants.

The most important region with B-positive cells is well-defined, a dome-like population in the hearth of the embryo corresponding to the shoot apex. This meristematic tissue shelters the undifferentiated cells, which will develop into all aerial organs of the adult plant (Fahn, 1990), whose B chromosome status is heterogeneous. Conclusively, another events of B chromosome elimination occur during the morpho-physiological specialization of the embryonic shoot apex into the vegetative and generative apices – the organizing centres for the aerial part of the plant (Lie *et al.*, 2018; Walla *et al.*, 2020). The process of B chromosome elimination in wild sorghum is thus not restricted only to embryonal development but also continues in postembryonal growth.

Our research explores the timing of B chromosome elimination during plant growth, shaping its tissue-specific distribution pattern. However, the cellular mechanism behind the B chromosome elimination process in wild sorghum remains unclear. Elimination occurs likely due to segregation defects of B chromatids during metaphase/anaphase transition as previously reported in Aegilops or sciarid flies (Escriba *et al.*, 2011; Ruban *et al.*, 2020). The

activation signal for this process and the molecular mechanisms causing selective B chromosome delay are also unresolved. Epigenetic histone modifications have been associated with chromosome elimination in sciarid flies, leading to delayed sister chromatid separation (Escriba & Goday, 2013). Alternatively, the disability of the B chromosome to be move to the pole of the mitotic spindle can be caused by defective kinetochore assembly. In Zebra finches, chromosome elimination occurs due to the failure of microtubule attachment to the kinetochore (Schoenmakers *et al.*, 2010). Similarly, unequal microtubule attachment is underlying the elimination of one of parental genomes in interspecific plant hybrids *Festuca* × *Lolium* (Majka *et al.*, 2023).

The purpose of programmed B chromosome elimination remains to be unravelled. Considering that B chromosomes act like a genomic parasite, their elimination from unwanted cells is a meaningful balancing mechanism. As the replication of extra B chromosome DNA imposes significant energy demands on the host (Lynch & Marinov, 2015), effectively removing this parasitic DNA from most cells serves as an efficient energy regulation method. In wild sorghum, only 'germ-line' cells and endosperm consistently possess B chromosomes, and no elimination occurs here. If the delineated theory is considered, then the B chromosome of *S. purpureosericeum* could reach the maximal level of effective maintenance when it persists in gametes, ensuring the transmission to the progeny and simultaneously minimizing the negative effect to the host.

Two alternative accumulation pathways occur in developing +B pollen

We found two ways how B chromosomes are maintained during wild sorghum pollen maturation: one is nondisjunction during the first pollen mitosis and another is initiation of extra division of pollen nucleus. The second accumulation mechanism is unique. Our research suggests that B chromosome, when regularly transmitted during the first pollen mitosis, activates an alternative pathway for its accumulation. This involves extra mitosis, resulting in additional pollen cells (see Figs 5h, 7).

Pollen nuclei can divide further if stimulated, as observed in various studies (Steward, 1970; Vasil, 1984). This capability is crucial for androgenesis – the development of embryos from pollen. Immature bicellular pollen is responsive to stress and can be induced to undergo *in vitro* androgenesis (Touraev *et al.*, 1996; Reynolds, 1997). Similarly, the apparently 'unsuccessful' accumulation during the first pollen division could be perceived as stressful by the B chromosome, altering the pollen developmental programme and inducing additional divisions. Considering the unstable frequency of standard accumulation process in this species, the existence of supplementing mechanism of B chromosome maintenance might be critical for its survival. Since the efficiency of nondisjunction is estimated to vary individually between 3 and 95% (Table S3), it alone may not be sufficient to preserve the B chromosome in the population. Analogically, the drive of maize B chromosome is also complex (Birchler & Yang, 2021). Nondisjunction of the B chromosome occurs uniquely at the second pollen mitosis and thus one of the SCs is transmitting two copies of the B chromosome(s) while the other none (the success rate of inheritance is 50%). In this case, the process of nondisjunction has to be coupled with preferential fertilization of B-containing sperm what increase the transmission of the B chromosome to 75% (Roman, 1948).

B chromosome manipulates the developmental programme of male gametophyte

Remarkably, our examination of the second accumulation pathway reveals another feature of the sorghum B chromosome. It surprisingly leads to the production of two equivalent SCs through an extraordinary mechanism that likely involves extra DNA replication event in additional nucleus without subsequent division (see Figs 5, 7).

The alternative pathway of pollen development is presumably initiated by the normal segregation of the B chromosome during the first pollen division. When both daughter nuclei carry a B chromosome (see Fig. 5c), one of them undergoes an additional division(s) in order to accumulate the B chromosome. This observation suggests that DNA replication must have occurred before chromosome segregation. Our observation indicates that the other nucleus (or its B chromosome) replicates simultaneously with the dividing nucleus, resulting in a doubling of B-signals (Fig. 5d), but does not divide. At this stage, immature pollen contains three nuclei: VCN (B-free), GCN (B chromosome doubled) and the 'extra nucleus' (B chromosome doubled, Fig. 5f). GCN, with two copies of the B chromosome, subsequently undergoes replication (B-signal doubles again, as shown in Fig. 5f,g) and then divides to form two identical SCs, while the extra nucleus with B chromosomes remains unchanged.

To decipher the direction of pollen nucleus divisions and clarify the replication process, we must address a crucial question regarding the span of replication. The impact of two potential modes of extra replication (whole nucleus or B chromosome itself) is essential for understanding the composition of mature pollen nuclei. The stage where immature pollen contains two +B nuclei is perplexing (Fig. 5c), and the fate of these nuclei remains uncertain.

Possibly, two alternative mechanisms can explain the observed numbers of the B chromosome signals in pollen nuclei. The first hypothesis involves the selective replication of the B chromosome, occurring concurrently with the replication of one nucleus at the bicellular stage. In this scenario, the replicated nucleus becomes diploid, while the other remains haploid with only the B chromosome doubled. The replicated nucleus then undergoes division, yielding two haploid nuclei – VCN (B-free) and GCN with a doubled number of Bs. Subsequently, GCN replicates before the final division to produce two identical haploid SCs.

Alternatively, the same distribution pattern of B chromosomes in bicellular pollen could result from the replication of both nuclei. In this case, both nuclei would be diploid, but only one would divide, giving rise to VCN and GCN. At the bicellular stage of pollen, both nuclei are sensitive to the replication signal and double their genetic content. While the division of one nucleus is accompanied by nondisjunction of the B

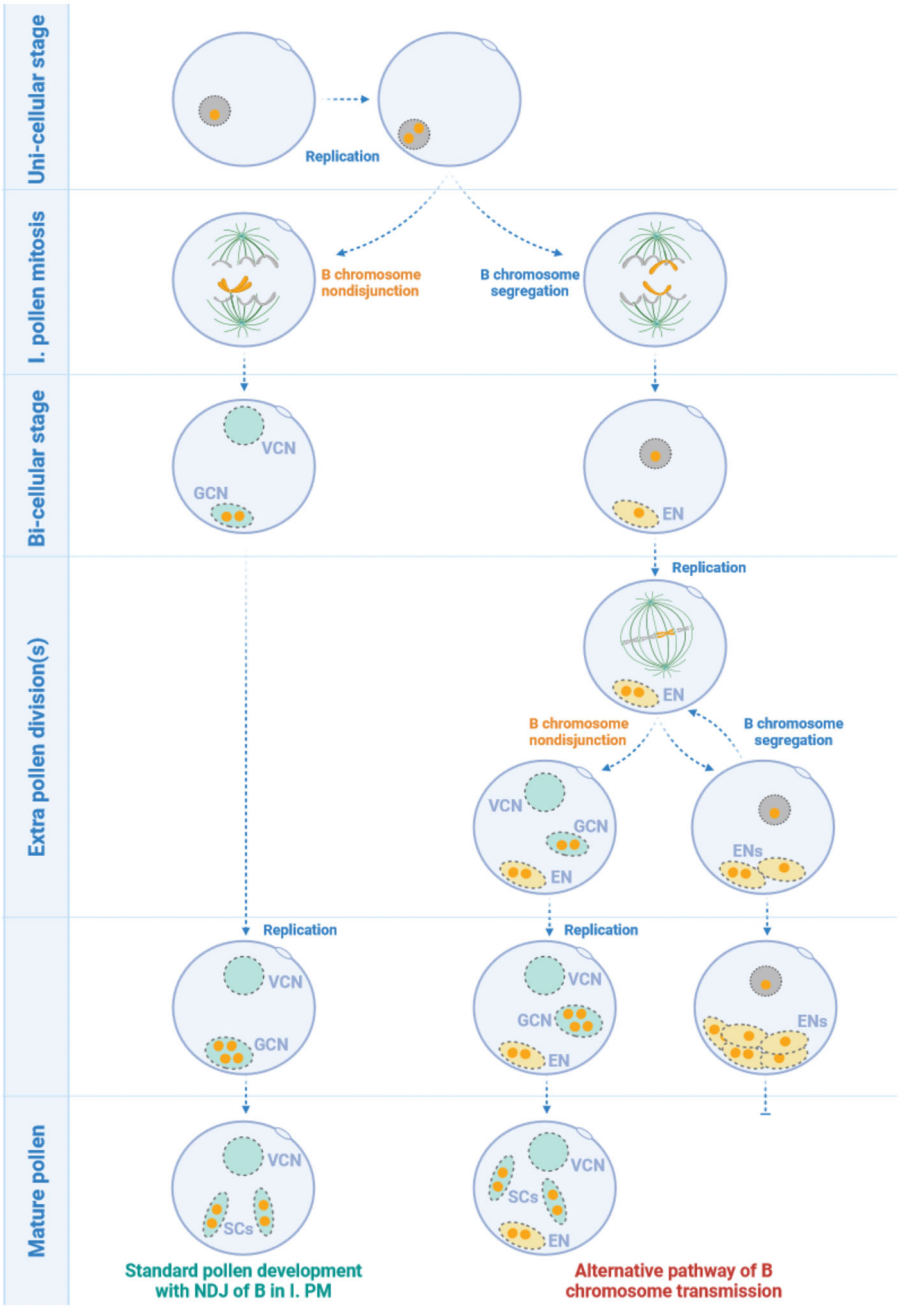

Fig. 7 Proposed model of transmission pathways of the B chromosome through pollen development in wild sorghum *Sorghum purpureosericeum*. When the B chromosome is accumulated via nondisjunction at the first pollen mitosis, the pollen development progresses as expected (left part of the scheme). The segregation of the B chromosome at first pollen division switches on the alternative pathway of extra pollen mitosis (right part of the scheme). Triggering of an extra pollen division, B chromosome makes a second attempt to accumulate. If managed, pollen mature in a regular manner, producing extracellular pollen. If it repeatedly fails, multiple copies of extra nuclei are produced and pollen is aborted. B chromosome(s) are represented as orange dots; EN, extra nucleus (yellow); VCN, vegetative nucleus; GCN, generative nucleus, and pollen nucleus is shown in grey.

chromosomes, producing two haploid nuclei, the other nucleus remains diploid and may become an extra nucleus.

The selective replication of a single chromosome would be challenging, requiring a specific replication origins and regulatory factors recognizing the B chromosome. Such model of replication has not been described to our knowledge in any eukaryotes so far. The second scenario, involving the replication of an entire extra nucleus, appears more probable. In our study, we proved that the extra nucleus most likely does not participate in fertilization and their increased ploidy would not be detrimental.

The outlined hypothesis of the wild sorghum B chromosome progression through pollen development implies that the B chromosome can 'reset' the developmental programme of daughter nuclei produced during the first pollen division. If the B chromosome fails to accumulate during this division, it is given another chance. Even if two nuclei are present in the pollen, they likely do not retain the VCN and GCN identities. The B chromosome probably alters the epigenetic profiles of these nuclei, resembling the unicellular stage. One of the nuclei is then directed back to the starting point to undergo a second attempt at the 'first pollen mitosis', ensuring nondisjunction occurs (Fig. 7).

Alternative pathway produces early germinating pollen

The purpose behind evolving the alternative accumulation mechanism remains unknown and is even more intriguing given

the uniformity of sperm produced by both pathways. The analysis of pollen tube growth reveals novel aspects of the wild sorghum B chromosome. Our observation of faster germination of abnormal pollen suggests that the extra nucleus may provide some advantage to the pollen, possibly aiding in the fertilization process. This unusual trait of extracellular pollen could potentially skew the transmission rate in favour of sperm delivered from extracellular pollen into the pollen tube. However, on initial examination, sperm from standard three-nuclei and extracellular pollen appear identical, making it unclear why sperm from extracellular grains might be preferred.

Nevertheless, the behaviour of the B chromosome depicted in this study emphasizes that accumulation pathways are the sole mechanisms leading to viable pollen, where SCs consistently carry more B chromosomes than their parent. This could lead to continuous B chromosome accumulation in progeny, ultimately resulting in lower fitness and sterility over generations. However, we did not observe a strong accumulation effect in our seed stock progeny, suggesting that the number of B chromosomes in the next generation is likely balanced through female gametes. The transmission of the B chromosome during female megasporogenesis has yet to be explored in wild sorghum, but it is worth noting that accumulation mechanisms are absent in divisions of embryo sac cells in maize (Roman, 1947).

Conclusions

We provide new, unprecedented B chromosome-associated features. Our analysis of B chromosome distribution demonstrates that B chromosome elimination is a mechanism accompanying sorghum plant growth from developing embryo to the reproductive stage and excessively affects most of the plant tissues. Furthermore, we describe a complex mechanism of B chromosome accumulation. First, the B chromosome in wild sorghum possesses a drive mechanism during the first pollen mitosis. When this accumulation process fails, the B chromosome responds by activating an alternative developmental pathway, securing the B chromosome accumulation during the extra division(s) of the pollen nucleus. Even with the limited information available, it is evident that the B chromosome of wild sorghum is extraordinary in many aspects, thus deserving scientific attention and in-depth investigation.

Acknowledgements

We thank the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) for providing the original seed stock of *Sorghum purpureosericeum*. We thank Jitka Weisserová and Helena Tvardíková for their technical assistance, Petr Smýkal and Lucie Kobrlová (Palacký University Olomouc, Czech Republic) for allowing access to the cryostat and Jana Čížková for histogram conversion. The work was supported by The Czech Science Foundation (grant no.: 22-02108S, to MK), the Czech Academy of Sciences (project no.: DAAD-22-02, to MK), the China Scholarship Council (CSC202006850005, to JCh), Deutsche Forschungsgemeinschaft (HO1779/30-1 and 30-2, to AH) and DAAD (57601714, to AH). Open access publishing facilitated by the Institute of Experimental Botany of the Czech Academy of Sciences, as a part of Wiley - CzechLib agreement. Open access publishing facilitated by Ustav experimentalni botaniky Akademie ved Ceske republiky, as part of the Wiley - CzechELib agreement.

Competing interest

None declared.

Author contributions

MK and JB designed the experiments. MK, JB, AH, JC and DH interpreted data and wrote the manuscript. MK, MS, TB, NH and VK performed all experiments. AD conducted the confocal microscopy. All authors approved the final version of the manuscript.

ORCID

Jan Bartoš https://orcid.org/0000-0002-4154-8895
Tereza Bojdová https://orcid.org/0009-0004-7257-270X
Jianyong Chen https://orcid.org/0000-0002-6996-1032
Alžběta Doležalová https://orcid.org/0000-0002-1926-9443
David Honys https://orcid.org/0000-0002-6848-4887
Andreas Houben https://orcid.org/0000-0003-3419-239X
Miroslava Karafiátová https://orcid.org/0000-0003-1177-6472
Vinod Kumar https://orcid.org/0000-0002-2537-1209
Magdaléna Stejskalová https://orcid.org/0009-0005-4972-3909

Data availability

The data that support the findings of this study are available in the main article and Tables S1–S5 of this article.

References

Alexander MP. 1969. Differential staining of aborted and non-aborted pollen. *Stain Technology* **44**: 117–122.

Baenziger H. 1962. Supernumerary chromosomes in diploid and tetraploid forms of crested wheatgrass. *Canadian Journal of Botany* **40**: 549–561.

Becher H, Powell RF, Brown MR, Metherell C, Pellice J, Leitch IJ, Twyford AD. 2021. The nature of intraspecific and interspecific genome size variation in taxonomically complex eyebrights. *Annals of Botany* **128**: 639–651.

Bednářová M, Karafiátová M, Hřibová E, Bartoš J. 2021. B chromosomes in genus Sorghum (Poaceae). *Plants* **10**: 1–13.

Bernardino AC, Cabral-de-Mello DC, Machado CB, Palacios-Gimenez OM, Santos N, Loreto VB. 2017. B chromosome variants of the grasshopper Xyleus discoideus angulatus are potentially derived from pericentromeric DNA. *Cytogenetic and Genome Research* **152**: 213–221.

Biederman MK, Nelson MM, Asalone KC, Pedersen AL, Saldanha CJ, Bracht JR. 2018. Discovery of the first germline-restricted gene by subtractive transcriptomic analysis in the zebra finch *Taeniopygia guttata*. *Current Biology* **28**: 1620–1627.

Birchler JA. 2015. Promises and pitfalls of synthetic chromosomes in plants. *Trends in Biotechnology* **33**: 189–193.

Birchler JA, Yang H. 2021. The supernumerary B chromosome of maize: drive and genomic conflict. *Open Biology* **11**: 210197.

Borodin P, Chen A, Forstmeier W, Fouché S, Malinovskaya L, Pei Y, Reifová R, Ruiz-Ruano F, Schlebusch SA, Sotelo-Muñoz TA *et al.* 2022. Mendelian nightmares: the germline –restricted chromosome of songbirds. *Chromosome Research* **30**: 255–272.

Chen J, Birchler JA, Houben A. 2022. The non-Mendelian behaviour of plant B chromosomes. *Chromosome Research* **30**: 229–239.

Cuevas A, Ravinet M, Sætre GP, Eroukhmanoff F. 2021. Intraspecific genomic variation and local adaptation in a young hybrid species. *Molecular Ecology* **30**: 791–809.

Darlington CD, Thomas TRS, Thomas PT. 1941. Morbid mitosis and the activity of inert chromosomes in Sorghum. *Proceedings of the Royal Society B: Biological Sciences* **130**: 127–150.

Doležel J, Binarová P, Lucretti S. 1989. Analysis of nuclear DNA content in plant cells by flow cytometry. *Biologia Plantarum* **31**: 113–120.

Ebrahimzadegan R, Fuchs J, Chen J, Schubert V, Houben A, Mirzaghaderi G. 2023. Meiotic segregation and post-meiotic drive of the Festuca pratensis B chromosome. *Chromosome Research* **31**: 26.

Escriba MC, Giardini MC, Goday C. 2011. Histone H3 phosphorylation and nondisjunction of the maternal X chromosomes during male meiosis in sciarid flies. *Journal of Cell Sciences* **124**: 1715–1725.

Escriba MC, Goday C. 2013. Histone H3 phosphorylation and elimination of paternal X chromosomes at early cleavages in sciarid flies. *Journal of Cell Sciences* **126**: 3214–3222.

Fahn A. 1990. *Plant anatomy, 4th edn.* Oxford, UK: Pergamon Press.

Fröst S. 1957. The inheritance of accessory chromosomes in Centauria scabiosa. *Hereditas* **43**: 403–422.

Fröst S. 1969. The inheritance of accessory chromosomes in plants, especially in *Ranunculus acris* and *Pheleum nodosum. Hereditas* **61**: 317–326.

Goday C, Esteban MR. 2001. Chromosome elimination in sciarid flies. *BioEssay* **23**: 242–250.

Gutiérrez ML, Rodríguez-González R, Fuentes I, Gálvez-Prada F, Kovařík A, Garcia S. 2023. First update to B-Chrom: a database on B-chromosomes. In: Garcia S, Nualart N, eds. *Plant genomic and cytogenetic databases. Methods in molecular biology, vol. 2703.* New York, NY, USA: Humana, 227–236.

Häkansson A. 1954. Transmission of accessory chromosomes in *Poa alpina. Hereditas* **40**: 523–526.

Hariprasanna K, Patil JV. 2015. Sorghum: origin, classification, biology and improvement. In: Madhusudhana R, Rajendrakumar P, Patil J, eds. *Sorghum molecular breeding.* New Delhi, India: Springer, 3–20.

Houben A. 2017. B chromosomes – a matter of chromosome drive. *Frontiers in Plant Science* **15**: 210.

Houben A, Ma W, Banaei-Moghaddam AM. 2021. The B chromosome of rye. In: Rabanus-Wallace MT, Stein N, eds. *The Rye genome. Compendium of plant genomes.* Cham, Switzerland: Springer, 63–76.

Janaki-Ammal EK. 1940. Chromosome diminuition in plant. *Nature* **146**: 839–840.

Jones RN, Gonzáles-Sanchez M, Gonzáles-García M, Vega JM, Puertas MJ. 2008. Chromosomes with a life of their own. *Cytogenetic and Genome Research* **120**: 265–280.

Jones RN, Rees H. 1982. *B chromosomes.* London, UK: Academic Press.

Karafiátová M, Bartoš J, Kopecký D, Ma L, Sato K, Houben A, Stein N, Doležel J. 2013. Mapping nonrecombining regions in barley using multicolor FISH. *Chromosome Research* **21**: 739–751.

Karafiátová M, Bednářová M, Said M, Čížková J, Holušová K, Blavet N, Bartoš J. 2021. B chromosome of *Sorghum purpureosericeum* reveals the first pieces of its sequence. *Journal of Experimental Botany* **72**: 1606–1616.

Kato A, Lamb JC, Birchler JA. 2004. Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. *Proceedings of the National Academy of Sciences, USA* **101**: 13554–13559.

Lie Y, Fu X, Thao M, Zhang W, Li B, An D, Li J, Zhang A, Liu R, Liu X. 2018. A genome-wide transcriptome dynamics during early spike development in bread wheat. *Scientific Reports* **8**: 15338.

Lynch M, Marinov GK. 2015. The bioenergetic costs of a gene. *Proceedings of the National Academy of Sciences, USA* **112**: 15690–15695.

Majka J, Glombik M, Doležalová A, Kneřová J, Ferreira MTM, Zwierzykowski Z, Duchoslav M, Studer B, Doležel J, Bartoš J *et al.* 2023. Both male and female meiosis contribute to non-Mendelian inheritance of parental chromosomes in interspecific plant hybrids (*Lolium* × *Festuca*). *New Phytologist* **238**: 624–636.

MsLeisch J. 1954. B-chormosomes in *Ranunculus ficaria. Annual Report – John Innes Horticultural Institution* **45**: 21–22.

Müntzing A, Nygren A. 1955. A new diploid variety of *Poa alpina* accessory chromosomes at meiosis. *Hereditas* **41**: 405–422.

Němečková A, Koláčková V, Vrána J, Doležel J, Hřibová E. 2020. DNA replication and chromosome positioning throughout the interphase in three-dimensional space of plant nuclei. *Journal of Experimental Botany* **71**: 6262–6272.

Nygren A. 1957. *Poa timoleontis* Heldr., a new diploid species of the section Bolbophorum and Gr. with accessory chromosomes only in meiosis. *Kungliga Lantbrukshögskolans Annaler* **23**: 489–495.

Östergren G, Frost S. 1962. Elimination of accessory chromosomes from the roots in *Haplopappus gracilis. Hereditas* **48**: 363–366.

Pereira HS, Delgado M, Viegas W, Rato JM, Barao A, Caperta AD. 2017. Rye (Secale cereal) supernumerary (B) chromosomes associated with heat tolerance during early stahes of male sporogenesis. *Annals of Botany* **119**: 325–337.

Plowman A, Bougourd S. 1994. Selectively advantageous effects of B chromosomes on germination behaviour in *Allium schoenoprasum* L. *Heredity* **72**: 587–593.

Rees H. 1974. B chromosomes. *Science Progress* **61**: 535–554.

Reynolds TL. 1997. Pollen embryogenesis. *Plant Molecular Biology* **33**: 1–10.

Roman H. 1947. Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. *Genetics* **32**: 391–409.

Roman H. 1948. Directed fertilization in maize. *Proceedings of the National Academy of Sciences, USA* **34**: 36–42.

Ruban A, Schmutzer T, Wu DD, Fuchs J, Boudichevskaia A, Rubtsova M, Pistrick K, Melzer M, Himmelbach A, Schubert V *et al.* 2020. Supernumerary B chromosomes of *Aegilops speltoides* undergo precise elimination in roots early in embryo development. *Nature Communications* **11**: 2764.

Said M, Hřibová E, Danilova TV, Karafiátová M, Čížková J, Friebe B, Doležel J, Gill BS, Vrána J. 2018. The *Agropyron cristatum* karyotype, chromosome structure and cross-genome homeology as revealed by fluorescence *in situ* hybridization with tandem repeats and wheat single-gene probes. *Theoretical and Applied Genetics* **131**: 2213–2227.

Sánchez L. 2014. Sex-determining mechanisms in insects based on imprinting and elimination of chromosomes. *Sexual Development* **8**: 83–103.

Schmid M, Ziegler CG, Steinlein C, Nanda I, Haaf T. 2002. Chromosome banding in Amphibia: XXIV. The B chromosomes of *Gastrotheca espeletia* (Anura, Hylidae). *Cytogenetic and Genome Research* **97**: 205–218.

Schoenmakers S, Wassenaar E, Laven JS, Grootegoed JA, Baarends WM. 2010. Meiotic silencing and fragmentation of the male germline restricted chromosome in zebra finch. *Chromosoma* **119**: 311–324.

Stevens JP, Bougourd SM. 1994. Unstable B-chromosomes in a European population of *Allium schoenoprasum* L. (Liliaceae). *Biological Journal of the Linnean Society* **52**: 357–363.

Steward FC. 1970. From cultured cells to whole plants: the induction and control of their growth and morphogenesis. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **175**: 1–30.

Touraev A, Ilham A, Vicente O, Heberle-Bors E. 1996. Stress induced microspore embryogenesis in tobacco – an optimized systems for molecular studies. *Plant Cell Reports* **15**: 561–565.

Vasil IK. 1984. *Cell culture and somatic cell genetics of plants. Vol 1. Laboratory procedures and their applications.* New York, NY, USA: New York Academic Press.

Walla A, Wilma van Esse G, Kirschner GK, Guo G, Brünje A, Finkemeier I, Simon R, von Korff M. 2020. An Acyl-CoA N-acyltransferase regulates meristem phase change and plant architecture in barley. *Plant Physiology* **3**: 1088–1109.

Wang J, Davis RE. 2015. Programmed DNA elimination in extra-cellular organism. *Current Opinion in Genetics & Development* **27**: 26–34.

Wu DD, Ruban A, Fuchs J, Macas J, Novák P, Vaio M, Zhou YH, Houben A. 2019. Nondisjunction and unequal spindle organization

accompany the drive of *Aegilops speltoides* B chromosomes. *New Phytologist* **223**: 1340–1352.

Wu TP. 1984. B–chromosomes in *Sorghum purpureosericeum*. *Proceedings of National Science Council, Republic of China* **8**: 198–209.

Zufall RA, Robinson T, Katz LA. 2005. Evolution of developmentally regulated genome rearrangements in eukaryots. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **304**: 448–455.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Verification of CL135 probe specificity for B chromosome of wild sorghum *Sorghum purpureosericeum*.

Fig. S2 Tissues and organs of *Sorghum purpureosericeum* studied for the presence of the B chromosome.

Fig. S3 Flow histograms of seven tissues of *Sorghum purpureosericeum* with no significant +B population detected.

Fig. S4 Flow histograms of tissues evaluated as B-positive.

Fig. S5 Flow cytometric analysis of developing seed 10 DAP in 0B and +B plant.

Fig. S6 PCR-based B chromosome status of selected tissues.

Fig. S7 FISH-based detection of nuclei carrying B chromosome in tissues evaluated as B-positive.

Fig. S8 Atypical behavioural patterns of the B chromosome in meiotic division of 1B, 2B and 3B *Sorghum purpureosericeum* plant.

Fig. S9 Analysis of pollen viability in parental plants of *Sorghum purpureosericeum* harbouring from 0 up to 4B chromosomes.

Methods S1 Supplementary methods.

Table S1 Proportion of nuclei carrying B chromosome in tissues of *Sorghum purpureosericeum* plant evaluated as B-positive.

Table S2 Observation of the B chromosome transmission during the pollen development of 1B pollen of *Sorghum purpureosericeum*.

Table S3 Frequency of individual transmission pathways in the development of 1B pollen of *Sorghum purpureosericeum*.

Table S4 Counts for pollen viability assessment in plants of *Sorghum purpureosericeum* carrying up to 4B chromosomes.

Table S5 Evaluation of the effect of extra nucleus formation on early germination of the pollen grain of *Sorghum purpureosericeum*.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.