Nucleus (December 2015) 58(3):199–206
DOI 10.1007/s13237-015-0151-2

ORIGINAL ARTICLE

The D-genome plays a critical role in the formation of haploid *Aegilops tauschii* through *Imperata cylindrica* mediated uniparental chromosome elimination

Yasuhiko Mukai[1] · Gentatsu Okamoto[1] · Shiho Kiryu[1] · Satoru Takemoto[1] · Santosh Kumar Sharma[1] · Go Suzuki[1] · Maki Yamamoto[2]

Received: 5 October 2015 Published online: 23 December 2015

Abstract Doubled haploid (DH) breeding eventually offers unique advantages for rapid genetic improvement of wheat in a single generation by achieving absolute homozygosity and enhanced selection efficiency. A new perennial invasive weed i.e., *Imperata cylindrica* has already proved its importance in haploid wheat induction through *Imperata*-mediated chromosome elimination process. Whereas, cytological evidences and the actual mechanism of haploid induction through chromosome elimination in wheat x *I. cylindrica* hybrids are well known, but which genome(s) of wheat (AA/BB/DD) are actually involved in such haploid formation has remained elusive. Therefore, in order to identify the genome-specific triggering of *Imperata*-mediated chromosome elimination in development of wheat haploids, 11 wheat genotypes including *Ae. tauschii* and D-genome chromosome substitution lines of the durum wheat variety 'Langdon' were crossed with *I. cylindrica* and examined with the prime aim of producing haploids of *Ae. tauschii*. The crosses suggest that only bread wheat, *Ae. tauschii* and Triticale (containing D genome) were successful to develop haploid plants through chromosome elimination process. Higher frequencies of embryo formation were obtained primarily in substitution lines with chromosomes 7D. The detailed anatomical and cytological analyses suggest that : (i) genome/genotypic specificity plays a key role in haploid induction through *Imperata*-mediated chromosome elimination process, (ii) D-genome triggers the chromosome elimination and haploid production in wheat x *Imperata,* (iii) some D-genome chromosomes (primarily 7D) substituted lines in wheat genetic background may enhance crossability with *Imperata* in the formation of haploid wheat. To the best of our knowledge, this is the first report of haploid induction in *Ae. tauschii*.

Keywords *Aegilops tauschii* · Haploid · *Imperata* · Chromosome elimination · FISH

Introduction

Doubled haploid (DH) breeding system in wheat offers opportunity to realize complete homozygosity in hybrid plants in a span of just in one generation [3, 4, 16]. Production of doubled haploids is an important methodology to speed the process of breeding and development of mapping populations in plants, thereby facilitate crop improvement, genetic manipulation, and genome and gene mapping. Durum wheat (*Triticum durum*, $2n = 4x = 28$; AABB genomes) and common wheat (*Triticum aestivum*, $2n = 6x = 42$; AABBDD genomes) are two important cereal crops for human consumption.

Conventionally, the development of durum and common wheat was mainly implemented through backcross selection, pedigree selection, bulk selection and single-seed descent methods [13]. Nevertheless, such conventional breeding methods require several generations (at least five) of self-pollination to achieve homozygous lines. In contrast, the double haploid breeding method allows wheat breeders to develop 'completely homozygous' lines in one (F1 or F2) generation [13]. In this context, the induction of haploids via wheat x maize (*Zea mays*) hybridization involving uniparental chromosome elimination phenomenon is a popular approach for

✉ Yasuhiko Mukai
ymukai@cc.osaka-kyoiku.ac.jp

[1] Laboratory of Plant Molecular Genetics, Division of Natural Sciences, Osaka Kyoiku University, 4-698-1 Asahigaoka, Kashiwara, Osaka 582-8582, Japan

[2] Faculty of Allied Health Science, Kansai University of Welfare Science, Osaka 582-0026, Japan

breeding common wheat and for developing mapping populations in both durum and common wheat. In the past several years, DH method has been successfully employed for induction of haploid wheat and mapping populations. Interestingly, the combination of parental species mainly determines the degree of chromosome elimination. For example, in wheat x barley [17, 18], paternal chromosomes were eliminated partially whereas wheat x *Hordeum bulbosum*, maize, pearl millet [1, 11, 12] showed complete loss of paternal chromosomes. Although the wheat x maize system of haploid production is quite successful, but for its practical implementation it is desirable that the maize flowering regime is coincidental with wheat flowering. As such, the maize crop is required to be grown in greenhouse so that the flowering in maize is concurrent with the flowering in wheat. Recently, intergeneric hybridization between wheat and a wild weedy species, *Imperata cylindrica* resulted in the recovery of a high frequency of wheat haploids, which were obtained through the elimination of *I. cylindrica* chromosomes [3, 4, 10]. The utility of *I. cylindrica* in wheat haploid production has already been confirmed and successfully carried out using bread wheat by Chaudhary et al. [4]. The important advantages of using *I. cylindrica* involves the natural coincidence of flowering period of wheat under the sub-temperate condition that ultimately enables to obtain fresh pollen of *I. cylindrica* for hybridization, high-frequency of haploids and genotype non-specific crossability with wheat [4, 10]. It is important to note that cytological evidences and the actual mechanism of haploid induction through chromosome elimination in wheat x *I. cylindrica* hybrids are completely known [10]. However, which genome of wheat (AA/BB/DD) is actually involved in such haploid formation is still elusive.

In the present study, various species of wheat tribe having diversified genotypes and their relatives were chosen to induce haploid production through the chromosome elimination of *Imperata* cylindrica. Further, an attempt has been made to identify the genome-specific triggering of chromosome elimination in *Aegilops tauschii* x *Imperata cylindrica* hybrids. The crossability of wheat genotypes including *Ae. tauschii* with *I. cylindrica* as well as D-genome chromosome substitution lines of the durum wheat variety 'Langdon' are examined with the prime aim of producing haploids of *Ae. tauschii*. Subsequently, the individual chromosomes specific to the genome that eventually triggers the uniparental chromosome elimination and control the haploid induction in wheat x *Imperata* hybrid have also been identified. The present investigation ultimately serves as a fundamental platform to understand the genome-specific chromosomal elimination phenomenon in double haploid production in plants, and in particular offer broad scope of generation of haploid wheat having genome-specific quality traits in near future.

Materials and methods

Plant materials

Hexaploid wheat i.e., *Triticum aestivum* cv. 'Chinese Spring' (AABBDD, $2n=42$) and *I. cylindrica* ($2n=2x=20$) were used as primary female and pollen parents, respectively. Further, ten more plants species belonging to family Poaceae including wheat tribe (Triticeae) having diverse genome(s) viz. durum wheat (*Triticum durum*, AABB), wild emmer wheat (*Triticum dicoccoides*, AABB), diploid wheat (*T. monococoum,* AA), *Aegilops tauschii* (DD), *Ae. speltoides* (SS), rye (*Secele cereale*, RR), barley (*Hordeum vulgare*, HH), Triticale (AABBD/RD/R) as well as oat (*Avena sativa*) and rice (*Oryza sativa*) were replaced simultaneously as female parents too. The information about the wheat species with their genomic details that were crossed with *I. cylindrica* has been provided in the Table 1. Further, a D-genome chromosome substitution line of the durum wheat variety 'Langdon' in which a pair of durum wheat homoeologues (either AA or BB) replaces with a pair of D-genome chromosomes also crossed with *I. cylindrica* to investigate the genome-specific chromosome elimination in induction of haploid *Ae. tauschii*.

Crossing procedure

Crossings were performed as described by Chaudhary et al. [4]. The *Ae.tauschii* spikes were emasculated before anthesis and pollinated with fresh pollen of *I. cylindrica* after 3 days. Embryos were excised from immature seeds 13 to 16 days after pollination (DAP) and grown at 20 °C for several weeks on the MS medium with essential supplements as described in Komeda et al. [10]. The regenerated plantlets were transferred to the field after substantial growth.

Paraffin sectioning and histological analyses of ovules

A standard and established method of paraffin embedding, sectioning analysis was followed to study the embryo sac development by examining histological serial sections of the ovules at different maturity period. Female florets at different stages of maturity were collected and fixed in FAA for 24 h and then transferred to 70 % ethanol. Pistils were dissected, dehydrated and embedded in paraffin wax, subsequently sectioned at approximately 10 μm and double-stained with a Safranin–Fastgreen combination and then mounted in Canada balsam.

Molecular cytological analyses

The root tips were collected for chromosome analyses from regenerated plantlets of *Ae. tauschii* x *I. cylindrica*. The pretreatment and fixation procedures of the root tips have been

Table 1 The list of members of wheat tribe (Triticeae) having diverse genome(s) that were crossed with *Imperata cylindrica* to obtain haploid plantlets following the chromosome elimination

S. No.	Scientific name	Common name	Genome	Chromosome elimination	Development of haploid plant
1.	*Triticum aestivum* cv. Chinese Spring	bread wheat	AABBDD	Yes	Yes
2.	*T. durum* cv. Langdon	durum wheat	AABB	Yes	No
3.	*T. dicoccides* KU8736A	wild emmer wheat	AABB	No	No
4.	*T. monococoum* Early mutant	diploid wheat	AA	No	No
5.	*Ae. speltoides* KU1-3	–	SS	No	No
6.	*Aegilops tauschii var.strangulata*	–	DD	Yes	Yes
7.	*Secele cereale* cv. Imperial	rye	RR	No	No
8.	*Hordeum vulgare* cv. Betzes	barley	HH	No	No
9.	Triticale (hexaploid)	–	AABBD/RD/R	Yes	Yes
10.	*Avena sativa*	oat	–	No	No
11.	*Oryza sativa*	rice	–	No	No

described elsewhere [10]. Chromosome preparations were made as described by Mukai et al. [14]. Subsequently, the cross-section analysis of dividing embryos from 1 to 10 DAP was also performed. A Clone pTa71, a 9-kb EcoR1 fragment from common wheat, containing the coding sequences from the 18S, 5.8S and 26S rRNA genes and the intergenic spacer sequence, was utilized as FISH probe. The molecular cytogenetic analysis using rDNA probe (pTa71) along with a binary cosmid clone including centromeric region of *Ae. tauschii* i.e., CENT14 [5] were employed to the metaphase chromosomes of root tips obtained after 28 days following embryo rescue from *Ae. tauschii* x *I. cylindrica* plantlets using FISH technique. Further, the same probes were also employed to the nucleus of embryo at stage of 12 DAP. The fixation and squashing procedures of embryos have been described elsewhere [10]. The fluorescent signals were detected with a Zeiss Axioscope fluorescence microscope equipped with a cooled CCD camera (Hamamatsu Photonics, 4880). The images were pseudo-colored and merged by Photoshop 5.0 (Adobe).

Results

D-genome triggers the chromosome elimination and haploid production in *wheat* x *Imperata*

The detailed analysis of chromosome elimination and haploid production has been summarized in Table 1. The crossing of wheat and related species with *Imperata* suggests that only those having D genome (bread wheat, *Ae. tauschii* and Triticale) were successful to develop haploid plants through chromosome elimination process (Table 1). Interestingly, the durum wheat (AABB) also showed chromosome elimination, but it did not induce the haploid production (Table 1). Such crossing experiments along with analysis of chromosome elimination clearly indicated the role of D-genome in chromosome elimination process during haploid induction.

Ae. tauschii x *I. cylindrica:* fertilization frequency and embryo formation

A total of 928 florets of *Ae. tauschii* pollinated with *Imperata* pollens produced 659 pseudo-seeds (71 %) in DAP ranging from 12 to 21 (Table 2). The dissection of the pseudo-seed showed the presence of aqueous solution, without the solid endosperm that is the characteristic feature of a true seeds (Fig. 1a–b). Such embryos of pseudo-seeds were rescued and germinated under in vitro conditions (Fig. 1c). After successful germination, the haploid plantlets were transferred to soil to grow naturally (Fig. 1d). The cross-sectional analysis of developing embryos from 1 DAP to 10 DAP *Ae. tauschii* x *Imperata* evidently showed various stages of nuclear divisions to form a multicellular embryo (Fig. 2). Percent frequency of embryo formation was quite low when compared to the formation of pseudo-seeds. A total of 105 (11 %) embryos were rescued out of the 928 florets crossed. The 14 and 16 DAP plantlets showed highest frequency of embryo formation (15

Table 2 The crossability of *Aegilops tauschii* x *Imerata cylindrica* and formation of haploid *Ae. tauschii* through *Imperata*-mediated chromosome elimination

S. No.	Days after pollination (DAP)	Number of florets crossed	Pseudo-seed (%)	Embryo obtained (%)	Haploid plants (%)
1.	12	94	69(73)	8(9)	0(0)
2.	14	126	96(76)	19(15)	5(4)
3.	16	351	229(65)	47(13)	15(4)
4.	17	156	112(72)	13(8)	2(1)
5.	18	48	36(75)	3(6)	1(2)
6.	20	129	99(77)	14(11)	3(2)
7.	21	24	18(75)	1(4)	1(4)
		928	659(71)	105(11)	27(3)

and 13 %, respectively (Table 2). A total of 27 haploid plants of *Ae. tauschii* were obtained (Fig. 3a–d), out of which highest (15) were produced by 16 DAP plantlets (Table 2).

Molecular cytogenetic analysis confirms the formation of haploid *Ae. tauschii*

The detailed cytogenetic analysis including conventional mitotic and meiotic behavior, followed by confirmation of chromosome elimination through GISH analysis in formation of wheat haploids has already been discussed by Komeda et al. [10]. In the present study, molecular cytogenetic analysis using FISH technique was performed on the mitotic chromosomes of *Ae. tauschii* x *I. cylindrica*. CENT 14 probe including centromeric region of *Ae. tauschii* eventually showed 7 centromeric signals and confirmed the haploid nature of the obtained plantlets. Similarly, a nucleus of 12 DAP embryo also reveled the presence of haploid genome of *Ae. tauschii* only by showing the 7 centromeric signals. Only a single signal of rDNA also confirmed the haploid induction of *Ae. tauschii* (Fig. 4a–b).

The 7D chromosome has important role in triggering the haploid induction in *Ae. tauschii* x *I. cylindrica*

The detailed anatomical and cytological investigations were performed on the haploid embryos formed by crossing between durum wheat disomic substitution lines 'Langdon'

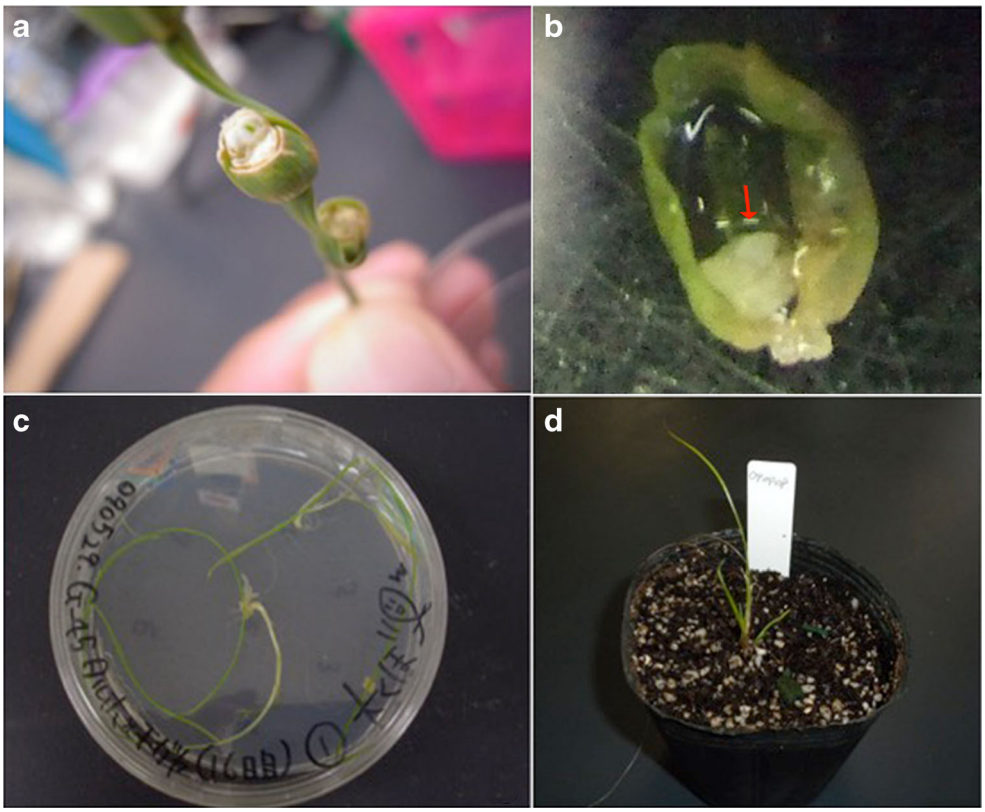

Fig. 1 Haploid production of *Aegilops tauschii* crossed with *Imperata cylindrica*. **a** *Ae. tauschii* embryos at 16 days after pollination(DAP) **b** dissected pseudo-seeds shows the absence of endosperm and contain only watery substance **c** the rescued embryo transformed into plantlet in vitro on nutrient medium at 28 DAP **d** plantlets after transferring to the soil

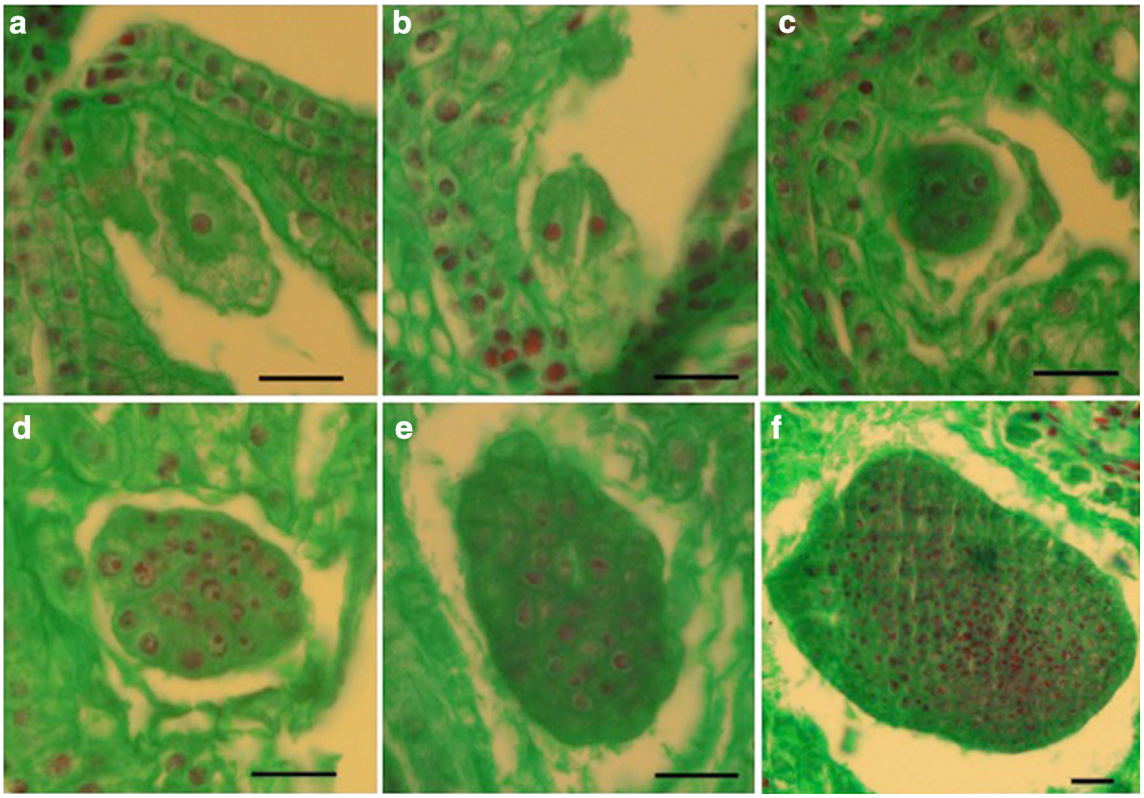

Fig. 2 Histological analysis of cross-sections of the developing ovule of the *Aegilops tauschii* x *I. cylindrica* at (**a**) 1 DAP with fertilized egg cell (**b**) 2 DAP with the two-celled stage embryo (**c**) 3 DAP (**d**) 5 DAP (**e**) 7 DAP and (**f**) 10 DAP with haploid embryo; Scale bar = 20 μm

and *I. cylindrica.* Critical analysis of the anatomical and cytological data suggests that the substitution lines with 7D (7A) and 7D (7B) successfully induce the haploid formation in *Ae. tauscii* (Fig. 5). Further, infrequent and a low number of haploid embryos were also obtained with substitution line with 1D (1A), 1D (1B), 2D (2A) and 5D (5B) chromosomes.

Fig. 3 Haploid plant of *Ae. tauschii.* (**a**) complete morphology of the haploid plantlet (**b**) comparison of the haploid (*left*) and diploid (*right*) *Ae. tauschii* plants; in haploids, anther does not come out of the florets and pollen is complete sterile

Discussion

Imperata cylindrica: A boon for wheat breeding programs

Though haploid production through wheat x maize crossing is quite successful under sub-temperate conditions, however, the maize crop needs to be grown in greenhouse as it is non-tolerant to the low temperature of the winter season thereby increasing the cost of haploid production [3, 4]. In the recent years, *Imperata cylindrica*, mediated chromosome elimination system has been emerged as an efficient alternative to the widely used maize- mediated system in terms of natural coincidence for flowering commensurate with the wheat, abundant pollen supply for longer span and developing significantly higher frequency of doubled haploids from winter and spring wheat ecotypes [4, 10]. The *I. cylindrica*- mediated system has evinced appreciable performance for the triticale × wheat derivatives where maize-mediated system remained unsuccessful [9]. Further, several reports [2, 3, 9, 16] also favor the utilization of the *I. cylindrica* as alternative to maize in haploid wheat production. Wheat haploid induction using *I. cylindrica* pollen has been reported in India and Japan so

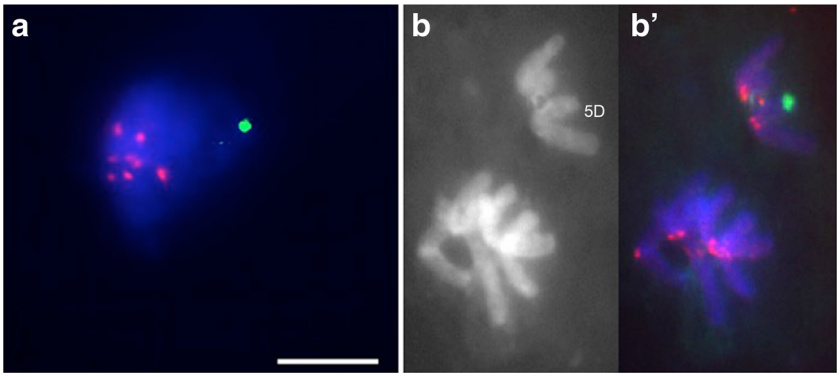

Fig. 4 Confirmation of haploid nature of the plantlets through molecular cytogenetic analysis of the *Ae. tauschii* x *I. cylindrica* using a centromeric-specific repeat CENT14 and a rDNA probes (**a**) a embryo nucleus showing seven signals of centromeric repeat and a single signal of rDNA (**b**) root tip mitotic metaphase in DAPI (**b'**) same metaphase showing seven signals of CENT14 along with single rDNA signal on chromosome 5D confirming the haploid nature of the *Ae. tauschii.* Scale bar = 10 μm

far [3, 4, 10, 15, 16]; however, efforts are also in progress in other countries too [2] to utilize the potential of *I. cylindrica* in haploid wheat production. The occurrence of uneven genotypes of *I. cylindrica* distributed in other regions of the world should be examined/exploited for haploid induction in wheat and allied genera too.

Genome/genotypic specificity plays a key role in haploid induction of *Ae. tauchii* through *Imperata*-mediated chromosome elimination process

The combination of parental species mainly determines the degree of chromosome elimination in inter-generic hybrids as evidenced by oat x maize, and wheat x barley [17, 18] where paternal chromosome were not eliminated completely. In the present study, various members of poaceae family having diversified genotype were chosen to identify the effect of genome specificity in chromosome elimination of *Imperata cylindrica* and subsequent formation of haploid plants. It is noteworthy to mention that only a few genotypes successfully produced the haploid plants through *Imperata*-mediated chromosome elimination. The critical analyses of the female parents revealed that only the genotypes having D-genome i.e., Bread wheat (AABBDD), *Ae. tauschii* (DD) and Tritacale (AABBD/RD/R) were able to induce the haploid formation by *Imperata*-mediated chromosome elimination process. This crossability variation is attributed to the absence of the D genome in wheat cultivars, and thus limiting the application

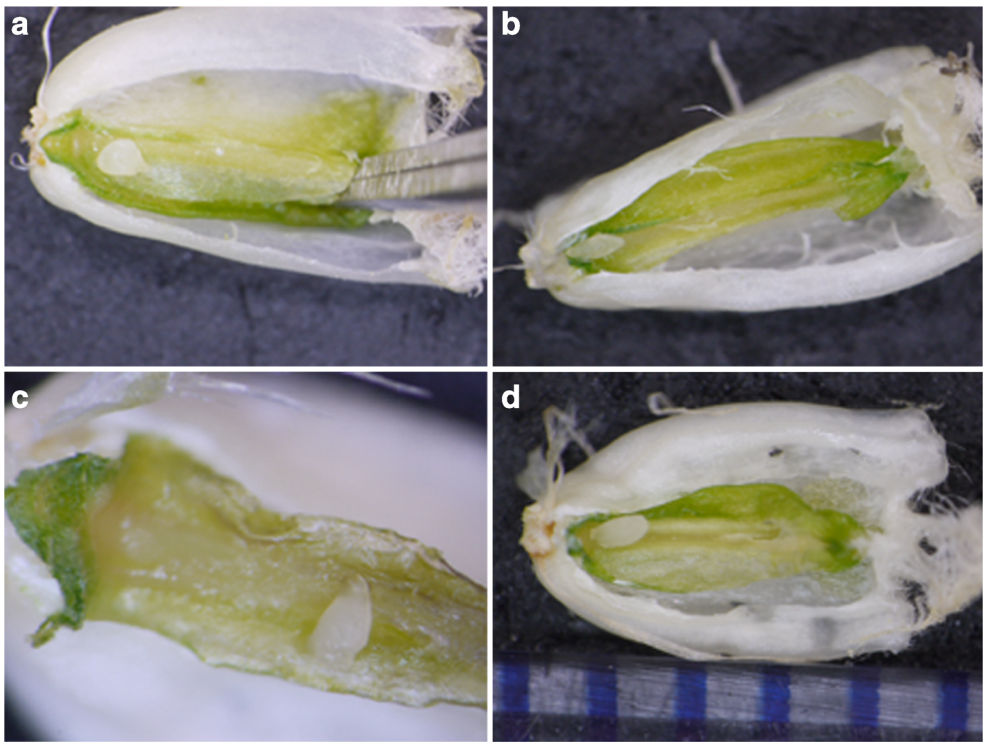

Fig. 5 The cross section analysis of crosses between a D-genome chromosome substitution line of the durum wheat variety 'Langdon' in which a pair of durum wheat homoeologues (either AA or BB) replaces with a pair of D-genome chromosomes with *I. cylindrica* in order to investigate the genome-specific chromosome elimination in induction of haploid *Ae. tauschii* and subsequent embryo formation. Haploid embryos obtained with 'langdon' substitution line (**a**) with 1D (1B) chromosomes (**b**) with 5D (5B) chromosomes (**c**) with 7D (7A) and (**d**) 7D (7B) chromosomes

of haploid production in breeding programs. Recently, Celiktaş et al. [2] reported the haploid production of durum wheat through *Imperata*-mediated chromosome elimination process. Interestingly, our report suggests that crosses of durum wheat (AABB) x *Imperata* do induce the chromosome elimination process, but fail to develop the haploid of durum wheat. In this context, the study conducted by Rather et al. [16] may be worth mentioning. They [16] assessed four Indian and one Japanese accession of *I. cylindrica* for their influence upon haploid of 21 wheat crosses (winter × spring, spring × spring and winter × winter) to find an efficient pollen source for haploid induction, which would enhance doubled haploid breeding in bread wheat. Interestingly, the frequency of haploid induction was found to be influenced differently by the wheat and the *I. cylindrica* genotypes, indicating both maternal and paternal genetic influence on haploid induction. In this context, Inagaki et al. [7] also suggested earlier that distinct genotypic variation in female parent affect the crossability of durum wheat with maize. Here, it is proposed that the genome/genotypic specificity have important role in chromosome elimination mediated haploid induction in wheat as suggested earlier by Inagaki et al. [7]. Therefore, utilization of D-genome chromosomes substituted line of wheat may enhance crossability with *Imparata* and subsequent haploid development in future.

Effect of D-genome chromosomes on haploid production in *Ae. tauschii*

The subsequent crosses of D-genome chromosome substitution lines of durum wheat 'Langdon' with *Imparata* showed seed development and embryo formation. It is quite interesting that 1D, 2D, 5D and 7D-substituted chromosome were found to have increased crossability of durum wheat 'Langdon' with *Imperata*. However, substituted chromosomes i.e., 1D, 2D, and 5D) showed less frequency of seed and haploid formation compared to 7D-substituted chromosomes. Earlier, Inagaki et al. [8] also suggested that substituted chromosomes 1D, 3D, 4D and 7D of bread wheat 'Chinese Spring' had the effect of increasing crossability of durum wheat 'Langdon' with maize. Overall observations suggest that some D-genome chromosomes may enhance crossability in combination with homoeologous chromosomes of the A or B genome. Haploids of bread wheat carrying D-genome chromosomes were also produced from diverse bread wheat genotypes by crossing with maize. The results obtained from *Ae. tauschii x I. cylindrica* crosses, evidently pinpoint that the genes that triggers chromosome elimination of *Imperata* genome and induce the haploid embryo formation in *Ae. tauschii* are located primarily on chromosome 7D (7A or 7B). Some of the previous studies also advocated that 'Langdon' chromosome substitution line 7D showed the highest crossability with rye [6], and hexaploid triticale (x *Triticosecale* Wittmack) 'Rhino' substituted by D-genome chromosomes (in particular, chromosomes 2D and 4D) showed higher crossability with maize [7]. Further, infrequent and a very low number of haploid embryos that obtained with substitution line with 1D, 2D and 5D chromosomes suggest the possible presence of low copy number of the genes responsible for chromosome elimination of *Imperata* genome that need to be analyzed using high-throughput technique in near future.

Acknowledgments This work was supported in part by Grants-in-Aid for Scientific Research (C) (No. 22580004 to YM). We thank Japan Society for the Promotion of Science (JSPS), Japan and Department of Science and Technology, India for providing research grant through Indo-Japan collaborative science program. Sincere thanks are also due to Dr. HK Chaudhary, CSK Himachal Pradesh Agricultural University, India and all members of Plant Molecular Genetics Laboratory and Osaka Kyoiku University, Osaka, Japan for their constant help and support.

References

1. Barclay IR. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature. 1975;256:410–1.
2. Celiktaş N, Tiryakioğlu M, Can E, Kutlay D, Hatipoğlu R. Production of dihaploids in durum wheat using *Imperata cylindrica* L. mediated chromosome elimination. Turk J Agric For. 2015;39:48–54.
3. Chaudhary HK. Dynamics of wheat x *Imperata cylindrica*-a new chromosome elimination mediated system for efficient haploid induction in wheat. In: Appels R, Eastwood R, Lagudah E, Langridge P, Mackay M, McIntyre L, Sharp P, editors. 11th international wheat genetics symposium. Sydney: University of Sydney Press; 2008. p. 647–50.
4. Chaudhary HK, Sethi GS, Singh S, Pratap A, Sharma S. Efficient haploid induction in wheat by using pollen of *Imperata cylindrica*. Plant Breed. 2005;124:96–8.
5. Fukui KN, Suzuki G, Lagudah ES, Rahman R, Appels R, Yamamoto M, et al. Physical arrangement of retrotransposon-related repeats in centromeric regions of wheat. Plant Cell Physiol. 2001;42:189–96.
6. Genç I, Özkan H, Yagbasanlar T. Crossability of D-genome chromosome substitution lines of durum wheat (*Triticum turgidum* ssp. *turgidum* conv. durum) with *Secale cereale* and *Aegilops squarrosa*. Wheat Inf Serv. 1996;83:1–6.
7. Inagaki MN, Pfeiffer WH, Mergoum M, Mujeeb-Kazi A, Lukaszewski AJ. Effects of D-genome chromosomes on crossability of hexaploid triticale (x *Triticosecale* Wittmack) with maize. Plant Breed. 1997;116:387–9.
8. Inagaki MN, Pfeiffer WH, Mergoum M, Mujeeb-Kazi A. Variation of the crossability of durum wheat with maize. Euphytica. 1998;104:17–23.
9. Kishore N, Chaudhary HK, Chahota RK, Kumar V, Sood SP, Jeberson S, et al. Relative efficiency of the maize- and *Imperata cylindrica*-mediated chromosome elimination approaches for induction of haploids of wheat-rye derivatives. Plant Breed. 2011;130:192–4.
10. Komeda N, Chaudhary HK, Suzuki G, Mukai Y. Cytological evidence for chromosome elimination in wheat x *Imperata cylindrica* hybrids through fluorescence in situ hybridization. Genes Genet Syst. 2007;82:241–8.

11. Laurie DA, Bennett MD. Cytological evidence for fertilization in hexaploid wheat x sorghum crosses. Plant Breed. 1988;100:73–82.
12. Laurie DA, Bennett MD. The production of haploid wheat plants from wheat · maize crosses. Theor Appl Genet. 1989;76:393–7.
13. Mergoum M, Singh PK, Anderson JA, Peiia RJ, Singh RP, Xu SS, et al. Spring wheat breeding. In: Carena MJ, editor. Handbook of plant breeding, Cereals, vol. 3. New York: Springer; 2009. p. 127–56.
14. Mukai Y, Endo TR, Gill BS. Physical mapping of the 5S rDNA multigene family in common wheat. J Hered. 1990;81:285–90.
15. Pratap A, Sethi GS, Chaudhary HK. Relative efficiency of different Gramineae genera for haploid induction in triticale and triticale x wheat hybrids through the chromosome elimination technique. Plant Breed. 2005;124:147–53.
16. Rather SA, Chaudhary HK, Kaila V. Influence of different wheat and *Imperata cylindrica* genetic backgrounds on haploid induction efficiency in wheat doubled haploid breeding. Czech J Genet Plant Breed. 2014;50:195–200.
17. Riera-Lizarazu O, Mujeeb-Kazi A. Polyhaploid production in the Triticeae: wheat x *Tripsacum* crosses. Crop Sci. 1993;33:973–6.
18. Thomas HM, Pickering RA. Chromosome elimination in *Hordeum vulgare* x *H. bulbosum* hybrids 1. Comparisons of stable and unstable amphidiploids. Theor Appl Genet. 1983;66:135–40.