Wheat Inf. Serv. 119, 2015. www.shigen.nig.ac.jp/ewis

# Wheat Information Service

## Electronic Newsletter for Wheat Researchers

## No. 119

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## Research Information

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**Research Information**

# Chromosome 1H of Betzes barley in Chinese Spring wheat causes meiotic abnormality by pre-meiotic duplication of chromosomes

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**Abstract**

Addition of chromosome 1H from barley cultivar Betzes causes sterility in Chinese Spring wheat. We observed severe distortion in meiotic metaphase I configurations in the monosomic 1H addition line. Number of chromosome increased greatly indicating several rounds of chromosome duplication in premeiotic mitosis. The meiotic cell division was arrested at the anaphase I, which was visualized by the stretched chromosomes connected at the telomeric ends. Immunostaining of the meiotic cells using an α-tubulin antibody indicated that premature cytokinesis took place before separation of homologous chromosomes and that the bipolar spindles are formed in the irregularly formed dyads.

Key words: wheat-barley addition, chromosome 1H, sterility, meiotic arrest

Barley (*Hordeum vulgare* L.; 2n = 2x = 14, genome formula HH) chromosome addition lines of common wheat (*Triticum aestivum* L., 2n = 6x = 42, genome formula AABBDD) have been established to broaden the spectrum of genetic diversity for improvement of wheat. Beyond their practical importance in agriculture, barley chromosome addition lines are good research resources in Triticeae genetics and genomics. In a wheat-barley addition line, each barely chromosome is sorted in wheat background, thus the addition line allows allocation of barley genes and molecular markers to each barley chromosome (for review, see Molnár-Láng et al. 2014). Recently published draft genome sequence of barely, aimed to reveal all gene-associated sequences in barley, also relies on the wheat-barley addition lines in generation of chromosome-specific survey sequences (the International Barley Genome Sequencing Consortium, the IBGSC, Mayer et al. 2012).

Islam and his colleagues did pioneering studies of wheat-barley addition. They produced six wheat-barley disomic addition lines of common wheat for barley chromosomes 2H to 7H by crossing Chinese Spring (CS) wheat and Betzes barley (Islam et al. 1981; Islam 1983). Islam and Shepherd (2000) produced the seventh addition line of the CS wheat-Betzes barley combination carrying one intact and the short arm

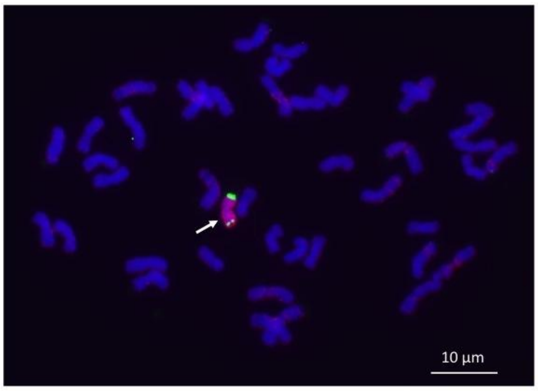

**Fig. 1.** Mitotic metaphase chromosomes of the 1H monosomic addition line (42 wheat chromosome plus one barley chromosome). Barley chromosome 1H (indicated by a white arrow) is detected by GISH with barley genomic DNA probe (red). The green signals are FISH signals of the barley specific subtelomeric sequence HvT01 (Belostotsky and Ananiev 1990). Chromsome 1H of barley has a bright FISH signal on the short arm and slightly reduced signal on the long arm. Chromosomes are counter-stained by 4’,6-diamidino-2-phenylindole dihydrochloride (DAPI, 1.5 µg/ml). White bar indicates 10 µm.

of chromosome 1H together with a pair of 6H chromosomes. Interestingly, only this chromosomal constitution allowed the fertile addition of chromosome 1H to common wheat. Islam and Shepherd (2000) demonstrated that single addition of 1H caused sterility, which could be rescued by the presence of 6H. The sterility of 1H addition is suspected to be due to meiotic abnormality.

In the season 2012-2013 we screened 35 plants of the progeny o f the cross double monosomic addition of Betzes barley chromosomes 1H and 6H in CS (21”W + 1’1H + 1’6H, female) and CS (male). Chromosome constitutions in root tip cells were tested by the FISH/GISH protocol as described elsewhere (Ishihara et al. in press). We found one plant that had chromosome 1H monosomically (Fig. 1). Other progeny possessed 6H (12 plants), 1H and 6H (2 plants), 1H and the long arm of 6H (1 plant), and neither of 1H and 6H (19 plants). In the season 2013-2014, we screened 59 plants of the progeny and found that seven had chromosome 1H, 12 had only chromosome 6H, seven had both 1H and 6H, and 33 had neither 1H nor 6H. These monosomic 1H addition plants grew normally to reproductive stages.

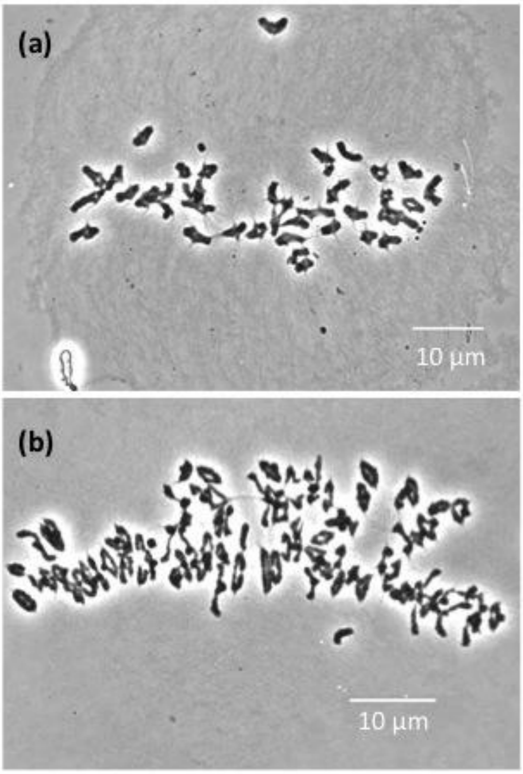

**Fig. 2.** Meiotic metaphase I of the pollen mother cell in the 1H monosomic addition line. Chromosome numbers are apparently more than 21” +1’ which is expected for the alien monosomic addition in wheat. The cell in (b) has more chromosomes than in cells in (a). Note that in both (a) and (b), chromosomes are forming mostly bivalents and they are aligned to the metaphase plates. Centromeres of the bivalents seem to be pulled to opposite poles. Bars indicate 10 µm.

Selfed- and crossed-seed fertilities of the 1H monosomic addition were very low. When it was selfed, ninety-seven florets bore no seed. When manually pollinated with CS pollen, it bore two seed out of 289 florets (0.7%), contrasting to the crossed-seed fertility of the double monosomic addition line 1H and 6H (30.3%, 47 seed out of 155 florets). The sterility of the monosomic 1H addition was on both female and male sides. Cytological observation of the meiotic metaphase I (MI) of the pollen mother cells (PMCs) in the monosomic 1H addition line revealed that numbers of chromosomes were increased (Fig. 2). Large numbers of chromosomes prevented us to correctly determine the meiotic chromosome configurations in MI, however, it was clear that chromosome numbers were more than the expected 21” + 1’ configuration and that the chromosome numbers varied greatly. Interestingly, most of chromosomes formed bivalents, indicating that random increase of individual chromosomes, which results in the increase of univalents, was not the case. Since the MI plates were normally formed, we speculate that increase of chromosome happened in premeiotic mitosis.

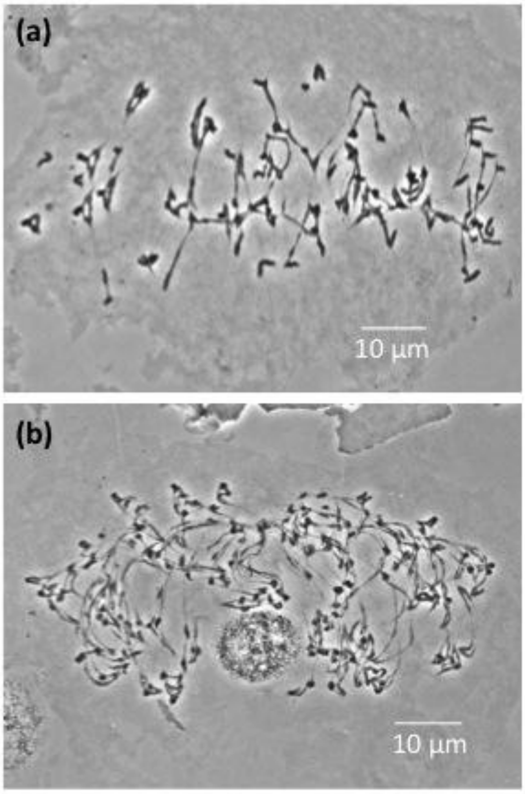

**Fig. 3.** At the onset of anaphase I, meiosis is arrested in the barley chromosome 1H addition line. Homologous chromosomes are pulled towards opposite poles but chiasmata are not dissolved at the telomeric ends. Thus, the chromosomes are irregularly stretched. The number of chromosomes differed form PMC to PMC; (a) has smaller number of chromosomes than (b). Bars indicate 10 µm.

Increase in chromosome number and/or chromosome doubling is not necessarily causes sterility but could produce fertile gamete with aneuploidy. In the case of 1H monosomic addition line, the progress of meiosis was blocked at anaphase I (AI) where the stretched bivalents were often observed (Fig. 3). The bivalents seemed to be under tention between two polls. We examined organization of spindles by immunostaining of the spindle by the α-tubulin antibody (methodology of the immunostaining can be found in Matsuoka et al. 2013). We could not find clear bipolar spindle in MI (Fig. 4). Cytokinesis happened precociously before the completion of anaphase I. The resulting irregular dyads contained nonseparated homologues where the bipolar spindles formed. These observations indicated that increase in chromosome number before entering to MI and abnormal separation of chromosomes at AI might be the cause of sterility.

One of the fertile progeny of the 1H monosomic addition crossed with CS had septaploid chromosome number, indicating that the 1H monosomic addition line formed unreduced hexaploid gamete (data not shown). We also observed the expected 21” + 1’ MI configuration very rarely in the PMC, which could also give rise of fertile gamete (data not shown). The very scarce crossed seed fertility in the 1H monosomic addition could be the results of hybridization of those very rare female gametes.

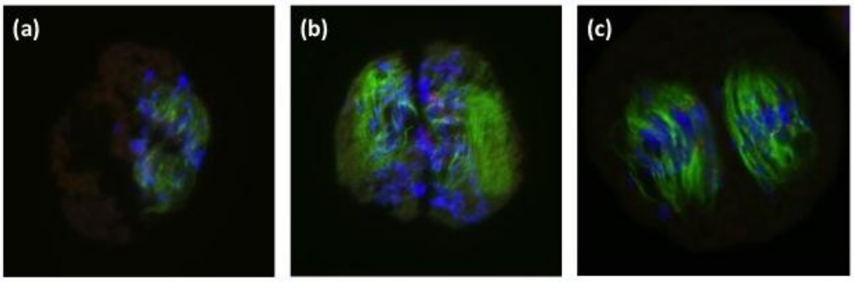

**Fig. 4.** Immunostaining of the PMC in the 1H monosomic addition line. Cells were stained by anti-α -tublin antibody to detect spindles (green) and anti-CENH3 antibody to detect active centromeres (red). Chromsomes are counter-stained by DAPI. In panel (a) stretched chromosomes in the transition stage from MI to AI are visible but the spindle is so amorph as not to form bipolar spindle. Precocious cytokinesis is visible in (b) that is indicated by a ditch lacking spindle signals. In the irregular dyads, bipolar spindles seems to be organized in each cell (c).

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**Wheat Inf. Serv. 119:7-10, 2015. www.shigen.nig.ac.jp/ewis**

**Research Information**

# Allelic diversity of puroindoline genes at the *Ha* locus in the core-collection of hexaploid wheat accessions conserved by NBRP-Wheat

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## Abstract

Allelic diversity of puroindoline genes *Pina-D1* and *Pinb-D1* was investigated by nucleotide sequencing of the PCR-amplified genes in the core-collection of 163 hexaploid wheat accessions conserved by NBRP-Wheat. Of the total, 99 accessions possessed both of wild-type gene sequences, *Pina-D1a*/*Pinb-D1a*. One and 5 of the previously reported mutations were found in the *Pina-D1* and *Pinb-D1* genes, respectively. The most prevalent mutation was *Pinb-D1b*, being present in one third of accessions with mutant alleles. The *Pinb-D1p* allele was found predominantly in Afghanistan. All puroindoline alleles found in *Triticum aestivum* were shared by the other *Tiriticum* species.

Key words: hexaploid wheat, grain hardness, allelic diversity, puroindoline

## Introduction

Grain hardness is an important end-use quality characteristics of wheat. This trait is mainly controlled by a single locus, called *Hardness* (*Ha*) (Symes 1965; Baker 1977), which is located on the short arm of chromosome 5D (Mattern *et al*. 1973; Law *et al*. 1978). The genes encoding friabilin were tightly linked to the *Ha* locus (Jolly *et al*. 1993; Sourdille *et al*. 1996; Campbell *et al*., 1999). Friabilin are composed mainly of two proteins, puroindoline a and b, which are encoded by the *Pina-D1* and *Pinb-D1* genes (Morris, 2002). All soft wheat varieties examined so far carry the wild-type puroindoline alleles that have been designated *Pina-D1a* and *Pinb-D1a* (Giroux and Morris, 1997, 1998; Morris, 2002; Bhave and Morris, 2008a, b). Hard wheat varieties have specific mutations in either of the *Pina-D1* or *Pinb-D1* genes, or they lack the genes (Morris, 2002; Morris and Bhave, 2007; Wang *et al*., 2008). In this study, we investigated puroindoline genotypes of the core-collection of hexaploid wheat accessions developed by the National BioResource Project-Wheat.

## Materials and methods

We examined 163 accessions of the core-collection of hexaploid wheat (*Triticum aestivum*, *T. compactum*, *T. macha*, *T. spelta*, *T. sphaerococcum*, *T. vavilovii*) develpped and conserved by National BioResource Project-Wheat, Japan (Takenaka *et al*., in preparation, Table 1). Total genomic DNA was extracted from young leaves by the CTAB method (Kim *et al*., 1997) and used as template for PCR.

Table 1. Distribution of puroindoline haplotypes of 163 hexaploid wheat accessions based on geographic origin

| | Puroindoline haplotypes (*Pina-D1*/*Pinb-D1*) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collection/breeding country | a/a | a/b | a/c | a/d | a/p | a/ab | a/null$^*$ | l/a | null$^*$/a | null$^*$/b | null$^*$/null$^*$ | Total |
| Afghanistan | 4 | 0 | 0 | 0 | 5 | 1 | 1 | 0 | 0 | 0 | 0 | 11 |
| Australia | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Bhutan | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Canada | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| China | 12 (Tc: 1) | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| DDR | 7 (Tspe: 7) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| Egypt | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Ethiopia | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1 (Tc: 1) | 8 |
| Georgia, USSR | 1 (Tm: 1) | 1 (Tm: 1) | 0 | 0 | 0 | 0 | 1 (Tm: 1) | 0 | 0 | 0 | 0 | 3 |
| Greece | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| India | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Iran | 8 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 1 (Tspe: 1) | 15 |
| Iraq | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| Italy | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Japan | 25 (Tc: 1) | 3 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 32 |
| Jordan | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Nepal | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 5 |
| Pakistan | 3 | 0 | 0 | 0 | 0 | 1 | 1 (Tsph: 1) | 0 | 2 (Tsph: 1) | 0 | 0 | 7 |
| Romania | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 5 |
| Spain | 6 (Tspe: 3) | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| Syria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Tanzania | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Turkey | 4 | 3 | 0 | 0 | 0 | 1 (Tv: 1) | 0 | 0 | 0 | 1 (Tm: 1) | 1 | 10 |
| UK | 4 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 6 |
| USA | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 4 |
| USSR | 1 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 6 |
| Unknown | 3 (Tspe: 1; Tsph: 1) | 0 | 0 | 0 | 0 | 1 (Tv: 1) | 0 | 0 | 0 | 0 | 0 | 4 |
| Total | 99 | 19 | 1 | 3 | 9 | 6 | 9 | 3 | 7 | 2 | 5 | 163 |

[*] 'Null' allele means that an expected single band was not amplified by *Pina-D1* or *Pinb-D1* primer set (Gautier *et al*., 1994).

Numbers in parentheses show numbers of non-*T.aestivum* species (Tc: *T. compactum*, Tm: *T. macha*, Tspe: *T. spelta*, Tsph: *T. sphaerococcum*, Tv: *T. vavilovii*).

The PCR primers used to amplify the puroindoline genes were those reported by Gautier *et al.* (1994) as follows: 5'-ATGAAGGCCCTCTTCCTCA-3' and 5'-TCACCAGTAATAGCCAATAGTG-3' for the amplification of the full-length (447 bp) *Pina-D1* gene, and 5'-ATGAAGACCTTATTCCTCCTA-3' and 5'-TCACCAGTAATAGCCACTAGGGAA-3' for the amplification of the full-length (447 bp) *Pinb-D1* gene. PCR amplification was performed using *TaKaRa Ex Taq* DNA polymerase (2.5 U, TaKaRa) in 100 µl of reaction buffer (TaKaRa, 2 mM $MgCl_2$) containing 100 ng of genomic DNA, 200 µM of each dNTP and 40 pmol of each primer. The PCR conditions were 93ºC for 4 min followed by 35 cycles of 94ºC for 60 s, 53ºC for 90 s and 72ºC for 120 s. A final cycle with an extension of 10 min at 72ºC completed the reaction. The DNA amplification was performed using a C1000$^{TM}$ Thermal Cycler (Bio-Rad). A 10 µL aliquot of the product was separated in 1.5% (w/v) agarose gel, stained with ethidium bromide and visualized using UV light. A 1 µL aliquot of the product was used for direct DNA sequencing on a DNA sequencer (Applied Biosystems 3130xl Genetic Analyzer) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequencing primers were those described above for primary gene amplification (Gautier *et al.*, 1994). The nucleotide sequence results were called by Sequence Scanner v1.0 (Applied Biosystems) and further analyzed using GENETYX v12 (GENETYX).

## Results and Discussion

*Pina-D1* and *Pinb-D1* primer sets successfully amplified an expected single band (447 bp) in 149 and 149 out of 163 accessions, respectively. The rest 14 and 14 accessions were assumed to have null alleles of *Pina-D1* and *Pinb-D1*, respectively. Ninety-nine accessions possessed wild-type alleles, *Pina-D1a*/*Pinb-D1a*. DNA sequencing of the *Pina-D1* gene identified one of the well characterized mutations, *Pina-D1l* (Gazza *et al.*, 2005). As for *Pinb-D1*, five previously reported mutations were identified (*Pinb-D1b* (Giroux and Morris, 1997), *Pinb-D1c* (Lillemo and Morris, 2000), *Pinb-D1d* (Lillemo and Morris, 2000), *Pinb-D1p* (Xia *et al.*, 2005) and *Pinb-D1ab* (Tanaka *et al.*, 2008)). All accessions were classified according to their puroindoline haplotypes and geographic origin (Table 1). The *Pinb-D1b* allele was the most prevalent mutation, being present in one third of accessions with mutations. Interestingly, this allele is the most frequent mutation among wheat varieties of all over the world (Cane *et al*., 2004; Xia *et al*., 2005; Ikeda *et al*., 2005; Chang *et al*., 2006; Chen *et al*., 2006; Lillemo *et al*., 2006; Pickering and Bhave, 2007; Tanaka *et al*., 2008). The *Pinb-D1p* allele was found predominantly in Afghanistan. Tanaka *et al*. (2008) reported that the distribution of the allele *Pinb-D1p* may be associated with the so-called 'Silk Road', the ancient trade and cultural transmission route between China and the Mediterranean Sea. This allele was also present in Pakistan and Afghanistan but was not found in Japan. In this study, the allele *Pinb-D1p* was found in one accession in China but was not found in Japan, which is in accordance with the previous study (Tanaka *et al.* 2008). The absence of the allele in Japan could be explained by assuming that the common wheat in small population size with a limited amount of genetic diversity was introduced to Japan. All of the puroindoline alleles found in this study, have been reported in *T. aestivum* (genome constitution AABBDD) and/or *Aegilops tauschii* (DD). We found these alleles in the other *Triticum* species. Therefore, if the polyphylogenetic origin of hexaploid wheat species is assumed, these alleles should have been established in *Ae. tauschii* before the allopolyploidization event in *Triticum* species. Alternatively, if we assume limited number of hexaploidization events, recurrent mutations in *Pina-D1* and *Pinb-D1* genes have occurred both in *Ae. tauschii* and hexaploid wheat.

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## Others

# Instructions to Authors

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(4) **Others:** Any other information useful for wheat researchers

Title, Affiliation and Abstract

In the title page(s), the manuscript category (as mentioned above), a title, the names of the author(s), affiliation(s) and address(es) of the authors, and the e-mail address, telephone, and fax numbers of the corresponding author must be clearly indicated.

The Abstract (100-250 words) may not contain references.

References

References should be cited in the text by the author(s) and year, and listed at the end of the text with the names of authors arranged alphabetically. When an article has more than two authors, only the first author's name should appear, followed by "et al.", in the text. The references should be formatted as follows.

Journal articles:

Payne PI, Holt LM, Law CN (1981) Structural and genetical studies on the high molecular weight subunits of wheat glutenin. Theor Appl Genet 60:229-236.

Book chapters:

Peacock WJ, Dennis ES, Gerlach WJ (1981) Molecular aspects of wheat evolution: repeated DNA sequences. In: Evans LT and Peacock WJ (eds.) Wheat Science -

Today and Tomorrow. Cambridge Univ. Press, Cambridge, UK, pp. 41-60.

Books:

Knott DR (1989) The Wheat Rusts - Breeding for Rust Resistance. Springer-Verlag, New York, USA.

Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., K. Tsunewaki personal communication).

Abbreviations

Abbreviations should be explained at first occurrence.

Symbols and Units

Gene names and protein names must carefully be discriminated. Gene names and loci should be italicized; protein should be upright. The SI units (http://physics.nist.gov/Pubs/SP330/contents.html ) should be used throughout.

Nomenclature

Nomenclature of genes and chromosomes should follow the 'Catalogue of gene symbols for wheat' (McIntosh et al.: 10th Int. Wheat Genet. Symp. 2003).

Nucleotide sequences

The DDBJ/EMBL/GenBank accession numbers must be provided for newly reported nucleotide sequences.

Tables

Tables must be numbered consecutively. For Table writing, Microsoft Word is recommended. Prepare a separate file for each table. Refer to the latest eWIS articles for format.

Figures

Figures must be numbered consecutively. Prepare a separate file for each figure.

**Outline of the publication process**

Authors of accepted manuscripts are informed by e-mail that a temporary URL has been created from which they can obtain their proof. Proofreading is the responsibility of the author. Authors should make proof corrections and send them to Editorial Office by e-mail. After online publication, corrections can only be made in exceptional cases when Editorial Office permits the necessity.

The final version of accepted manuscripts will be published in the 'Online First' section of the eWIS web page upon receipt of proof corrections. Editorial Office biannually gathers the accepted manuscripts published in the 'Online First' into a volume. In 'Archive' of eWIS, all manuscripts are collected as PDF format, and open to all wheat researchers.

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**10 March 2015**
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