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mollis Double Disomic Addition Line with Stripe Rust
Resistance**

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Molecular Cytogenetics Identification of a Wheat-*Leymus mollis* Double Disomic Addition Line with Stripe Rust Resistance

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Abstract *Leymus mollis* (Trin.) Pilger ($2n = 4x = 28$, **NsNsXmXm**) possesses a number of valuable genes against biotic and abiotic stress, which could be transferred into common wheat background for wheat improvement. In the present study, we determined the karyotypic constitution of a wheat-*L. mollis* double disomic addition line M11003-4-4-1-1 selected from the F_5 progeny of a stable wheat-*L. mollis* derivative M39 ($2n = 56$) $\times$ *Triticum aestivum* cv. 7182, by morphological and cytogenetic identification, GISH (genomic *in situ* hybridization), FISH (fluorescent *in situ* hybridization), molecular markers analysis and stripe rust resistance evaluation. Cytological studies demonstrated that M11003-4-4-1-1 had a chromosome karyotype of $2n = 46$ with 23 bivalents, while GISH and FISH analysis indicated that this line contained 42 common wheat chromosomes and two pairs of *L. mollis* chromosomes. DNA markers showed that the alien chromosomes from *L. mollis* belonged to homeologous group 5 and group 6. Evaluation of the agronomic traits revealed that M11003-4-4-1-1 was resistant to stripe rust at the adult stage, while the plant height was reduced and the 1000-grain weight was increased significantly. Therefore, the new line M11003-4-4-1-1 could be exploited as an important bridge material in chromosome engineering and wheat breeding.

Keywords Wheat • *Leymus mollis* • Double disomic addition line • Stripe rust resistance

Introduction

Leymus mollis (Trin) Hara ($2n = 4x = 28$, **NsNsXmXm**) is a very important tetraploid species with a long evolutionary distance from wheat (Mohammed et al. 2013). It possesses exceptionally large spikes, strong rhizomes, multiple spikelets, tolerance of abiotic stresses (such as salts, cold and drought) (McGuire and Dvorak. 1981), and resistance to various fungal diseases (such as leaf rust, stripe rust and powdery mildew) (McGuire and Dvorak. 1981; Faith 1983; Kishii et al. 2003). Thus, *L. mollis* could be used as a useful germplasm resource for the improvement of wheat agronomic traits and disease resistance capacity. The genomic formula of *L. mollis* is **NsNsXmXm**, and the **Ns** genome originates from *Psathyrostachys huashanica* Keng, whereas the **Xm** genome remains unknown (Wang and Jensen. 1994; Wang et al. 2006; Zhou et al. 2010).

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating and widespread wheat diseases in the world (Line 2002; Dong et al. 2013). China has the largest epidemic region of wheat stripe rust in the world in terms of wheat-growing area (Wellings 2011) and the disease has become a major risk in wheat

production because of the appearance of various new virulent races (Wan et al. 2004). Therefore, it is very important to obtain and identify new durable resistance genes and pyramid the genes into wheat cultivars (Liu et al. 2014). Wide hybridization is an effective approach for introducing valuable alien genes from wild relatives into wheat varieties in order to enrich wheat germplasm (Niu et al. 2011; Sepsi et al. 2008).

Wide hybridization between wheat and *L. mollis* has been conducted successfully since the 1960s (Tsitsin 1965), and various wheat-*L. mollis* derivatives, such as octoploid amphiploids and some derivate lines (Wu et al. 2003; Song et al. 2008; Yang et al. 2010; Wang et al. 2012; Pang et al. 2014) have been subsequently produced with many desirable characters, including those of strong rhizomes, high tillering ability, good fecundity, a high number of seeds per spike, especially the high resistance to stripe rust and powdery mildew, as well as other fungal diseases (Merker 1992; Anamthawat-Jónsson et al. 1997; Habora et al. 2012). It is worth mentioning that the octoploid *Tritileymus*, extensively used as bridge materials for backcrossing with common wheat, played an important role in wheat breeding (Yang et al. 2015). In China, Fu et al (1993) first developed the octoploid *Tritileymus* M842, derived from the F₁ hybrids between common wheat cv.7182 and *L. mollis* by embryo rescue and colchine treatment. Consequently M842 has been widely

applied to the development of wheat germplasm resources. Three addition lines and two substitution lines were developed from hybridization between M842 and wheat **4D** and **5A** nullisomic lines (Fu et al. 1996, 1997). Zhao et al (2012) reported a multiple alien substitution line 05DM6, which was selected among F₅ progenies of octoploid *Tritileymus* M842-12 and *T. durum* L. cv. Trs372, carrying 3 pairs of chromosomes from *L. mollis* and 18 pairs of chromosomes from wheat. A wheat-*L. mollis* **3Ns(3D)** disomic substitution line isolated from the F₅ progeny of octoploid *Tritileymus* M842-16 × *T. durum* cv. D4286, which was designated as 10DM57, showed high resistance to leaf rust (Pang et al. 2014). In our laboratory, an octoploid *Tritileymus* M47 highly resistant to stripe rust and powdery mildew at the adult stage was developed (Yang et al. 2014). And M39 was a stable derivative of 7182 and *L. mollis* with 2n = 56, containing 44 common wheat chromosomes and 12 alien chromosomes from *L. mollis* **Ns** genome, including 2 extra **4B** chromosomes (data are unpublished).

In this study, we developed and identified a novel wheat-*L. mollis* double disomic addition line M11003-4-4-1-1, derived from the BC₁F₅ progeny of common wheat cv. 7182 and M39 (2n=56). The objectives of this study were to identify the chromosome composition of M11003-4-4-1-1 using GISH, FISH and DNA markers, and to evaluate its agronomic traits

and stripe rust resistance.

Materials and methods

Materials

The materials in this study included two common wheat cultivars ($2n = 6x = 42$, **AABBDD**) 7182 and Huixianhong (HXH), *Leymus* ($2n = 4x = 28$, **NsNsXmXm**), M39 ($2n = 56$), *P. huashanica* Keng ($2n = 2x = 14$, **NsNs**) and a wheat-*L. mollis* double disomic addition line M11003-4-4-1-1, provided by College of Agronomy, Northwest A&F University. M11003-4-4-1-1 was selected from BC_1F_5 progeny of common wheat cv. 7182 and M39, with 7182 and *L. mollis* as the two parents of the derivative, and 7182 regarded as a control in the agronomic trait evaluation and also in the electrophoretic analyses, while HXH was used as the susceptible control in the stripe rust resistance tests. The *Pst* races employed in this study were composed of equal amount of CYR32 and CYR33, and conserved in College of Plant Protection, Northwest

A&F University.

Methods

Cytological characterization

All the samples of roots and young spikes were obtained at the suitable time, respectively, and the pre-treatment of root tips and anthers were proceed as described previously (Yang et al. 2015). An Olympus BX-43 microscope (Japan) was used to photograph metaphase I cells which had a complete chromosomes numbers.

GISH and FISH

The total genomic DNA of *L. mollis*, *P. huashanica* and 7182 were extracted from seedling leaves according to a modified

CTAB method (Cota-Sanchez et al. 2006). With high-quality DNA for GISH probe, one additional step with chloroform was performed to purify the DNA. The total genomic DNA of *L. mollis* and *P. huashanica* was labeled with a Dig-Nick Translation Mix (Roche, Germany), respectively, without blocking DNA. The root tips were digested in 2% cellulase + 1% pectinase at 37°C for about 55-58 min (digestion times were varied among different materials), with the slides and GISH process accomplished on the basis of the previous method (Yang et al. 2015). FISH was performed to distinguish wheat **A**-, **B**-, and **D**-genome and further characterized the existence of the additional chromosomes. Oligonucleotide probes pSc-119.2 (green) and pTa-535 (red) were used to analyze the chromosome constitutions, following the procedures of Tang et al (2014a, b). The chromosome slides were washed in $2 \times$ SSC solution and counterstained by 4, 6-diamidino-2-phenyl-indole (DAPI) before observation. Then Olympus BX53 (Japan) with a Photometrics SenSys CCD camera DP 80 was used for fluorescence signal detection and photography.

SSR and EST-STS analysis

EST-STS markers (http://wheat.pw.usda.gov/SNP/new/pcr_primers.shtml) and SSR markers (Pestsova et al. 2000; Röder et al. 1998) were all synthesized by AuGCT SYN DNA- Biotechnology Co., Ltd of Beijing. Totally 70 pairs of SSR primers and 70 pairs of EST-STS primers for homoeologous groups 1-7 of wheat chromosomes were used in PCR amplification. The polymerase chain reaction (PCR) was performed in an ABI-9700 thermo-cycler. A 10 µL reaction mixture consisted of 1.0 µL of DNA template (50-100 ng/µL), 1.0 µL of 10 × PCR Buffer (Mg⁺), 0.8 µL of dNTP mixture (2.5 mmol/L), 0.1 µL of *Taq* DNA polymerase (2.5 U/ µL), 1.0 µL of each primer pair (5 µmol/µL) and 6.1 µL ddH₂O. PCR amplifications were conducted with 3 min of denaturation at 94°C, then amplifications were programmed for 35 cycles, each consisting of 30 s at 94°C, 45 s at 50 to 60°C (depending on the individual primer pair), 50 s at 72°C and followed by a 10 min extension at 72°C before cooling to 4°C. After amplification, the PCR products were resolved in 8% non-denaturing polyacrylamide and silver-stained for visualization.

PLUG analysis

70 pairs of PLUG (PCR-based landmark unique gene) markers (Ishikawa et al. 2009) for homeologous groups 1-7 of wheat chromosomes were selected to perform PCR and all of the primers were synthesized in AuGCT DNA-SYN Biotechnology Co., Ltd of Beijing. PCR assays were conducted as described above. PCR amplifications were leaded with 4 min of denaturation at 94°C, then amplifications were programmed for 38 cycles, each consisting of 45 s at 94°C, 45 s at 60°C, 2 min at 72°C and followed by a 10 min extension at 72°C before cooling to 4°C. 7.5 µL of the product was digested with *Hae*III (37°C) for 3 h or *Taq* I (65°C) for 2 h in order to obtain different polymorphism fragments, then they were fractionated by electrophoresis on 2% agarose gel.

Agronomic traits and stripe rust resistance evaluation

Eight agronomic traits of the plant materials were assessed, including plant height, plant type, spike length, spikelets number per spike, florets number per spikelet, kernels number per spike, 1000-grain weight and awnedness. After harvesting, morphological traits of M11003-4-4-1-1 and its parents were examined and compared using Duncan's multiple range

procedure ($P < 0.05$).

A mixture of *Pst* races composed of CYR32, CYR33 were used to evaluate resistance to stripe rust at the adult stage. *L. mollis*, M39, 7182, M11003-4-4-1-1 and the susceptible control cultivar HXH were tested at the College of Agronomy, Northwest A&F University. After artificial inoculation, infection types (IT) were recorded when rust was fully developed on the susceptible control HXH. IT were based on a 0-4 scale described by Bariana and McIntosh (1993).

Results

Cytological characterization

M11003-4-4-1-1 was selected from BC₁F₅ progenies of wheat cv. 7182 and M39 (2n = 56). The mitotic observation of M11003-4-4-1-1 indicated that 32 root tip cells (RTCs) had a chromosome number of 2n = 46 (Fig. 1a), meanwhile the meiotic analysis of pollen mother cells (PMCs) at metaphase I (M I) of the first meiotic division showed 23 bivalents

(Fig. 1b). We counted 159 PMCs, with the mean frequency of bivalents, univalents and quadrivalents at metaphase I as 22.90 (21-23), 0.16 (0-4) and 0.01 (0-1), respectively, while no trivalents were observed at M I , and no chromosomes lagged at anaphase I (Fig. 1c), indicating cytogenetic stability of M11003-4-4-1-1.

GISH and FISH analysis

GISH analysis was performed on M11003-4-4-1-1 chromosomes at mitotic metaphase with the labeled genomic DNA of *L. mollis* (Fig. 2b) and *P. huashanica* (Fig. 3) as the probes, respectively. Mitotic GISH results showed that the root tip cells of M11003-4-4-1-1 contained 4 distinctive signals with $2n = 46$, which represented four exogenous chromosomes from *Ns* genome of *L. mollis*.

FISH analysis was performed subsequently in order to further determine the introduced *L. mollis* chromosomes in M11003-4-4-1-1. Repetitive sequences pSc-119.2 and pTa-535 were used to distinguish the whole set of common wheat 42 chromosomes. Using probes Oligo-pSc119.2 and Oligo-pTa535, the standard FISH karyotype patterns of cv. 7182 had been

painted by Yang et al (2015). Compared with the standard FISH painting, it revealed that M11003-4-4-1-1 had a full set of common wheat 42 chromosomes which showed the same karyotypes as 7182 , and meanwhile there were 4 other karyotypes that were completely different from the parent, with two of them as the same while the other two also had no difference. We observed that green signals from pTa-119.2 were absent among the four alien chromosomes, two chromosomes showed red signals in two ends, the other two displayed red signals on one half of two chromosomes. Summarily, two pairs of *L. mollis* chromosomes were added into the 7182 background (Fig. 2a).

SSR, EST-STS, PLUG markers analysis

To confirm the chromosomes introduced from *L. mollis*, 70 SSR primer pairs, 70 EST-STS primer pairs and 70 PLUG primer pairs located on seven wheat chromosomes homeologous groups were tested to compare polymorphisms in cv. 7182, M11003-4-4-1-1 and *L. mollis*. The results indicated that three SSR markers, three EST-STS markers and two PLUG markers, i.e., *Xwmc417*, *Xgpw7574*, *Xgpw7425*, *BF293016*, *BQ167013*, *CD452568*, *TNAC1674-Taq I* , *TNAC1674-HaeIII*,

TNAC1618-Taq I (Table 1, Fig. 4, Fig. 5) could amplified clear specific *L. mollis* bands in M11003-4-4-1-1 while these bands were inexistent in the wheat parent cv. 7182. *Xwmc417*, *BQ167013*, *CD452468* and *TNAC1674* were mapped onto the sixth homeologous group while the other four markers were mapped onto the fifth homeologous group. These results showed that 8 markers could be used as unique markers of *L. mollis*. Furthermore, two pairs of chromosomes added from *L. mollis* in M11003-4-4-1-1 suggested they were homeologous with the fifth and sixth homeologous group chromosomes of common wheat, respectively. Therefore, M11003-4-4-1-1 is wheat-*L. mollis* double disomic addition line with Lm#5Ns and Lm#6Ns chromosomes.

Morphology characterization and stripe rust resistance evaluation

The average plant height of M11003-4-4-1-1 was about 20 cm shorter than that of its common wheat parent cv. 7182, but higher than that of *L. mollis*. The plant type, spikelets number per spike, florets number per spike and awnedness were all similar to those of cv. 7182. However, comparing with cv. 7182, the spike length and kernels number per spike were

reduced, whereas the 1000-grain weight was higher than that of cv. 7182. The results of Duncan’s multiple range procedure showed that the spike length, kernels number per spike, plant height and 1000-grain weight were significantly different from those of cv. 7182 ($P < 0.05$) (Table 2, Fig. 6).

At the adult stage, field tests demonstrated that the wheat parent 7182 (IT=3) and the control variety HXH (IT=4) were susceptible to stripe rust with the mixed *Pst* races CYR32 and CYR33, whereas *L. mollis* (IT=0) and M39 (IT=0;) was immune, and M11003-4-4-1-1 was highly resistant to stripe rust (IT=1), indicating that the stripe rust resistance in M11003-4-4-1-1 maybe come from M39, traceble to *L. mollis* (Fig. 7).

Discussion

In this study, we identified the genomic constitution of M11003-4-4-1-1 by GISH, FISH and molecular markers analysis. GISH and FISH analyses are accurate and powerful tool for tracking alien chromosomes or fragments from different intergeneric hybrids (Nakazawa et al. 2011; Danilova et al. 2014). However, the traditional cytogenetic techniques could not

determine the homoeologous groups of alien chromosomes. In contrast, DNA markers, including SSR, EST and PLUG markers make it more convenient and accurate to identify exogenous chromosomes and segments in wheat (Wang et al. 2009), and they are widely used for testing chromosome addition lines, substitution lines and translocation lines in wide hybridization breeding (Ishikawa et al. 2007; Kong et al. 2008; Kang et al. 2011; Zhao et al. 2012; An et al. 2015). In this study, FISH-GISH analysis could only show that M11003-4-4-1-1 was an addition line containing four *L. mollis* chromosomes, however, molecular markers could further show the details of introduced chromosomes. We obtained eight DNA markers that could amplify polymorphism *L. mollis* bands in M11003-4-4-1-1. In terms of the previous specific markers of 5Ns/6Ns chromosomes and these new markers, the introduced 5Ns/6Ns chromosomes from *L. mollis* could be detected more easily. Thus, while *L. mollis* was used as gene donors, these markers could be used as unique tools for tracking alien *L. mollis* chromosomes in common wheat background, and for efficient introgression of useful genes from wheat-related species into common wheat and comparative gene mapping.

It was found that four introduced chromosomes in M11003-4-4-1-1 were from Lm#Ns genome. In the previous study, different derived lines with Ns chromosome (s) possessed different phenotypes, e.g., a 1Ns addition line carried LMW-GS,

HMW-GS and gliadin genes from *P. huashanica* (Zhao et al. 2009), and derivatives which contained 2Ns, 3Ns, 4Ns or 7Ns chromosomes, respectively, all with high resistance to stripe rust (Du et al. 2014a; Du et al. 2014b; Pang et al. 2014; Yang et al. 2015). M11003-4-4-1-1 was also highly resistant to stripe rust at the adult stage, which suggested the resistance gene(s) had been transferred into 7182 background with the extra chromosomes added from *L. mollis*. Du et al (2013b) reported a 6Ns addition line carried gene(s) controlling twin spikelet and multiple kernels. Wang et al (2015) developed a wheat-*P. huashanica* Keng 6Ns addition line with early maturation. Du et al (2013a) found a wheat-*P. huashanica* Keng 5Ns disomic addition line that was resistant to stripe rust. While there is no relevant reporters about stripe rust resistance from 6Ns chromosomes, we speculated that the resistance of M11003-4-4-1-1 was from Lm#5Ns chromosome.

However, in order to confirm the accurate chromosomes which carried resistance gene (s), more genetic analysis of resistance in M11003-4-4-1-1 should be performed. Dong et al (2013) developed the segregating populations from the cross between H9014-14-4-6-1 and Mingxian 169, and then identified the stripe rust resistance gene *YrH9014*, derived from *P. huashanica* Keng. Yang et al (2016) used a combination of classic genetic analysis, molecular and cytogenetic methods to characterize resistance in an F₂ population, which was derived from Chuanmai45 and the susceptible Chuanmai42. They

identified two resistant genes from Chuanmai45 which were located on a 1RS·1BL translocation and chromosome 4B, respectively. According to the previous studies, genetic analysis and DNA markers analysis were efficient methods for locating resistant genes. Therefore, in the following study, we plan to develop segregating populations from the cross between M11003-4-4-1-1 and HXH, and combine genetic analysis, ISH analysis with molecular analysis, we can map the resistance gene(s).

In conclusion, M11003-4-4-1-1 is a wheat-*L. mollis* double disomic addition line with Lm#5Ns and Lm#6Ns chromosomes from *L. mollis*, which possesses traits such as shorter height, higher 1000-grain weight and higher resistance to stripe rust. It can be used as a donor to derive disomic addition lines and translocation lines to transfer valuable genes to common wheat by chromosomal manipulation strategies.

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Disclosure of potential conflict of interest The authors declare that they have no conflict of interest.

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Table 1 SSR, EST-STS and PLUG polymorphic markers located on the fifth and sixth homoeologous groups applied to linkage analysis of the Lm#5Ns and Lm#6Ns

Marker	Primer(5'-3')	Location	Gel type/Restriction enzyme	Tm(°C)/t (h)
<i>Xwmc417</i>	F: GTTCTTTTAGTTGCGACTGAGG R: CGATGTATGCCGTATGAATGTT	6AL 6BL	8% non-denaturing polyacrylamide gel / -	60/-
<i>Xgpw7574</i>	F: CCGATTGCTACACCGAAGTT R: TGTTTGCAATTCTCACAGGTAGC	5BL	8% non-denaturing polyacrylamide gel / -	60/-
<i>Xgpw7425</i>	F: CTGAACTCGAAGAAGGCCAA R: CCTCGATAGGCTCTGTCCTG	5BL	8% non-denaturing polyacrylamide gel / -	60/-
<i>BF293016</i>	F: CGTCCTCAAGTCCCTCCTCT R: GTAGAACGGTCTCTGTTGGTC	5AS 5BS 5DS	8% non-denaturing polyacrylamide gel / -	60/-
<i>BQ167013</i>	F: GCCTCTTTCAGCCCTTCTTT R: ATTTTGAGTGGCCTGAGGTG	6AS 6BS 6DS	8% non-denaturing polyacrylamide gel / -	60/-
<i>CD452568</i>	F: TTTGCATTTTCGTCTGCAAG R: TCGACACGAGCAAGATTCAC	6AL 6BL 6DL	8% non-denaturing polyacrylamide gel / -	58/-
<i>TNAC1674</i>	F: CCACCACAGAAGCAGATGAAT R: GCTAGATGGCACACCAAGTG	6AS 6DS	2% agarose gel / <i>Taq</i> I / <i>Hae</i> III	60/2 or 3
<i>TNAC1618</i>	F: GTTGGCTGTTGATGGTAAGGA R: GGAGGCCACCAACTAATGTTT	5AL 5BL 5DL	2% agarose gel / <i>Taq</i> I	60/2

- indicates no data recorded

Table 2 Agronomic traits of wheat parent cv. 7182, *L. mollis*, and double disomic addition line M11003-4-4-1-1

Material	Plant height (cm)	Plant type	Spike length (cm)	Spikelets/spike	Florets/spikelet	Kernels/spike	1000-grain weight (g)	Awtnedness
7182	82.3±1.5a	Tighten	10.9±0.4a	22±1a	5.0±0.0a	60±6a	38.6±0.5a	long awn
<i>L.mollis</i>	52.3±1.5b	Drooping	13.2±0.3b	22±1a	–	–	–	awnless
M11003-4-4-1-1	62.3±0.6c	Tighten	9.8±0.8c	20±2a	4.5±0.6a	37±7b	45.8±0.3b	long awn

Significant differences in the means are indicated at the $P < 0.05$ levels, according to Duncan’s multiple range procedure

- indicates no data recorded

Legends to Figures

Fig. 1 Mitotic (**a**), meiotic I (**b**) and anaphase I (**c**) chromosome characteristics of M11003-4-4-1-1, **a** $2n = 46$, **b** $2n = 23 \text{ II}$, **c** $2n = 23+23$.

Fig. 2 FISH (Fluorescence *in situ* hybridization) analysis using Oligo-pTa535 (red), Oligo-pSc119.2 (green) as probes on root tip metaphase chromosomes of M11003-4-4-1-1(**a**), GISH (Genomic *in situ* hybridization) analysis using *L. mollis* genomic DNA (green) as probe (**b**) on root tip metaphase chromosomes of M11003-4-4-1-1. Chromosomes were counterstained with DAPI (blue). The arrows indicate the alien *L. mollis* chromosomes in M11003-4-4-1-1.

Fig. 3 GISH (Genomic *in situ* hybridization) analysis using *P. huashanica* genomic DNA (green) as probe.

Fig. 4 SSR and EST-STS markers results of M11003-4-4-1-1. An arrow indicates a *L. mollis* specific band. M DL2000, 1 7182, 2 *L. mollis*, 3 M11003-4-4-1-1 **a** *Xwmc417*, **b** *Xgpc7425*, **c** *Xgpc7574*, **d** *BF293016*, **e** *BQ167013*, **f** *CD452568*.

Fig. 5 PLUG markers results of M11003-4-4-1-1. An arrow indicates a *L. mollis* specific band. All PCR products are digested by *Taq* I or *Hae*III. M DL2000, 1 7182, 2 *L. mollis*, 3 M11003-4-4-1-1. **a** *TNAC1618-Taq* I , **b** *TNAC1674-Taq* I , **c** *TNAC1674- Hae*III.

Fig. 6 Morphological comparison of adult plants, spikes, spikelets, and seeds from wheat-*L. mollis* derivative line

M11003-4-4-1-1 and its parents, common wheat cv.7182 and *L.mollis*. a adult plants, b spikes, c spikelets and seeds. 1 7182, 2 *L. mollis*, 3 M11003-4-4-1-1.

Fig. 7 Infection type for *Pst* of susceptible control variety Huixianhong(HXH) (1), 7182 (2), *L. mollis* (3), M39 (4) and M11003-4-4-1-1 (5) at the adult stage.

Draft

Fig. 1 Mitotic (a), meiotic I (b) and anaphase I (c) chromosome characteristics of M11003-4-4-1-1, a $2n = 46$, b $2n = 23 \text{ II}$, c $2n = 23+23$.

Fig. 1
77x30mm (300 x 300 DPI)

Draft

Fig. 2 FISH (Fluorescence in situ hybridization) analysis using Oligo-pTa535 (red), Oligo-pSc119.2 (green) as probes on root tip metaphase chromosomes of M11003-4-4-1-1(a), GISH (Genomic in situ hybridization) analysis using *L. mollis* genomic DNA (green) as probe (b) on root tip metaphase chromosomes of M11003-4-4-1-1. Chromosomes were counterstained with DAPI (blue). The arrows indicate the alien *L. mollis* chromosomes in M11003-4-4-1-1.

Fig. 2

75x37mm (300 x 300 DPI)

GISH (Genomic in situ hybridization) analysis using *P. huashanica* genomic DNA (green) as probe.
Fig. 3
59x58mm (300 x 300 DPI)

SSR and EST-STS markers results of M11003-4-4-1-1. An arrow indicates a *L. mollis* specific band. M DL2000, 1 7182, 2 *L. mollis*, 3 M11003-4-4-1-1 a Xwmc417, b Xgpw7425, c Xgpw7574, d BF293016, e BQ167013, f CD452568.

Fig. 4

106x93mm (300 x 300 DPI)

Fig. 5 PLUG markers results of M11003-4-4-1-1. An arrow indicates a *L. mollis* specific band. All PCR products are digested by Taq I or HaeIII. M DL2000, 1 7182, 2 *L. mollis*, 3 M11003-4-4-1-1. a TNAC1618-Taq I , b TNAC1674-Taq I , c TNAC1674- HaeIII.

Fig. 5
52x18mm (300 x 300 DPI)

Draft

Morphological comparison of adult plants, spikes, spikelets, and seeds from wheat-*L. mollis* derivative line M11003-4-4-1-1 and its parents, common wheat cv.7182 and *L.mollis*. a adult plants, b spikes, c spikelets and seeds. 1 7182, 2 *L. mollis*, 3 M11003-4-4-1-1.

Fig. 6
137x98mm (300 x 300 DPI)

Fig. 7 Infection type for Pst of susceptible control variety Huixianhong(HXH) (1), 7182 (2), *L. mollis* (3), M39 (4) and M11003-4-4-1-1 (5) at the adult stage.

Fig. 7
72x39mm (300 x 300 DPI)