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Molecular cytogenetic identification of a wheat—*Aegilops geniculata* Roth 7M^g disomic addition line with powdery mildew resistance

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Abstract *Aegilops geniculata* Roth is an important germplasm resource for the transfer of beneficial genes into common wheat (*Triticum aestivum* L.). A new disomic addition line NA0973-5-4-1-2-9-1 was developed from the BC_1F_6 progeny of the cross wheat cv. Chinese Spring (CS)/*Ae. geniculata* SY159//CS. We characterized this new line by morphological and cytogenetic identification, analysis of functional molecular markers, genomic in situ hybridization (GISH), fluorescence in situ hybridization (FISH), and disease resistance evaluation. Cytological observations suggested that NA0973-5-4-1-2-9-1 contained 44 chromosomes and formed 22 bivalents at meiotic metaphase I. The GISH investigations showed that the line contained 42 wheat chromosomes and a pair of *Ae. geniculata* chromosomes. EST-STS multiple loci markers and PLUG (PCR-based landmark unique gene) markers confirmed that the introduced *Ae. geniculata* chromosomes belonged to homoeologous group 7. FISH identification suggested that NA0973-5-4-1-2-9-1 possessed an additional pair of 7M^g chromosomes, and at the same time, there were structural differences in a pair of 6D chromosomes between NA0973-5-4-1-2-9-1 and TA7661 (CS-AEGEN DA 7M^g). After inoculation with powdery mildew (*Blumeria graminis* f. sp. *tritici, Bgt*) isolates E09, NA0973-5-4-1-2-9-1 exhibited a powdery mildew resistance infection type different from that of TA7661, and we conclude that the powdery mildew resistance of NA0973-5-4-1-2-9-1 originated from its parent *Ae. geniculata* SY159. Therefore, NA0973-5-4-1-2-9-1 can be used as a donor source for introducing novel disease resistance genes into wheat during breeding programs with the assistance of molecular and cytogenetic markers.

Keywords *Aegilops geniculata* Roth · Disomic addition line · Molecular cytogenetics · Powdery mildew · *Triticum aestivum*

Yajuan Wang and Wei Quan have contributed equally to this article.

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Introduction

Aegilops geniculata Roth (syn. *Ae. ovata* L. *pro parte*) is an annual self-fertilizing tetraploid (2n = 4x = 28) with the genomic formula U^{g}U^{g}M^{g}M^g, where the U^g genome was derived from the U genome of the diploid species *Ae. umbellulata* Zhuk. (2n = 2x = 14, UU), and the M^g genome originated from the M

genome of *Ae. comosa* Sm. in Sibth. & Sm. (2n = 2x = 14, MM) (Kilian et al. 2011; Friebe et al. 1999). The species, which has a wide distribution, is native to the Middle East, the Mediterranean, and southern parts of Russia and Ukraine. *Ae. geniculata* is a valuable source of genes for pest and disease resistance (Zaharieva et al. 2001; Gill et al. 1985), salt tolerance (Siddiqui and Yosufzai 1988), high-grain protein content, and early maturity (Bochev et al. 1982). Because *Ae. geniculata* is highly crossable with common wheat, it will be an important germplasm for wheat improvement (Zhang et al. 1996). Additionally, *Ae. geniculata* is one of the most widespread species of the genus (Van Slageren 1994) and is a potentially useful genetic resource for improving cultivated bread or common wheat (*Triticum aestivum* L.; 2n = 6x = 42, AABBDD) (Friebe et al. 1996).

The identification of exogenous chromosome(s) or chromosome segments is very important after alien genetic material has been introduced successfully into wheat. Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) are used routinely, because those are efficient and accurate techniques to directly and precisely detect the alien chromosomes or introgressed alien segments (An et al. 2013; Fu et al. 2014, 2015); the constitution of chromosome complements can thus be analyzed. Moreover, diverse functional molecular markers are also powerful techniques, and expressed sequence tags (EST) are a class of simple sequence repeats (SSR) that originate from the expressed regions of genes and these have developed into powerful molecular markers because of their low cost and the capacity for in silico analysis using EST databases (Zhang et al. 2005a, b). Thus, the EST markers from bread wheat can be used to analyze collinearity and homoeology of chromosomes derived from distantly related species. In addition, PLUG (PCR-based landmark unique gene) markers can detect polymorphisms between wheat A, B, and D genomes due to intron differences (Ishikawa et al. 2009), reveal collinearity relationships and sequence polymorphisms among different Triticeae species, and hence be used to allocate unknown alien chromosomes to one of the seven homoeologous chromosome groups (Hu et al. 2011).

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (*Bgt*), is one of the most globally devastating diseases of wheat (Mohler et al. 2013; Wang et al. 2016). A long-established and increasingly important approach to enhance the genetic diversity of common wheat involves the incorporation of beneficial genes from related species by wide hybridization. The first step toward improving the disease resistance of wheat involves screening of large germplasm collections followed by the transfer of and combining of resistance genes to several diseases. The genus *Aegilops* has been the source of several highly effective disease resistance genes for wheat. The first successful transfer of an alien chromosome segment to wheat by irradiation involved the *Lr9* gene from *Ae. umbellulata* (Sears 1956), and the first transfer to wheat using homoeologous chromosome pairing (*Ph1* suppressor) incorporated the *Yr8* gene from *Ae. comosa* (Riley et al. 1968). The powdery mildew resistance gene *Pm29* was first introduced from a *Triticum aestivum* cv. Poros-*Ae. geniculata* alien addition line (Zeller et al. 2002). These initial positive results indicate further possibilities for the use of the genus *Aegilops* as a donor of disease resistance genes through distant hybridization with wheat.

In the current study, we developed and selected a wheat–*Ae. geniculata* disomic addition line (NA0973-5-4-1-2-9-1) with powdery mildew resistance from the BC_1F_6 progeny of crosses between wheat Chinese Spring (CS) and *Ae. geniculata* SY159. Thus, the objectives of this study were to: (a) identify the addition line by cytogenetic and GISH and FISH analyses; (b) develop specific EST-STS markers and PLUG markers for the alien chromosome in the addition line; and (c) evaluate the powdery mildew and agronomic traits of the addition line.

Materials and methods

Materials

Ae. geniculata (2n = 4x = 28, UUMM), accession SY159, was kindly provided by Dr. Lihui Li of the Chinese Academy of Agricultural Sciences, Beijing, China. The wheat–*Ae. geniculata* disomic addition line NA0973-5-4-1-2-9-1 was developed and selected from the BC_1F_6 progeny of common wheat Chinese Spring (CS)/*Ae. geniculata* SY159//CS. CS was a parent, and Shaanyou 225 was the powdery mildew-susceptible control variety. All genotypes were

maintained at the College of Agronomy, Northwest A&F University, China. The wheat–*Ae. geniculata* disomic addition line TA7661 (CS-AEGEN DA 7M^{g}) and addition line TA7667 (CS-AEGEN MA 7U^{g} MtA 7U^{g}, including Chinese Spring 42 chromosomes, a 7U^{g} mono and a 7U^{g} monotelosomic chromosome) were kindly provided by Dr. Friebe BR and Dr. Jon Raupp of the Department of Plant Pathology (Friebe et al. 1999), Throckmorton Plant Sciences Center, Kansas State University, Manhattan, USA.

Cytogenetic analysis

Seeds were placed on moistened filter paper in petri dishes at room temperature for approximately 1 day and then germinated in a constant temperature incubator at 23 °C in the dark. When the roots were 2–3 cm in length, the root tips were removed and pretreated with ice water at 0–4 °C for 24 h, fixed in Carnoy's fixative fluid (a 3:1 ethanol–acetic acid mixture) at 4 °C for at least 2 days, and then stored in 70 % ethanol at −20 °C for later use. The root tips were stained with 1 % (w/v) aceto-carmine solution for 2–4 h and squashed in 45 % (v/v) acetic acid. Young spikes were excised at the appropriate stage and fixed in 6:3:1 ethanol–chloroform–acetic acid mixture for at least 2 days. The anthers were squashed on a slide in 1 % aceto-carmine solution, and metaphase I cells with a complete chromosome complement were photographed with an Olympus BX43 microscope (Japan) equipped with a Photometrics SenSys CCD camera.

GISH and FISH

The total genomic DNA of common wheat CS and SY159 was isolated from seedling leaves using a modified CTAB method (Doyle and Doyle 1987), with one additional purification step using chloroform to obtain high-quality DNA. The total genomic DNA of SY159 was used as the labeled probe DNA, and sheared genomic DNA of CS was used as blocking DNA. The root tips were digested in 2 % cellulase and 1 % pectinase at 37 °C for 52–58 min (different digestion time should be needed in various materials). The slides were prepared using the drop technique (Han et al. 2006). The GISH procedure was performed as described in Fu et al. (2014), and with minor modifications. Oligonucleotide probes Oligo-pSc119.2 and Oligo-pTa535, 5′ end-labeled with 6-carboxyfluorescein (6-FAM) or 6-carboxy-tetramethylrhodamine (TAMRA) were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (Shanghai, China), which were used for identifying wheat and *Ae. geniculata* chromosomes by FISH analysis. Probe labeling and in situ hybridization were performed according to Tang et al. (2014). Chromosomes were counterstained with DAPI (blue). Fluorescent signals were viewed, photographed (Olympus BX53, Japan), and equipped with a Photometrics SenSys CCD camera DP 80.

EST-STS and PLUG markers analysis

EST-STS markers (http://wheat.pw.usda.gov/SNP/new/pcr_primers.shtml) and PLUG markers for homoeologous groups 1 to 7 of wheat chromosomes were all synthesized in AuGCT DNA-SYN Biotechnology Co., Ltd of Beijing (Ishikawa et al. 2009). These different markers were used to further determine homoeologous group relationships of the introduced alien chromosomes for the wheat–*Ae. geniculata* disomic addition line NA0973-5-4-1-2-9-1. The PCR products of EST-STS markers were separated in 8 % non-denaturing polyacrylamide gel and with silver staining. The products of PLUG markers were analyzed by electrophoresis on a 1 % agarose gel, and to obtain high levels of polymorphism, a 7.5-μl subsample of the product was digested with TaqI (60 °C) for 2 h. Digested fragments were fractionated by electrophoresis on 2 % agarose gel in 1 % TAE buffer.

Disease resistance and agronomic trait evaluation

To evaluate resistance to powdery mildew at the seedling stage, CS, SY159, NA0973-5-4-1-2-9-1, TA7661, and the susceptible control variety Shaanyou 225 were separately tested in the greenhouse at the College of Agronomy, Northwest A&F University. The *Bgt* isolate E09 came from the College of Agronomy of Northwest A&F University. When the control variety Shaanyou 225 was fully infected after the artificial inoculation, the reactions to E09 were assessed on a scale from 0 to 4, as follows: 0, and 1 were considered to be resistant, 2 was recorded to be moderately resistant, and 3 and 4 were assessed to be moderately susceptible and susceptible, respectively

(Sheng 1988; Wang et al. 2016). Morphological traits of line NA0973-5-4-1-2-9-1 and its parents, CS, SY159, tillering, plant height, spike length, spikelets per spike, kernels per spikelet, kernels per spike, thousand-kernel weight, presence/absence of awns, self-fertility, and maturity, were all sampled randomly and recorded. All the materials were planted in the field for the growing season 2014–2015.

Results

Morphology and cytological characterization

In 2009, an F1 hybrid named NA0821 (2n = 35, ABDUM) was obtained from a cross between CS and SY159. Subsequent backcrossing with the maternal parent CS (No. NA0973) was undertaken, and a set of CS–*Ae. geniculata* progenies was later produced after several generations of self-pollination. The line NA0973-5-4-1-2-9-1 was derived from BC_1F_6 progenies. The plant height, spike length, and absence of awns of NA0973-5-4-1-2-9-1 closely resembled that of the wheat parent CS. This line also exhibited a high seed set similar to wheat cv. CS. However, NA0973-5-4-1-2-9-1 had slightly more kernels per spikelet, a significantly higher thousand-kernel weight, and earlier maturity than CS. Its tillering traits more closely resembled the *Aegilops* parent; SY159 had 75 ± 5; and NA0973-5-4-1-2-9-1 had 61 ± 5 (Supplementary Table 1; Fig. 1). The mitotic and meiotic observations of line NA0973-5-4-1-2-9-1 indicated that root tip cells (RTCs) had a chromosome number of 44 (Fig. 2a), pollen mother cells (PMCs) formed a pairing configuration of 22 ring bivalents (Fig. 2b). The average chromosome configuration at PMCs during metaphase I was 0.12 univalents, 1.62 rod bivalents, and 20.32 ring bivalents. No trivalents or quadrivalents were observed at metaphase I, meanwhile no chromosomes were observed lagging at anaphase I (Fig. 2c). Therefore, NA0973-5-4-1-2-9-1 exhibited high cytological stability.

GISH analysis

GISH analysis was conducted to determine the chromosome constitution of NA0973-5-4-1-2-9-1. Whole genomic DNA of *Ae. geniculata* SY159 was used as the labeled probe, and CS was used as a blocker DNA. The GISH screening of mitotic cell divisions showed that NA0973-5-4-1-2-9-1 had two chromosomes with bright green hybridization signals (Fig. 3). Therefore, NA0973-5-4-1-2-9-1 contained two chromosomes from *Ae. geniculata*. GISH analysis also showed that other chromosomes displayed blue signals counterstained with DAPI, indicating that these chromosomes originated from the wheat parent CS. These results clearly showed that NA0973-5-4-1-2-9-1 was a disomic addition line with 42 chromosomes from wheat and a pair of chromosomes from *Ae. geniculata* SY159.

Molecular markers analysis

In the present study, 155 pairs of EST-STS markers and PLUG markers, representing specific markers for all of homoeologous groups 1 to 7 of common wheat, were screened against CS and *Ae. geniculata* SY159. Of these, 50 were polymorphic, i.e., a ratio of 32 %. These polymorphic primers were used to amplify DNA samples from the disomic addition line NA0973-5-4-1-2-9-1 and its parents. One EST-STS marker and three PLUG markers (Fig. 4; Supplementary Table 2), i.e., BE637663, TNAC1829, TNAC1888, and TNAC1941 which mapped onto the seventh homoeologous group, amplified clear polymorphic bands in NA0973-5-4-1-2-9-1 and SY159 but not in the wheat parent CS. Hence, the introduced pair of SY159 chromosomes in NA0973-5-4-1-2-9-1 has been shown to be homoeologous with the group 7 chromosomes of wheat. This addition line was designated as wheat–*Ae. geniculata* disomic addition line (CS-AEGEN DA) $7M^g$ or (CS-AEGEN DA) $7U^g$.

FISH analysis

In order to further determine the identity of the *Ae. geniculata* chromosomes from SY159 in NA0973-5-4-1-2-9-1 line, FISH analysis was performed on the newly developed addition line NA0973-5-4-1-2-9-1, as well as the addition lines TA7661 ($+7M^g$) and TA7667 ($+7U^g$) sourced from the WGRC, USA. Probe Oligo-pTa535, mainly hybridizes to wheat D genome chromosomes, and Oligo-pSc119.2 are useful for identifying B genome chromosomes. We can successfully discriminate the whole set of common wheat 42 chromosomes by combining these two oligonucleotide probes. First, the standard FISH

Fig. 1 Evaluation morphological comparison in the field and resistance reactions after inoculation with E09 in the seedling stages. **a** Adult plants, **b** spikes, **c** spikelets, **d** seeds. **e** resistance reactions *1* CS, *2* NA0973-5-4-1-2-9-1, *3* SY159, *4* Shaanyou 225, *5* TA7661

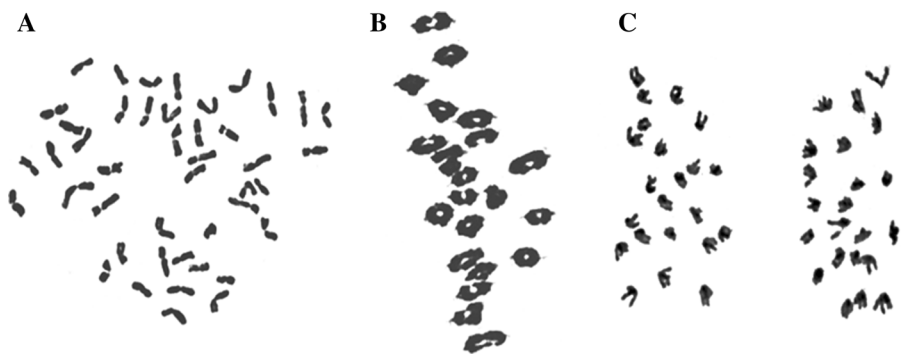

Fig. 2 Mitotic (**a**), meiotic metaphase I (**b**), and meiotic anaphase I (**c**) chromosome characteristics of NA0973-5-4-1-2-9-1. **a** 2n = 44, **b** 2n = 22Π, **c** 2n = 22I + 22I

karyotype patterns of wheat parent CS were painted using oligonucleotide probes Oligo-pTa535 (red) and Oligo-pSc119.2 (green) (Fig. 5a) according to Tang et al. (2014). This enabled us to study the structures of common wheat chromosomes in NA0973-5-4-1-2-9-1 compared with the addition lines TA7661 and TA7667 sourced from the USA. TA7661 was a wheat–*Ae. geniculata* $7M^g$ disomic addition line and showed 42 pairs of CS chromosomes and a pair of chromosomes with a red signal indicating the hybridization of probe Oligo-pTa535 (Fig. 5c), while TA7667 was a wheat–*Ae. geniculata* $7U^g$ addition line, and the $7U^g$ chromosomes showed the presence of the green signal of Oligo-pSc119.2 in a monosomic additions chromosome and a monotelosomic chromosome (Fig. 5b). The addition line NA0973-5-4-1-2-9-1 and TA7661 have the same FISH patterns using oligonucleotide probes Oligo-pTa535 (Fig. 5c, d). These results suggested that disomic addition line NA0973-5-4-1-2-9-1 contained an added pair of 7M chromosomes derived from *Ae. geniculata* SY159.

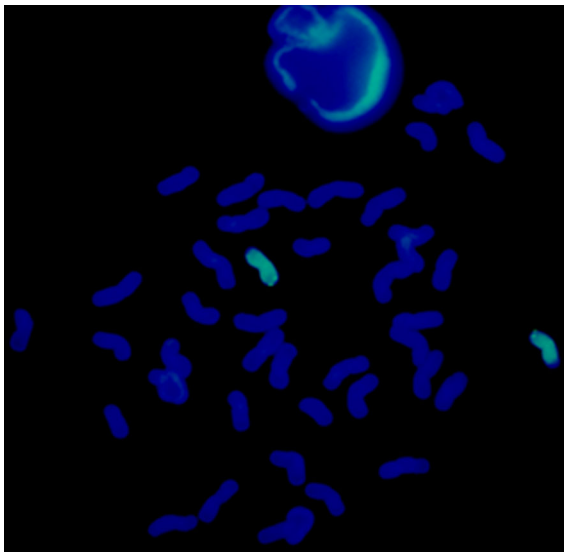

Fig. 3 Genomic in situ hybridization (GISH) analysis using SY159 genomic DNA (*green*) as probe on root tip metaphase chromosomes of NA0973-5-4-1-2-9-1. Chromosomes were counterstained with DAPI (*blue*)

More interestingly, FISH signal patterns of chromosomes of TA7661 were different from the parent line to some extent. In particular, there was structural variation in a pair of 6D chromosomes, where those from TA7661 had a green signal of Oligo-pSc119.2 on the telomeric ends of the short arm, while CS and NA0973-5-4-1-2-9-1 had hybridization of the red signal of Oligo-pTa535.

Disease resistance evaluation

For testing the powdery mildew reaction at the seedling stage, CS, SY159, NA0973-5-4-1-2-9-1, TA7661, and control variety Shaanyou 225 were inoculated with the *Bgt* isolate E09. SY159 and NA0973-5-4-1-2-9-1 showed immunity to E09 isolate with an IT score of 0-0. In contrast, CS and the susceptible control Shaanyou 225 were highly susceptible to E09 isolate with an IT score of 4, and TA7661 was moderately susceptible to E09 isolate with an IT score of 3 (Fig. 1e). The results indicated that NA0973-5-4-1-2-9-1 was different from the $7M^g$ disomic addition line TA7661, these results suggested that NA0973-5-4-1-2-9-1 was a wheat–*Ae. geniculata* $7M^g$ disomic addition line with new powdery mildew resistance, and this powdery mildew resistance gene was derived from the *Ae. geniculata* parent SY159.

Discussion

It is well known that alien chromosomes, carrying potentially useful agronomic traits that have been introduced into a common wheat background, have been useful bridge resources for wheat breeding. Addition lines have played a vital role during this process (Du et al. 2013; Wang et al. 1993; Taketa and Takeda 2001). Friebe et al. (1999) reported the development and identification of a complete set of *T. aestivum–Ae. geniculata* chromosome addition lines. In this study, NA0973-5-4-1-2-9-1 was a wheat–*Ae. geniculata* $7M^g$ disomic addition line. Its tillering trait was more prolific than other disomic addition. A superior spike trait is also essential for improving wheat yields and has previously been achieved by the introgression of chromosomes from wild species related to wheat (Wu et al. 2006). The addition line NA0973-5-4-1-2-9-1 had higher thousand-kernel weight and earlier maturity compared with CS, and these desirable characteristics most likely have been introduced to wheat from *Ae. geniculata* SY159. Thus, the disomic addition line NA0973-5-4-1-2-9-1 can be used as a donor source for introducing novel genes into wheat during breeding programs.

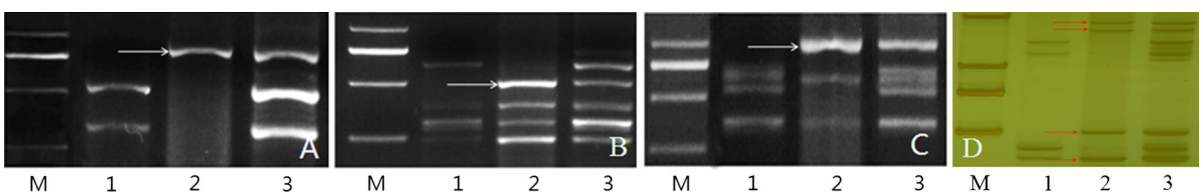

Fig. 4 PLUG marker and EST-STS marker results. The *arrow* indicates an *Ae. geniculata* SY159 specific band. *M* DL2000, *1* CS, *2 Ae. geniculata* SY159, *3* NA0973-5-4-1-2-9-1. **a** TNAC1829-*TaqI*, **b** TNAC1888-*TaqI*, **c** TNAC1941-*TaqI*, **d** EST-STS marker *BE637663*

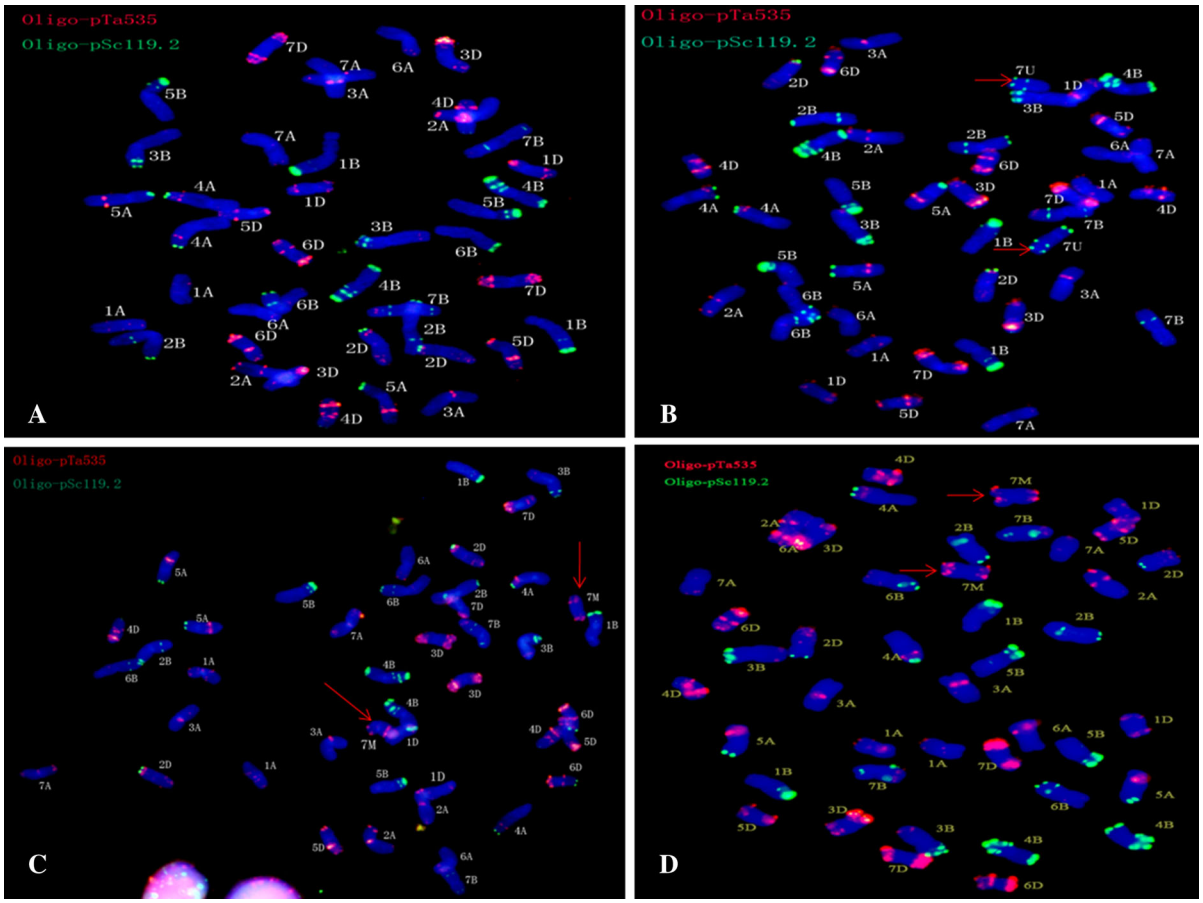

Fig. 5 Fluorescence in situ hybridization (FISH) analysis using Oligo-pTa535 (*red*), Oligo-pSc119.2 (*green*) as probes on root tip metaphase chromosomes of CS (**a**), TA7667 (**b**), TA7661 (**c**), and NA0973-5-4-1-2-9-1 (**d**). The *red arrows* indicate the introduced *Aegilops geniculata* chromosomes

Molecular markers could provide a simple and precise approach to track the alien DNA in a wheat background based on comparative genome analysis and could determine the homoeologous group relationship of alien chromosomes in a wheat background. Markers especially of value are those derived from the conserved genetic region, showing high levels of collinearity among the cereal genomes, such as rice, *Brachypodium*, and wheat (Heslop-Harrison 2000). EST-STS markers and PLUG molecular markers have been developed for the Triticeae to analyze wheat–alien species derivative lines, which can be used to distinguish alien chromosome homoeologous groups and for tracking alien chromosome(s) (Kong et al. 2008; Hu et al. 2011), and have been used extensively as effective tools for the genetic analysis of alien chromosomes including *Psathyrostachys huashanica* (Du et al. 2013), *Agropyron cristatum* (L.) Gaertn (Wu et al. 2006), *Leymus mollis* (Yang et al. 2015), *Secale cereale* L. (Wang et al. 2010), and *Thinopyrum ponticum* (Zhang et al. 2011; Chen et al. 2012). In this current study, we had screened one EST-STS marker and three PLUG markers based on homoeologous group 7 of the wheat chromosomes, and we deduced that the pair of *Ae. geniculata* chromosomes in NA0973-5-4-1-2-9-1 belonged to homoeologous group 7 (Fig. 4). Thus, these markers can be used to identify the addition line NA0973-5-4-1-2-9-1 and to assign the alien chromosomes as either 7M or 7U. These markers can also be used as unique tools for tracking alien *Ae. geniculata* in a wheat background and for comparative gene

mapping, chromosomal evolutionary analysis, and gene introgression during wheat improvement using *Ae. geniculata* accessions as gene donors.

Early reports of the production and identification of a complete set of intact *Ae. geniculata* chromosome and telosome additions to common wheat were described after C-banding (Friebe et al. 1999). Afterward, FISH has become a powerful technique for localizing highly repetitive DNA sequences, detecting specific sites in particular regions of individual chromosomes, and discriminating genome constitutions (Rayburn and Gill 1986; Leitch and Heslop-Harrison 1992). Many substitution, disomic addition and translocation lines involving the incorporation of alien chromosomes and segments into wheat have been identified using FISH analysis (Yang et al. 2014 and 2015; Fu et al. 2014; An et al. 2013). In this present study, in order to further characterize NA0973-5-4-1-2-9-1 line, the standard FISH karyotype patterns of wheat parent CS were painted using oligonucleotide probes Oligo-pTa535 (red) and Oligo-pSc119.2 (green), which enabled a comparison of the common wheat chromosomes in NA0973-5-4-1-2-9-1 and in TA7661 and TA7667. NA0973-5-4-1-2-9-1 was found to be a wheat–*Ae. geniculata* $7M^g$ disomic addition line. The $7U^g$ chromosomes in TA7667 showed the presence of a green signal on a monosomic addition and a monotelosomic chromosome corresponding to hybridization of probe Oligo-pSc119.2 (Fig. 5b). Interestingly, the FISH signal patterns of CS, TA7661, and NA0973-5-4-1-2-9-1 differed in the structure of a pair of 6D chromosomes. The FISH signal patterns were same for the 6D chromosomes of CS and NA0973-5-4-1-2-9-1 and showed the red signal of Oligo-pTa535. However, a pair 6D chromosomes of TA7661 had a green signal of Oligo-pSc119.2 on the ends of the short arms. This means that these wheat chromosomes have been extensively restructured as has previously been reported when crossing common wheat and other species (Friebe et al. 1999; Zhang et al. 2013; Tang et al. 2014; Li et al. 2015).

The development of alien addition lines could also facilitate the isolation of different alien chromosomes and the analysis of individual chromosome functions in a wheat background. Many genes related to powdery mildew resistance are derived from wild relatives of wheat including *Dasypyrum breviaristatum* (Yang et al. 2008), *Ae. geniculata* (Kuraparthy et al. 2007), *Haynaldia villosa* (L.) Schur. (Li et al. 2007), *Lophopyrum ponticum* (Zhang et al. 2005a, b), and *Thinopyrum ponticum* (Yin et al. 2006). In this study, a powdery mildew resistance test showed that derivatives of the disomic addition line NA0973-5-4-1-2-9-1 carrying the 7M chromosome of *Ae. geniculata* were resistant to powdery mildew. The wild species *Ae. geniculata* exhibited immunity, whereas CS and Shaanyou 225 were infected with powdery mildew. Thus, NA0973-5-4-1-2-9-1 had acquired resistance to powdery mildew due to the extra chromosomes added from its alien parent *Ae. geniculata* SY159. Mettin et al. (1977) reported on four different wheat–alien addition lines, in which a single pair of *Ae. ovata* chromosomes had been added to the chromosome complement of the wheat cultivar Poros. Among the four lines, the Poros-*Aegilops ovata* addition line VI ($2n = 44$) was highly resistant to powdery mildew. However, the resistance could not be traced back to a specific chromosome, because of the high structural variation in the chromosome complements of the A and B genomes of the addition lines (Friebe and Heun 1989). Friebe reported that the resistance gene *Pm29* was incorporated into wheat line Pova and was derived from a *Triticum aestivum* cv. Poros-*Aegilops ovata* alien addition line. The *Bgt* isolates used for the differentiation of the known major resistance genes were collected from different parts of Europe and selected from single spore progenies (Zeller et al. 2002), and TA7661 was one of the addition lines from Friebe et al. In our study, TA7661 and NA0973-5-4-1-2-9-1 were both infected with E09, and the result was that NA0973-5-4-1-2-9-1 was almost immune (Fig. 1e). Line TA7661 was susceptible to powdery mildew. The results indicated that NA0973-5-4-1-2-9-1 was different from disomic addition line TA7661; NA0973-5-4-1-2-9-1 was a wheat–*Ae. geniculata* disomic addition line with new powdery mildew resistance. This line could be a useful bridge for the transfer of powdery mildew resistance gene(s) from *Ae. geniculata* SY159 to common wheat.

We developed the *Ae. geniculata* $7M^g$ disomic addition line, NA0973-5-4-1-2-9-1, via an intergeneric hybrid that possessed improved agronomic characteristics compared with its parents, i.e., higher tiller number and thousand-kernel weight (Supplementary Table 1). There is always a positive correlation between the yield and the individual yield

components, so wheat breeding demands the coordination of these relationships. Thus, the disomic addition line NA0973-5-4-1-2-9-1 can be used as a donor source for introducing novel genes into wheat during breeding programs.

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