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NOTE

In situ hybridization analysis indicates that 4AL–5AL–7BS translocation preceded subspecies differentiation of *Triticum turgidum*

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Abstract: The important cyclic translocation 4AL–5AL–7BS is an evolutionary signature of polyploidy in wheat. This study aimed to determine its distribution within the subspecies of *Triticum turgidum* L., using genomic in situ hybridization and fluorescence in situ hybridization. As it exists in all eight subspecies, this translocation appeared before the differentiation of the subspecies of *T. turgidum*. This translocation probably first appeared in *T. turgidum* subsp. *dicoccoides* and was then transmitted into the other subspecies. Its existence in all of the analyzed subspecies suggests that this translocation may confer an adaptive advantage during the course of evolution.

Key words: Triticum turgidum L., translocation, wheat.

Résumé : L'importante translocation cyclique 4AL–5AL–7BS est une signature évolutive de la polyploïdie chez le blé. Cette étude visait à déterminer sa distribution au sein des sous-espèces du *Triticum turgidum* L. au moyen de l'hybridation génomique in situ et de l'hybridation in situ en fluorescence. Comme elle est présente au sein des huit sous-espèces, cette translocation serait apparue avant la séparation des sous-espèces du *T. turgidum*. Cette translocation est vraisemblablement apparue d'abord chez le *T. turgidum* subsp. *dicoccoides* et aurait été transmise ensuite aux autres sous-espèces. Sa présence au sein de toutes les sous-espèces examinées suggère que cette translocation pourrait présenter un avantage adaptatif au cours de l'évolution. [Traduit par la Rédaction]

Mots-clés : Triticum turgidum L., translocation, blé.

Introduction

Chromosome translocation is an important driving force in the evolutionary process that can immediately change gene linkages and gene expression (Rieseberg 2001; Gaeta and Pires 2010). It has been detected in newly resynthesized polyploids as well as natural polyploids (Xiong et al. 2011; Chester et al. 2012). Species-specific translocation is an important evolutionary signature for certain species. Numerous chromosome translocations have been observed in species of *Triticum* in the tribe Triticeae (Qi et al. 2006). 4AL–5AL–7BS is an important translocation in common wheat (*Triticum aestivum*, 2*n* = 6*x* = 42, genome AABBDD) (Naranjo et al. 1987, 1988; Chao et al. 1989; Liu et al. 1992; Nelson et al. 1995; Coriton et al. 2009; Berkman et al. 2012) and tetraploid emmer wheat (*Triticum turgidum* L., AABB) (Naranjo 1990; Jiang and Gill 1994; Blanco et al. 1998; Maestra and Naranjo 1999; Kawahara and Taketa 2000; Rodríguez et al. 2000; Marone et al. 2012). The 4AL–5AL terminal translocation happened at the diploid level as it exists in the A genome of the diploid species *Triticum urartu* and *Triticum monococcum* (King et al. 1994; Devos et al. 1995), and it may derive from a common ancestor as similar rearrangements were detected in other species within Triticeae, such as *Secale cereale* (Devos et al. 1993; King et al. 1994), *Leymus triticoides*, and *Leymus cinereus* (Larson et al. 2012). Then, the distal portion of the chromosome 5A segment on 4AL was exchanged with a terminal segment from chromosome 7BS in *T. turgidum* (Devos et al. 1995). According to van Slageren (1994), *T. turgidum* includes eight subspecies: *dicoccoides*, *dicoccon*, *carthlicum*, *durum*, *polonicum*, *turanicum*, *turgidum*, and *paleocolchicum*. This study aimed to determine the distribution of the 4AL–5AL–7BS translocation within the subspecies of *T. turgidum*.

Materials and methods

Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) were used to analyze 16 lines from the eight subspecies of *T. turgidum* (Table 1). GISH was performed as described previously (Hao et al. 2011). Biotinylated A genome (pink) of *T. urartu* was used as a probe, with unlabeled S genome (blue) of *Aegilops speltoides* used as a blocker (Fig. 1). To further identify the translocated chromosomes, FISH was performed with repeat sequence probes pSc119.2 (green), pTa71 (yellow), and Afa family (red) (Fig. 2).

Table 1. The *Triticum turgidum* wheat lines used in this study.

T. turgidum subsp.	Line (origin)
carthlicum	PI94751 (Georgia), AS2268 (Germany)
dicoccoides	AS285 (Germany), AS286 (France)
dicoccon	PI377655 (Yugoslavia), PI94655 (Bulgaria)
durum	Langdon (USA), AS2262 (Syria)
polonicum	PI14892 (Ethiopia), AS308 (China)
turanicum	PI184526 (Portugal), PI184543 (Portugal)
turgidum	AS2255 (China), AS2240 (China), AS313 (China)
paleocolchicum	AS365 (unknown)

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Fig. 1. GISH images of A- (pink) and B-genome (blue) chromosomes of seven lines from different subspecies of *Triticum turgidum*. (*a*) AS286. (*b*) AS2268. (*c*) Langdon. (*d*) AS365. (*e*) PI184526. (*f*) AS2240. (*g*) AS308. Chromosomes showing the 4A–7B translocation are indicated with arrows. Scale bar = 50 μm.

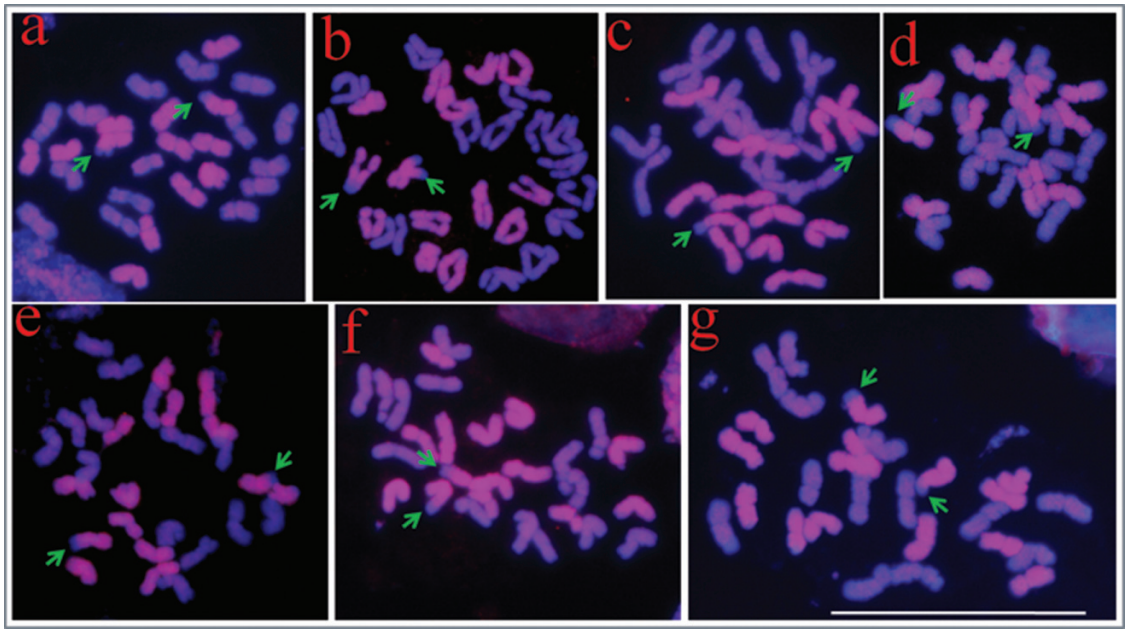
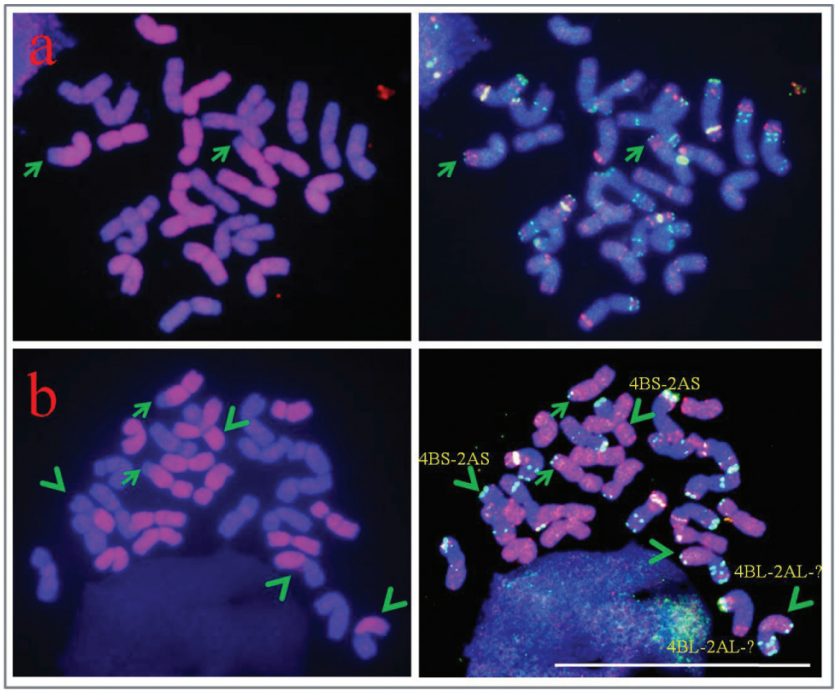


Fig. 2. GISH (left) and FISH (right) images of two *Triticum turgidum* lines. GISH was performed using A genome (red) as a probe, with unlabeled S genome used as a blocker. The same slide was then used for FISH using repeat sequence probes pSc119.2 (green), pTa71 (yellow), and Afa (red). (*a*) PI377655. (*b*) PI14892. Scale bar = 50 μm.



Results and discussion

One similar pair of A/B chromosome translocations was observed in all 16 lines (Fig. 1). The B-chromosome fragments had about 20% of the total length of the translocated chromosome arm. The pair of chromosomes were likely to be 4A, judged by the ratio of A and B segments (Devos et al. 1995; Miftahudin et al. 2004). Based on the FISH signals (Fig. 2; Sepsi et al. 2008; Uhrin et al. 2012), the translocated chromosomes mentioned above in the 16 lines were 4AL–5AL–7BS. Previous results suggest that the terminal segment on the current chromosome 7BS was derived from the original 5AL (Devos et al. 1995; Berkman et al. 2012). As the 5AL segment is much smaller than the 7BS segment on the modern 4AL, we failed to find any evidence showing any A-chromosome segment on any pair of the B chromosomes.

The existence of translocation 4AL–5AL–7BS in all eight subspecies of *T. turgidum* indicated that this translocation appeared

before the differentiation of the subspecies of *T. turgidum*. This translocation probably first appeared in *T. turgidum* subsp. *dicoccoides*, since it is the wild ancestor of the other subspecies. It could have occurred after the origin of *T. turgidum* subsp. *dicoccoides* or during its origin in the cross between *T. urartu* and *Ae. speltoides* or closely related species. Whenever it originated, it is expected that the original population was a mixture of cytotypes with and without this translocation, especially if we consider that *T. turgidum* subsp. *dicoccoides* originated more than once, although it is unclear whether or not cytotypes without this translocation also exist in the current *T. turgidum*. However, the translocation of *T. turgidum* subsp. *dicoccoides* was conserved in all 16 lines analyzed from the eight subspecies, which suggests that this translocation may confer an adaptive advantage during the course of evolution.

Besides the above mentioned translocation, Kawahara and Taketa (2000) found the presence of two pairs of translocations, i.e., 2AS–4BS and 2AL–4BL, in *T. turgidum* lines from Ethiopia by N-banding and GISH analysis. These translocations were also observed in *T. turgidum* subsp. *polonicum* line PI14892 from Ethiopia (Fig. 2*b*, arrowhead). The breakpoint for 2AS–4BS translocation was located near but not in the centromeric region, in which the centromere was inherited from the 4B chromosome (Fig. 2*b*). The 2AL–4BL translocation was a centric translocation. pSc119.2 signals absent for normal 2A, however, were observed on the terminal of 2AL. This suggested that another recombination event had happened between 2AL and another chromosome. However, the two pairs of translocations did not exist in *T. turgidum* subsp. *polonicum* line AS308 from China, indicating that they are not subspecies-specific translocations.

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