(GAA)n microsatellite as an indicator of the A genome reorganization during wheat evolution and domestication

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Abstract
Although the wheat A genomes have been intensively studied over past decades, many questions concerning the mechanisms of their divergence and evolution still remain unsolved. In the present study we performed comparative analysis of the A genome chromosomes in diploid (Triticum urartu Tumanian ex Gandilyan, 1972, T. boeoticum Boissier, 1874 and T. monococcum Linnaeus, 1753) and polyploid wheat species representing two evolutionary lineages, Timopheevi (T. timopheevii (Zhukovsky) Zhukovsky, 1934 and T. zhubkovskyi Menabde & Ericzjan, 1960) and Emmer (T. dicoccoides (Körnicke ex Ascherson & Graebner) Schueinfurth, 1908, T. durum Desfontaines, 1798, and T. aestivum Linnaeus, 1753) using a new cytogenetic marker – the pTm30 probe cloned from T. monococcum genome and containing (GAA)n microsatellite sequence. Up to four pTm30 sites located on 1AS, 5AS, 2AS, and 4AL chromosomes have been revealed in the wild diploid species, although most accessions contained one–two (GAA)n sites. The domesticated diploid species T. monococcum differs from the wild diploid species by almost complete lack of polymorphism in the distribution of (GAA)n site. Only one (GAA)n site in the 4AL chromosome has been found in T. monococcum. Among three wild emmer (T. dicoccoides) accessions we detected 4 conserved and 9 polymorphic (GAA)n sites in the A genome. The (GAA)n loci on chromosomes 2AS, 4AL, and 5AL found in of T. dicoccoides were retained in T. durum and T. aestivum. In species of the Timopheevi lineage, the...
only one, large (GAA)n site has been detected in the short arm of 6A chromosome. (GAA)n site observed in T. monococcum are undetectable in the A\textsuperscript{b} genome of T. zhukovskyi, this site could be eliminated over the course of amphiploidization, while the species was established. We also demonstrated that changes in the distribution of (GAA)n sequence on the A-genome chromosomes of diploid and polyploid wheats are associated with chromosomal rearrangements/ modifications, involving mainly the NOR (nucleolus organizer region)-bearing chromosomes, that took place during the evolution of wild and domesticated species.

**Keywords**

*Triticum monococcum, T. boeoticum, T. urartu, T. zhukovskyi, T. dicoccoides*, (GAA)n microsatellite, FISH

**Introduction**

The genus *Triticum* Linnaeus, 1753 comprises species at different ploidy levels, from diploid to hexaploid. Common wheat *T. aestivum* L., 1753 is natural allopolyploid with the genome BBAADD, which emerged about 8–10 thousand years ago (TYA) *via* the cross of tetraploid Emmer species (BBAA genome) with *Aegilops tauschii* Cosson, 1850 (DD genome). Another hexaploid wheat, *T. zhukovskyi* Menabde & Ericzjan, 1960 (genome GGA\textsuperscript{a}A\textsuperscript{a}A\textsuperscript{b}) was discovered in 1957, in the Zanduri region of Western Georgia and is regarded as natural allopolyploid of *T. timopheevii* (Zhukovsky) Zhukovsky, 1934 and *T. monococcum* L., 1753 growing in the same area (Jakubtsiner 1959, Tavrin 1964). As is currently assumed by the majority of researchers, tetraploid Emmer (*T. dicoccoides* ((Körnicke ex Ascherson & Graebner) Schweinfurth, 1908, *T. durum* Desfontaines, 1798, etc. genome BBAA) and Timopheevi (*T. araraticum* Jakubziner, 1947, *T. timopheevii*, and *T. militinae* Zhukovsky & Miguschova, 1969, genome GGA\textsuperscript{a}A\textsuperscript{a}) wheats occurred as a result of hybridization between the ancestral forms of *Ae. speltoides* Tausch, 1837 as a maternal parent and *T. urartu* Tumanian ex Gandilyan, 1972, as a paternal parent (Dvorak et al. 1988, Tsunewaki 1996, Huang et al. 2002). Although both evolutionary lineages of the tetraploid wheats originated *via* hybridization of closely related parental forms, their emergence occurred independently at different times and probably in different places. In particular, the origin of the tetraploid *T. dicoccoides* is dated back to over 500 TYA, versus *T. araraticum*, dated back to 50–300 TYA (Mori et al. 1995, Huang et al. 2002, Levy and Feldman 2002).

The diploid wheats are the most ancient members of the genus *Triticum*. Among them taxonomists recognize three species, namely, cultivated *T. monococcum* and two wild species, *T. boeoticum* Boissier, 1874 and *T. urartu* (Goncharov, 2012). Two different types of the A genome, A\textsuperscript{a} (*T. urartu*) and A\textsuperscript{b} (*T. boeoticum* and *T. monococcum* L., 1753), have been discriminated among the diploid wheats. According to the current concept, the A\textsuperscript{a} and A\textsuperscript{b} genomes diverged approximately one million years ago (Huang et al. 2002). Morphologically *T. urartu* and *T. boeoticum* are very similar (Filatenko et al. 2002), and differ distinctly only in the leaf pubescence pattern (velvety *vs.* bristly), controlled by allelic genes (Golovnina et al. 2009). These wild species have overlapping distribution ranges, and in some cases accessions belonging to either one of the species are identified incorrectly.
Despite morphological similarity, the level of genome divergence between *T. urartu* and *T. boeoticum* is very high. First of all it is indicated by the sterility of hybrids between *T. urartu* and *T. boeoticum* and/or *T. monococcum*, although in certain combinations of accessions and crossing direction hybrid fertility was elevated from zero to 4.5% (Fricano et al. 2014). Analysis of a broad sample of diploid A-genome species using multilocus markers, such as SSAP (sequence specific amplification polymorphism) and AFLP (amplified fragment length polymorphism) demonstrated considerable genetic differentiation among the accessions; and two super-clusters of diploid wheats have been discriminated among them (Konovalov et al. 2010, Fricano et al. 2014). The first super-cluster contains *T. urartu* and the second one, domesticated species *T. monococcum* and its wild progenitor, *T. boeoticum*. Importantly, intermediate forms between two super-clusters are detectable independently of the approach used for analysis; moreover, solitary accessions morphologically affiliated with *T. urartu* fall either within the opposite cluster or close to it.

Genome rearrangements, such as translocations, inversions, and the emergence of large blocks of repeats via amplification, are of considerable importance for the reproductive isolation of species. Such large-scale rearrangements are detectable by meiotic chromosome pairing analysis, comparative genome mapping, and FISH with repetitive probes. The data obtained so far suggest that the emergence of two evolutionary lineages of polyploid wheats, Emmer and Timopheevi, was accompanied by several species-specific translocations (Rodriguez et al. 2000, Salina et al. 2006a). Only one of these translocations, 4AL/5AL, which was inherited by polyploid wheat species from their diploid A genome progenitor, is characteristic of both *T. urartu* and *T. monococcum* (King et al. 1994, Devos et al. 1995). No information concerning the detection of other intraspecific and interspecific translocations in diploid wheats is available from literature.

One of the approaches for the identification of chromosomal rearrangements is cytogenetic analysis. Despite significant progress in sequencing and mapping of cereal genomes, this method is still most powerful for detection of chromosome aberrations; however, it needs a sufficient pool of cytogenetic markers. The number of cytogenetic markers used for the analysis of A genome chromosome is currently rather few. Single hybridization signals can be obtained with probes pSc119.2, pAs1, pTa71 (45S RNA genes), and pTa794 (5S rRNA genes) (Dubcovsky and Dvořák 1995, Schneider et al. 2003, Megyeri et al. 2012, Uhrin et al. 2012). Several (GAA)n sites have been detected on the A genome chromosomes of polyploid wheat, although signals are located predominantly on the B and G genome chromosomes of wheats and in the S-genome chromosomes of their diploid progenitor *Aegilops speltoides* (Gerlach and Dyer 1980). Hybridization with the (GAA)n probe not always produces stable signals on the A genome chromosomes, since either a synthetic probe or PCR fragments amplified from the wheat or rye genomic DNA were used (Kubaláková et al. 2005, Megyeri et al. 2012).

The goal of this work was to study the rearrangement of the A genome chromosomes of wheats during the evolution based on the distribution of (GAA)n microsatellite on the chromosomes.
Materials and methods

Plant material

The following diploid Triticum species were used in our work (see Table 1 for the complete list): *T. boeoticum* (2n = 2x = 14, A\textsuperscript{b}A\textsuperscript{b}) – six accessions; *T. monococcum* (2n = 2x = 14, A\textsuperscript{b}A\textsuperscript{b}) – six accessions, and *T. urartu* (2n = 2x = 14, A\textsuperscript{u}A\textsuperscript{u}) – seven accessions. For each species we selected accessions differing in the level of genomic divergence. In particular, according
to SSAP analysis based on the *BARE-1* and *Jeli* retrotransposons (Konovalov et al. 2010), all accessions of diploid wheats were divided into groups and super-clusters (Table 1).

Table 1. Accessions of the diploid and polyploid Triticum species used in the work.

| Accession/group | Species | Subspecies/variety (if available) | Centre of genetic resource | Accession number | Geographic origin |
|-----------------|---------|----------------------------------|---------------------------|------------------|-------------------|
| BO2/IG1         | *T. boeoticum* | subsp. thaoudar | Kyoto Univ. | KU8120 | Iraq |
| BO3/IG2         | *T. boeoticum* | – | VIR | K-25811 | Armenia |
| BO9/IG1         | *T. boeoticum* | – | ICARDA | IG116198 | Turkey |
| BO12/IG2        | *T. boeoticum* | subsp. boeoticum | VIR | K-18424 | Crimea |
| BO14/IG1        | *T. boeoticum* | – | USDA | PI427328 | Iraq |
| BO19/IG2        | *T. boeoticum* | subsp. boeoticum | VIR | K-33869a | Armenia |
| MO1/IG3         | *T. monococcum* | var. macedonicum | VIR | K-18140 | Azerbaijan |
| MO3/IG3         | *T. monococcum* | var. monococcum | VIR | K-20409 | Spain |
|                 | *T. monococcum* | – | VIR | K-18105 | Nagorno-Karabakh Autonomous Region |
|                 | *T. monococcum* | – | VIR | K-8555 | Crimea |
|                 | *T. monococcum* | – | USDA | PI119423 | Turkey |
|                 | *T. monococcum* | var. hornemannii, population Zanduri | VIR | K-46586 | Georgia |
| UR1/IIG4        | *T. urartu* | – | USDA | PI538736 | Lebanon |
| UR2/IIG4        | *T. urartu* | var. albinigricans | VIR | K-33869b | Armenia |
| UR3/IG3         | *T. urartu* | – | USDA | PI428276 | Lebanon |
| UR4/IG1         | *T. urartu* | – | ICARDA | IG116196 | Turkey |
| UR5/IIG2        | *T. urartu* | var. albinigricans | VIR | K-33871 | Armenia |
| UR6/IIG4        | *T. urartu* | – | ICARDA | IG45298 | Syria |
| UR44/IIG4       | *T. urartu* | – | USDA | PI428182 | Armenia |
|                 | *T. timopheevii* | population Zanduri | VIR | K-38555 | Georgia |
|                 | *T. zhukovskyi* | population Zanduri | VIR | K-43063 | Georgia |
|                 | *T. dicoccoides* | | ICARDA | IG46273 | Israel |
|                 | *T. dicoccoides* | | ICARDA | IG46288 | Israel |
|                 | *T. dicoccoides* | | ICARDA | IG139189 | Jordan |
|                 | *T. durum* | | VIR | K-1931 | Russia |
|                 | *T. aestivum* | | ICG | cv. Chinese Spring | China |
|                 | *T. aestivum* | var. lutescens | ICG | cv. Saratovskaya 29 | Russia |

* Designation of accessions and their clustering into groups (I/II, superclusters and G, groups) are according to Konovalov et al. (2010).
Current analysis included accessions from both well-differentiated super-clusters, *T. urartu* and *T. boeoticum/T. monococcum*, as well as three *T. urartu* accessions (UR3, UR4, UR5) for which the genome affinity determined by morphological traits was not confirmed by molecular analysis (Konovalov et al. 2010).

Polyploid wheat species belonging to either Timopheevi (*T. timopheevii*, 2n = 4x = 28, GGA:At, and *T. zhukovskyi*, 2n = 6x = 42, GGA:AAtAAb), or Emmer evolutionary lineage (*T. dicoccoides*, 2n = 4x = 28, BBAA, *T. durum*, 2n = 4x = 28, BBAA, and *T. aestivum*, 2n = 6x = 42, BBAAADD) were analyzed (Table 1).

The plants of all accessions used in our work were grown at the Joint Access Laboratory for Artificial Plant Cultivation for verification of species authenticity by morphological characters using a guide published by Goncharov (2012).

*T. zhukovskyi* authenticity was verified by electrophoresis of wheat storage proteins (gliadins) (Goncharov et al. 2007).

**DNA isolation and cloning**

The (GAA)n microsatellite sequence was cloned from einkorn wheat genome in order to increase the resolution of FISH analysis.

Total DNA was isolated from 5–7-day-old seedlings according to Plaschke et al. (1995). PCR for production of (GAA)n microsatellite was conducted according to Vrána et al. (2000) using (CTT) and (GAA), as primers and *T. urartu* (IG45298) and *T. monococcum* (PI119423) DNAs as templates. PCR comprised 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and synthesis at 72 °C for 1 min. The amplification products were cloned with a Qiagen kit. The clones differing in the length of the insert were selected and sequenced using ABI PRISM Dye Terminator Cycle Sequencing ready reaction kit (Perkin Elmer Cetus, USA). Sequencing was performed in an ABI PRISM 310 Genetic Analyzer (Perkin Elmer Cetus).

**Giemsa C-banding**

Giemsa C-banding was performed according to the protocol by Badaeva et al. (1994). The slides were examined with a Leitz Wetzlar microscope and recorded with a Leica DFC 280 CCD digital camera. The chromosomes were classified according to the standard nomenclature (Friebe and Gill 1996, Gill et al. 1991).

The work was performed at the Vavilov Institute of General Genetics, Russian Academy of Sciences.

**Fluorescence in situ hybridization**

Fluorescence in situ hybridization (FISH) was conducted as earlier described (Salina et al. 2006b). The probes were labeled with biotin or digoxigenin by nick translation.
Biotinylated probes were detected with fluorescein avidin D (Vector Laboratories). The digoxigenin-labeled probes were detected with antibodies to anti-digoxigenin-rhodamine, Fab fragments (Roche Applied Science).

The chromosomes were identified using the pSc119.2 (120 bp, rye repeats; Bedbrook et al. 1980) or pTa71 (45S RNA genes; Gerlach and Bedbrook 1979) probes.

The preparations were embedded into Vectashield mounting medium (Vector Laboratories), containing 0.5 µg/ml DAPI (4',6-diamidino-2-phenylindole, Sigma) for chromosome staining. The chromosomes were examined with an Axioskop 2 Plus (Zeiss) microscope and recorded with a VC-44 (PCO) CCD camera.

The work was performed at the Joint Access Center for Microscopic Analysis of Biological Objects with the Siberian Branch of the Russian Academy of Sciences.

Results

Cloning of (GAA)n microsatellite

Totally, four clones differing in the length of the insert were selected and sequenced. The clones obtained from *T. urartu* were designated pTu and from *T. monococcum*, pTm. All the clones contain (GAA)n microsatellite sequence, but differ in length: pTm30 has a length of 167 bp [(GAA)_{56}]; pTm17, 62 bp [(GAA)_{21}]; pTu33, 56 bp [(GAA)_{19}]; and pTu38, 36 bp [(GAA)_{12}]. The (GAA)_{56} microsatellite variant pTm30 generating most distinct signals was selected for further work.

Localization of (GAA)n probe on einkorn wheat chromosomes

The new probe pTm30 containing (GAA)_{56} sequence was hybridized to chromosomal preparations of *T. urartu*, *T. boeoticum* and *T. monococcum*; the pTa71 (45S rRNA genes) probe was used for chromosome identification. As was expected, the 45S ribosomal RNA genes in all species were localized to the nucleolar organizer region in the distal parts of the 1AS and 5AS chromosomes.

The accessions of wild species *T. boeoticum* and *T. urartu* displayed polymorphism in the distribution of (GAA)n microsatellite on the chromosomes. One to three pTm30 sites per haploid genome can be detected in these two species (Table 2, Fig. 1). As a whole, (GAA)n can be detected in four positions, on the 1AS, 2AS, 5AS, and 4AL chromosomes of *T. boeoticum* and *T. urartu*. The (GAA)n site on the 1AS is observed only in *T. urartu*, being detectable in four of the seven examined accessions. Both *T. boeoticum* and *T. urartu* carry (GAA)n sites on 2AS and 5AS chromosomes; however, the microsatellite is detectable at a higher rate on the 5AS of *T. urartu* and the 2AS of *T. boeoticum*. (GAA)n site on the 2AS in one accession of *T. boeoticum* was heteromorphic between homologous chromosomes (Fig. 1c). Some accessions of *T. boeoticum* and *T. urartu* carry a (GAA)n site on the 4AL chromosome.
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Figure 1. FISH with probes pTm30 (green signal) and pTa71 (red signal) on the chromosomes of diploid Triticum species: a T. monococcum MO1 b T. boeoticum BO3 c T. boeoticum BO14 and d T. urartu UR6.

Table 2. Localization of pTm30 probe on the chromosomes of diploid Triticum species.

| Chromosome (arm) | T. boeoticum | T. urartu | T. monococcum |
|-----------------|--------------|------------|---------------|
|                 | BO1 IG1 | BO3 IG1 | BO9 IG1 | BO12 IG1 | BO14 IG1 | BO19 IG4 | UR1 IIG4 | UR2 IIG4 | UR3 IIG3 | UR4 IIG1 | UR5 IIG2 | UR6 IIG4 | UR4 IIG4 |
| 1A(S)           |         | +       | +       | +       | +       | -       | +       | +       | +       | +       | +       | +       | +       |
| 2A(S)           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
| 5A(S)           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
| 4A(L)           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |

1 Designation of accessions and their clustering into groups (I/II, superclusters and G, groups) are according to Konovalov et al. (2010).
2 Characteristic of five examined T. monococcum accessions; no pTm30 hybridization sites are detected for PI119423.

The domesticated species T. monococcum differs from the wild species by an almost complete lack of polymorphism in the distribution of pTm30 probe. The (GAA)n site
in five of the six examined accessions is localized to the pericentromeric region of 4AL (Fig. 1a). No distinct hybridization sites of pTm30 have been found on chromosomes of accession PI119423.

**FISH analysis of Timopheevi wheats**

The examined accessions of *T. timopheevii* originated from the Zanduri population (Western Georgia), where the species *T. zhukovskyi* was first identified. *Triticum timopheevii* carries pSc119.2 signals predominantly on the G genome chromosomes and also on 1A'L and 5A'S; the pTa71 signals are present on 6A'S and 6GS chromosomes (Fig. 2). The pTm30 probe intensively hybridized to all G genome chromosomes and generates only one hybridization site on the chromosome 6A'S of the A' genome (Fig. 2).

Similar results were obtained for hexaploid *T. zhukovskyi* (2n = 42, GGA'TATbA' genome), a natural amphiploid resulting from interspecific hybridization between *T. timopheevii* (2n = 28, GGA'A' genome) and *T. monococcum* (2n = 14, AA genome). *T. zhukovskyi*, like *T. timopheevii*, carries the pTm30 site in the short arm of the 6A' chromosome and pSc119.2 on 1A'L (Fig. 2). The remaining A' genome chromosomes lack both pTm30 and pSc119.2 signals. The 45S RNA genes were detected on the 6A'S and 6GS chromosomes, as in *T. timopheevii*, and additionally on 1A'S and 5A'S, as in *T. monococcum* (Fig. 2). No pTm30 hybridization sites, which could have been donated by *T. monococcum*, have been detected.

**C-banding and FISH analysis of Emmer wheats**

Since karyotyping of the A genome of polyploid wheats by FISH alone is not precise, Giemsa C-banding was also used in order to identify all chromosomes of *T. dicoccoides*, *T. durum*, and *T. aestivum*. In addition, the distribution pattern of probe pSc119.2 was considered, when identifying the chromosomes.

Among three *T. dicoccoides* accessions we identified the conserved pSc119.2 sites in subtelomeric regions of 1AS and 4AL chromosomes (Fig. 3), whereas polymorphic pSc119.2 sites were detected on chromosomes 5AS (subtelomeric localization), 5AL (intercalary localization), and 2AL (intercalary localization).

A comparative analysis of the A genome chromosomes of *T. dicoccoides* by FISH with pTm30 probe revealed both conserved and polymorphic (GAA)n signals (Fig. 3). The 1A lacked (GAA)n site during tetraploid formation, while up to two polymorphic (GAA)n loci were detectable on the 3A, 6A, and 7A chromosomes. The highest number of pTm30 hybridization sites was observed on the 2A and 4A chromosomes; of them, the pericentromeric site on 2AS and proximal and distal sites on 4AL were conserved. Chromosome 5AL carried a conserved (GAA)n site in the proximal region; no polymorphic blocks were detected. Note also that the localization of (GAA)n, as was
expected, always coincided with the position of C-bands; however, for approximately 20% of the C-bands no corresponding (GAA)n regions have been detected.

The distribution of pTm30 hybridization sites on the A genome chromosomes of two hexaploid wheat cultivars, Chinese Spring and Saratovskaya 29, was similar (Fig. 3).

The absence of (GAA)n microsatellite on the 1A chromosome was the common feature for all *T. dicoccoides*, *T. durum*, and *T. aestivum* accessions. In addition, the conserved (GAA)n sites on *T. dicoccoides* chromosomes 2AS, 4AL, and 5AL were remained in *T. durum* and *T. aestivum*.

**Discussion**

**(GAA)n as a cytogenetic marker for einkorn wheat**

The goal of the search for cytogenetic markers for the wheat A genome dates back to the very first application of cytogenetic methods to analysis of chromosome structure and phylogeny of *Triticum* species. This was due to both a small number of C-bands detectable by Giemsa staining and the difficulties in FISH-based distinguishing between the A genome chromosomes. In particular, two cytogenetic markers, pSc119.2 and pAs1, are able to discriminate all B and D genomes chromosomes of Emmer wheat, but only three A genome chromosomes (Schneider et al. 2003). The pSc119.2 probe hybridized mainly to the B genome chromosomes of polyploid wheats belonging to Emmer evolutionary lineage, as has been demonstrated for hexaploid *T. aestivum* and tetraploid *T. durum* (Schneider et al. 2003; Kubaláková et al. 2005). In the A-genome of hexaploid wheat, the pSc119.2 hybridization sites were detected only on chromosomes 2AL, 4AL (subtelomeric localization), 5AS (subtelomeric localization), and 5AL (intercalary site). Note that different wheat cultivars display polymorphism
in distribution of pSc119.2 probe on the A-genome chromosomes (Schneider et al. 2003). Involvement of additional probes—pTa71 (45S RNA) and pTa794 (5S RNA genes), localized to 1A (pTa71 and pTa794) and 5A (pTa794) chromosomes—failed to improve the resolution of this assay. The (GAA)n microsatellite, detected in all A genome chromosomes of common and durum wheat except for 1A (Fig. 3), is mainly used for chromosome sorting in polyploid wheats (Pedersen and Langridge 1997; Vrána et al. 2000; Kubaláková et al. 2005). However the direct application of this probe to phylogenetic studies of the A genome of polyploid wheats is hardly possible, because it gives only few minor signals compared to numerous major hybridization sites on the B and G genome chromosomes (Fig. 2; Kubaláková et al. 2005; Cuadrado et al. 2008).

The situation with chromosome identification in einkorn wheat is even more complex. The probes that are frequently used in molecular cytogenetic analysis of polyploid wheats, such as pSc119.2 and pAs1, either do not hybridize to einkorn chromosomes at all, or give few fuzzy signals (Megyeri et al. 2012, Danilova et al. 2012, I.G. Adonina, unpublished data). The rDNA markers, pTa71 and pTa794, produce conserved hybridization sites on the 1AS and 5AS chromosomes and fail to distinguish the einkorn genomes. An Afa probe, PCR-amplified from the genomic DNA of common wheat (Megyeri et al. 2012), may be the most promising for the study of chromosome reorganization of the A genomes. The Afa probe produces numerous hybridization sites; however, so far this has been demonstrated for only one accession of T. monococcum.

The distribution of (GAA)n microsatellite on chromosomes of the A genome diploid species has not been studied until recently. Dvorak (2009) wrote in his review referring to the works of Peacock et al. (1981) and Pedersen (et al. 1996) that (GAA)n sequence is

Figure 3. Localization of probe pTm30 on the chromosomes of Emmer wheats and C-banding. Accessions of T. dicoccoides: pTm30 (red) and pSc119.2 (green); accessions of T. aestivum: pTm30 (green) and pSc119.2 (red).
absent in the diploid A genome donors of common wheat. However, karyotype analysis of individual *T. monococcum* and *T. urartu* accessions employing either (GAA)₉ oligonucleotide or GAA fragments amplified by PCR from wheat genomic DNA has been recently reported (Danilova et al. 2012, Megyeri et al. 2012). Megyeri et al. (2012) studied one accession of *T. monococcum* and discovered two chromosomes with major hybridization sites of the (GAA)n probe in their distal and pericentromeric regions, which were identified as 2AS and 6AL, respectively, based on the distribution of *Afa* family and pTa71 probe. Danilova et al. (2012) identified the chromosomes more precisely using FLcDNAs and defined the chromosomes carrying the major sites as 2AS and 4AL in *T. monococcum* and 1AS in *T. urartu*. In addition, one to three minor (GAA)n sites were detected in two studied accessions of diploid species. So far, no publications describing the (GAA)n distribution on *T. boeoticum* chromosomes has been reported.

As has been demonstrated here, the pTm30 produces up to four major hybridization sites on the A genome chromosomes of diploid wheats (1AS, 2AS, 5AS, and 4AL), while any minor hybridization sites are undetectable. All four major hybridization sites are present in *T. urartu* only, and the site on 1AL is absent in *T. monococcum* and *T. boeoticum*. Interestingly, *T. urartu* accessions belonging to super-cluster II (*urartu*) mainly display two (GAA)n sites, on the 1AS and 5AS chromosomes, also carrying the 45S RNA genes. The major (GAA)n site on 1AS and minor site on 5AS have been also detected in the *T. urartu* accession by Danilova et al. (2012). An interesting fact has been obtained by FISH analysis of the three *T. urartu* accessions (UR3, UR4, and UR5), which were regarded as intermediate forms according to comparison of morphological and SSAP data (Tables 1 and 2). All three accessions differ in the distribution of (GAA)n microsatellite. However, UR5 accession is attributed to super-cluster II (*urartu*) according to pTm30 [(GAA)₅₆₆] pattern, while UR3 and UR4 carry (GAA)n sites on the 2AS and 4AL chromosomes, which are mainly characteristic of *T. boeoticum* and *T. monococcum*. Cultivated einkorn *T. monococcum* displays the lowest polymorphism. We found pTm30 hybridization site on one chromosome pair only, designated 4AL.

Thus, it has been shown that the (GAA)n microsatellite can be used as marker for the 1AS, 2AS, 4AL, and 5AS chromosomes of einkorn wheat; however, it should be kept in mind that depending on species, the number of hybridization sites varies from zero to three in individual accessions. The (GAA)n site on chromosome 1AS is present only in *T. urartu*, while *T. boeoticum* and *T. monococcum* often carry (GAA)n site on the chromosome 4AL.

Evolutionary reorganization of the A genomes in diploid and polyploid wheat species

The evolution of diploid and polyploid wheat species is known to be accompanied by reorganization of the genomes. At the diploid level, genome divergence occurs via accumulation of DNA mutations, amplifications/deletions of tandem repeats, proliferation of mobile elements, and, in some cases, chromosomal rearrangements (Devos et
Polyploid wheat displays a high level of chromosome rearrangements (Jiang and Gill 1994, Rodriguez et al. 2000, Salina et al. 2006a, Badaeva et al. 2007). As any other tandem repeats, microsatellites frequently form large clusters on chromosomes, detectable with FISH. The polymorphism of satellite repeats most typically involve changes in the copy number, resulting in the appearance/elimination of large blocks of repeated sequences.

Study of the distribution of (GAA)n hybridization sites in diploid and polyploid wheats allows us to propose that several factors could have led to redistribution of regions housing this microsatellite. In particular, a decrease in the number of major microsatellite blocks in domesticated T. monococcum may only be a result genetic diversity shortage caused by bottleneck effect during domestication. Another important fact is that all studied accessions of polyploid wheat species T. dicocoides, T. durum, T. aestivum, and T. timopheevii lack hybridization sites on the short arms of their 1A and 5A chromosomes (Fig. 3), which are characteristic of T. urartu, a putative donor of the A genome. The most likely reason for such event is the involvement of (GAA)n loci in reorganization of the nucleolus organizer region on the A genome chromosomes during formation and stabilization of primary allotetraploids which took place about 500 TYA. This resulted in total loss of 45S DNA locus and (GAA)n site on the 5AS as well as in the significant reduction in the number of 45S RNA gene copies (Jiang and Gill 1994) and the loss of (GAA)n site on the 1AS chromosome (Fig. 3). Hexaploid species T. zhukovskyi (genome GGA'AA'Ab) was formed about 60YA or more via the cross of T. timopheevii (genome GGA'Ab) and T. monococcum (genome AbAb). According to our data, this species retained the 45S DNA loci on the 1AbS and 5AbS chromosomes, inherited from T. monococcum, however, the Ab genome chromosomes of T. zhukovskyi lacks (GAA)n sites observed in T. monococcum. This can be due to either the lack of such sites in the parental T. monococcum form, or elimination of (GAA)n loci over the course of amphiploidization, while the species was established.

As in the parental species T. timopheevii, T. zhukovskyi displays only one (GAA)n site in the short arm of chromosome 6At, near the nucleolus organizer region. It is known that the T6AS/1GS translocation took place during T. timopheevii speciation (Jiang and Gill 1994). Thus, it is likely that the (GAA)n site on appeared a result of this translocation. The (GAA)n sites of the einkorn wheat that are localized to more conserved chromosome regions, namely, pericentromeric regions of 2AS and 4AL, were inherited by all polyploid Emmer species (Fig. 3). No (GAA)n sites have been detected on the A's genome homoeologous chromosomes of Timopheevi wheats, thereby confirming that their origin is independent from the Emmer group.

Thus, amplification and cloning of the long fragment of (GAA)n sequence from T. monococcum genome allowed us to obtain the new DNA probe for analysis of the A-genome chromosomes in diploid and polyploid wheat. An increased sequence length provides for higher probe stability, which enhances resolution of hybridization. Using a new probe we defined differences between Ab and Au variants of the A-genomes, revealed variability of labeling patterns among T. boeoticum and T. urartu accessions,
and significant shortage of polymorphism in *T. monococum*, probably due to domestication. We suppose that distribution of (GAA)n sites in diploid and polyploid species reflects the chromosome reorganizations, mainly including the nucleolus organize region, that have taken place during the evolution of wild and domesticated species.

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