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Dynamic nucleolar activity in wheat × Aegilops hybrids: evidence of C-genome dominance

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Abstract

**Key message** NOR loci of C-subgenome are dominant in wheat × Aegilops interspecific hybrids, which may have evolutionary implications for wheat group genome dynamics and evolution.

After interspecific hybridisation, some genes are often expressed from only one of the progenitor species, shaping subsequent allopolyploid genome evolution processes. A well-known example is nucleolar dominance, i.e. the formation of cell nucleoli from chromosomes of only one parental species. We studied nucleolar organizing regions (NORs) in diploid Aegilops markgrafii (syn: Ae. caudata; CC), Ae. umbellulata (UU), allotetraploids Aegilops cylindrica (C⁵CcCcDcDc) and Ae. triuncialis (C⁵CtCtUtUt), synthetic interspecific F₁ hybrids between these two allotetraploids and bread wheat (Triticum aestivum, AABBDD) and in F₃ generation hybrids with genome composition AABBDDC₉tCtUtUt using silver staining and fluorescence in situ hybridization (FISH). In Ae. markgrafii (CC), NORs of both 1C and 5C or only 5C chromosome pairs were active in different individual cells, while only NORs on 1U chromosomes were active in Ae. umbellulata (UU). Although all 35S rDNA loci of the C⁵ subgenome (located on 1C⁵ and 5C⁵) were active in Ae. triuncialis, only one pair (occupying either 1C⁵ or 5C⁵) was active in Ae. cylindrica, depending on the genotype studied. These C-genome expression patterns were transmitted to the F₁ and F₃ generations. Wheat chromosome NOR activity was variable in Ae. triuncialis × T. aestivum F₁ seeds, but silenced by the F₃ generation. No effect of maternal or paternal cross direction was observed. These results indicate that C-subgenome NOR loci are dominant in wheat × Aegilops interspecific hybrids, which may have evolutionary implications for wheat group genome dynamics and allopolyploid evolution.

Keywords Nucleolar dominance · 35S rRNA gene · Polyploidization · Interspecific hybridization

Introduction

Eukaryotic genomes generally contain hundreds of copies of 35–48S ribosomal RNA (rRNA) genes organized in clustered tandem repeats at two or more chromosomal loci. It was found that animals have the 45S rDNA unit, while the 35S rDNA unit is characteristic for most of the plant species. rRNA loci were firstly described by McClintock (1934) as nucleolar organizing bodies and later were called as nucleolar organizing regions (NORs). However, only loci with active rRNA genes form nucleoli. NORs that are active during interphase remain relatively decondensed at metaphase, forming so-called secondary constrictions (Tucker et al. 2010). Active rRNA genes are transcribed by RNA polymerase I (Pol I) into 35–48S rRNA precursors
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L. (C tCtUtUt) are two widespread allotetraploid species in McStay 2006; Michalak et al. 2015; Pikaard 2000a; Tucker (Pikaard 2001; Turowski and Tollervey 2015). The proportion of active rRNA genes is regulated by a dosage control mechanism (Chandrasekhara et al. 2016; Lawrence et al. 2004) such that excess genes are silenced by repressive chromatin modifications (coarse control) (Pikaard 2000a) and the number of RNA Pol I enzymes engaged in transcription at each active gene is regulated (fine control) (Grummt and Längst 2013). Nucleolar dominance—originally termed differential amphiplasty by Navashin (1934)—is the deactivation of NORs of one subgenome due to the presence of NORs of another subgenome in an allopolyploid (McStay 2006; Pikaard 2000a; Tucker et al. 2010). Nucleolar dominance is an epigenetic phenomenon common among interspecific hybrids and allopolyploids of plants and animals (Jiang and Gill 1994; McStay 2006; Michalak et al. 2015; Pikaard 2000a; Tucker et al. 2010). In both natural and synthetic allopolyploid plants, nucleolar dominance has been shown to be related to DNA hypermethylation, repressive histone modification and noncoding RNAs in the promoter regions of silenced NORs after chromosome doubling (Pontvianne et al. 2012).

The major 35S rDNA loci in diploid wheat progenitors are distributed on the 1A, 5A, 1B, 6B, and 5D chromosomes (Appels et al. 1980; Frankel et al. 1987; Miller et al. 1983). NOR loci in Ae. markgrafii are located on 1C and 5C (Friebe et al. 1992) and in Ae. umbellulata on 1U and 5U (Friebe et al. 1995). Some of these loci are known to become inactive during the evolutionary processes of interspecific hybridization and allopolyploid speciation: this nucleolar dominance has previously been documented in the Poaceae family (Guo and Han 2014; Idziak and Hasterok 2008; Reeder 1974). As an example, in synthetic allopolyploid wheat both sets of parental NORs are expressed after hybridization, but one parental NOR is immediately silenced by chromosome doubling, an effect putatively related to biased sequence elimination of silenced NORs (Guo and Han 2014).

Species belonging to the genus Aegilops are important sources of genetic material for improving bread wheat, Triticum aestivum L. (2n = 6x = 42, AABBD). Ae. cylindrica Host (C°C-D°C genome) and Ae. triuncialis L. (C°C-U-U°) are two widespread allotetraploid species in this genus, originating from the hybridization of their diploid progenitors: Ae. umbellulata Zhuk. (UU), Ae. markgrafii (Greuter) Hammer (syn. Ae. caudata L., CC) and Ae. tauschii Coss. (DD) (Reeder 1974; Vanichananon et al. 2003). However, little is known about nucleolar dominance within these natural allopolyploids or in relation to the bread wheat subgenomes. Here, we analyzed NOR patterns and activity in Ae. cylindrica and Ae. triuncialis and their synthetic interspecific hybrids with T. aestivum using Ag-NOR staining and fluorescent in situ hybridization (FISH).

Materials and methods

Plant materials

The plant materials used were: Ae. cylindrica Host (accessions: 236 and 387), Ae. markgrafii (Greuter) Hammer (accessions: AE1418, AE1082), Ae. triuncialis L. (accession: S101), Ae. umbellulata Zhuk., T. aestivum L., T. aestivum × Ae. cylindrica F1 hybrids, T. aestivum × Ae. triuncialis F1 hybrids and F2 hybrids from reciprocal crosses between T. aestivum × Ae. triuncialis. The Ae. markgrafii accessions were obtained from the IPK germplasm collection, Germany. Other Aegilops accessions were collected from the Kurdistan province of Iran. Wheat cultivars were provided by the Seed and Plant Improvement Institute (SPII) of Iran.

Root pretreatment and slide preparation

Seeds were germinated on moist filter paper in Petri dishes for 2–3 days at room temperature (RT). Actively growing root tips of about 1–1.5 cm length were cut and pretreated in ice-cold water for 24 h. Roots were then fixed in Carnoy’s I solution (3:1 volume per volume absolute ethanol:glacial acetic acid solution) for two days, then were transferred to 75% ethanol and stored at −20 °C until use. Chromosome preparations from root tip cells were prepared using the drooping method. Two treated root tips of each seed or genotype were washed in ice-cold water twice for 5 min, then further washed in 0.01 M citrate buffer (0.01 M citric acid and 0.01 M sodium citrate, pH 4.8) twice for 5 min. Root sections containing dividing cells were cut and digested in 30 μl enzyme mixture containing 0.7% cellulase R10 (Duchefa C8001), 0.7% cellulase (CalBiochem 219466), 1% pectolyase (Sigma P3026) and 1% cytohelicase (Sigma C8274) dissolved in 0.01 M citrate buffer, pH 4.8. After digestion, enzyme mixture was removed and meristems were washed twice with citrate buffer and once with ethanol. Ethanol was replaced with 60 μl freshly prepared fixative (9:1 glacial acetic acid and absolute methanol). The meristems were carefully broken by using a needle to obtain a cell suspension. Seven μl of the cell suspension was dropped onto each glass slide in a box lined with wet paper towels (to have about 50% humidity inside the box) and left to dry slowly.
FISH procedure

After the slides were dried, they were fixed in 4% paraformaldehyde prepared in 1 × PBS (3 mM NaH2PO4, 7 mM Na2HPO4, 0.13 M NaCl, pH 7.4) for 10 min at room temperature, followed by washing in 2 × SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.4) and dehydrating in ethanol series (70, 90 and 100%). The hybridization mixture for FISH containing 50% formamide, 2 × SSC, 20% dextran sulfate, 1 μg sheared salmon testes DNA and 10–30 ng of each of labeled probes. In the case of genomic in situ hybridization (GISH), unlabeled, fragmented DNA from an appropriate genome (depending on genome constitution) was also added to the hybridization mixture as blocking DNA. 20 μl of the hybridization mixture was applied to each slide, covered with a coverslip and the coverslip was arrested with rubber cement. Slides were denatured at 80 °C for 2 min on a hot plate. Slides were then placed in a closed humidified container overnight at 37 °C for hybridization. After removing the coverslips in 2 × SSC, slides were washed in 2 × SSC for 20 min at 56 °C. The slide were dehydrated in ethanol series, dried at RT and mounted in 70% ethanol. Staining was applied based on Lacadena et al. (1984) with the following modifications. Slides were prepared from root tip cells using the dropping method. 50–100 μl of Carnoy’s I fixative was dropped onto the preparations and flame dried. A solution of 0.136 mM sodium citrate (C6H5Na3O7 · 2H2O) was prepared and the pH was adjusted to 3 with formic acid. 1 g AgNO3 was dissolved in 1 ml of the prepared sodium citrate solution. Twenty μl AgNO3 solution was placed onto the slide and covered with a coverslip. Slides were then incubated in a moist chamber at 60 °C for about 90–120 min, following which slides were rinsed thoroughly in distilled water and dried at RT. A drop of 45% acetic acid was added and a coverslip was applied. Slides were then observed under 1000× magnification using immersion oil. For sequential silver staining and FISH, coverslips on silver-stained slides were carefully flipped up, slides were washed in 2 × SSC, dehydrated in ethanol series and used in FISH.

Silver staining

In order to detect active NOR loci on chromosomes, silver staining was applied based on Lacadena et al. (1984) with the following modifications. Slides were prepared from root tip cells using the dropping method. 50–100 μl of Carnoy’s I fixative was dropped onto the preparations and flame dried. A solution of 0.136 mM sodium citrate (C6H5Na3O7 · 2H2O) was prepared and the pH was adjusted to 3 with formic acid. 1 g AgNO3 was dissolved in 1 ml of the prepared sodium citrate solution. Twenty μl AgNO3 solution was placed onto the slide and covered with a coverslip. Slides were then incubated in a moist chamber at 60 °C for about 90–120 min, following which slides were rinsed thoroughly in distilled water and dried at RT. A drop of 45% acetic acid was added and a coverslip was applied. Slides were then observed under 1000× magnification using immersion oil. For sequential silver staining and FISH, coverslips on silver-stained slides were carefully flipped up, slides were washed in 2 × SSC, dehydrated in ethanol series and used in FISH.

Results

Cytogenetic characterization of 35S rDNA loci in diploid species: Ae. markgrafii and Ae. umbellulata

FISH using 35S rDNA as a probe detected two pairs of NOR-bearing chromosomes in each of the diploid species Ae. markgrafii (CC genome) and Ae. umbellulata (UU genome). These chromosomes were 1C and 5C in Ae. markgrafii and 1U and 5U in Ae. umbellulata, respectively (Fig. 1). In each Ae. markgrafii accession, 8–10 cells from 3 different individuals were analyzed by Ag-NOR staining. Results showed that NORs of both 1C and 5C (in 50% of the cells) (Supplementary Fig. 1) or only 5C chromosome pairs (Fig. 1c), were active in the two studied accessions, while only NORs on 1U chromosome pair were active in Ae. umbellulata (UU genome). 1U chromosomes are distinguished by their size and morphology as they are smaller and more symmetric than the other satellited chromosome pair 5U.

Cytogenetic analysis of 35S rDNA loci in allotetraploid species Ae. cylindrica and Ae. triuncialis

NOR patterns varied in the natural allotetraploids as revealed by FISH and silver staining. Sequential GISH (with C-genome DNA as a probe) and FISH (with pTa71 as
a probe) showed four pairs of *Ae. triuncialis* chromosomes with rDNA signals of different sizes (Fig. 1h). Two major NORs were located in the short arms of C-genome chromosomes and two minor NORs were located in the short arms of U genome chromosomes. By referring to the FISH patterns of CTT oligonucleotide probes on the diploid progenitors (Fig. 1b, e), the NOR-bearing chromosomes in *Ae. triuncialis* could be assumed to be 1Cₜ, 5Cₜ, 1Uₜ and 5Uₜ, with a signal size intensity order of 1Cₜ > 5Cₜ > 5Uₜ > 1Uₜ. In silver staining, weak and non-reproducible signals sometimes appear on telomeric regions of non-satellited chromosomes, but should be disregarded: such nonspecific signals were observed in some replications (at low frequency) of *Ae. triuncialis* (Fig. 1i) and wheat-*Ae. triuncialis* amphiploids.

Both pairs of Cₜ subgenome NORs (located on the short arms of the 1Cₜ and 5Cₜ chromosomes) were active in *Ae. triuncialis* individuals, but only one pair (either 1Cₜ or 5Cₜ) was active in the studied accessions of *Ae. cylindrica*. In fact, the two accessions showed different NOR inactivation...
patterns: *Ae. cylindrica* accession 236 had active 1C<sup>c</sup> NORs, and accession 387 had active 5C<sup>c</sup> NORs as assessed by silver staining (Supplementary Fig. 2).

**Expression patterns of 35S rDNA loci in F<sub>1</sub> hybrids between bread wheat and allotetraploid *Aegilops* species**

In *F<sub>1</sub>* wheat-*Ae. cylindrica* hybrids (*2n = 5x = 35, ABDD<sup>c</sup>C<sup>c</sup>), NOR activity patterns depended on and mimicked the *Ae. cylindrica* accession used as a parent. Silver staining of mitotic metaphase cells revealed strong active NORs on 5Cc and 6B and a very weak signal on 1B in *T. aestivum* var. Pishgam × *Ae. cylindrica* accession 387 (Fig. 2c). In contrast, in *T. aestivum* var. Pishgam × *Ae. cylindrica* accession 236, wheat NORs were completely silenced and only a single very strong active NOR signal was observed for 1C<sup>c</sup> chromosomes (Supplementary Fig. 3).

Similar C-genome NOR expression patterns were observed in *F<sub>1</sub>* plants and *F<sub>3</sub>* amphiploids from wheat-*Ae. triuncialis* crosses. FISH using pSc119.2-1 and pTa535-1 as a probe combination was sufficient to identify all bread wheat chromosomes individually. With reference to chromosome morphology and pSc119.2-1 banding patterns, satellited chromosomes of *Ae. triuncialis* (1C<sup>t</sup> and 5C<sup>t</sup>) were also identified in this *F<sub>1</sub>* hybrid (Fig. 2a, b). Silver staining of mitotic metaphase cells of the *F<sub>1</sub>* hybrid of *T. aestivum* var. Pishgam × *Ae. triuncialis* (*2n = 5x = 35, ABDU<sup>c</sup>U<sup>c</sup>) revealed strong active NORs on 1C<sup>c</sup>, moderately active NORs on 5C<sup>c</sup> and weakly active NORs on 1B and 6B (Fig. 2b). NORs on chromosomes 1C<sup>c</sup> and 5C<sup>c</sup> remained relatively decondensed and formed secondary constrictions at metaphase (Fig. 2a).

**Expression patterns of 35S rDNA loci in *Ae. triuncialis* × *T. aestivum* *F<sub>3</sub>* amphiploids**

Wheat-*Aegilops cylindrica* *F<sub>1</sub>* hybrids were completely sterile, so no *F<sub>2</sub>* or *F<sub>3</sub>* seed was produced or was able to be analyzed (Fakhri et al. 2016). Therefore only the *F<sub>1</sub>* generation was analyzed from this cross type. However, sequential silver staining and FISH were applied to clearly identify the active NOR-bearing chromosomes in *Ae. triuncialis* × *T. aestivum* *F<sub>3</sub>* amphiploids (*2n = 10x = 70, AABBDDC<sup>c</sup>C<sup>t</sup>U<sup>t</sup>U<sup>t</sup>; both parental cross

![Fig. 2 FISH and silver staining on mitotic metaphase chromosomes preparations of *F<sub>1</sub>* wheat × *Aegilops* hybrids; a FISH using pTa535.1 and pSc119.2 probes in a *T. aestivum* × *Ae. triuncialis* (*2n = 5x = 35, ABDD<sup>c</sup>C<sup>c</sup>) *F<sub>1</sub>* hybrid identified chromosomes. Arrowheads point to the secondary constrictions belonging to the C-subgenome; b silver staining in a *T. aestivum* × *Ae. triuncialis* *F<sub>1</sub>* hybrid showing active 35S rDNA loci (NORs) on 1C<sup>c</sup> and 5C<sup>c</sup> and low-level activity on 1B and 6B chromosomes; c silver staining in a *T. aestivum* var. Pishgam × *Ae. cylindrica* accession 387 (*2n = 5x = 35, ABDD<sup>c</sup>C<sup>c</sup>) *F<sub>1</sub>* hybrid showing active 35S rDNA loci on chromosomes 5C<sup>c</sup> and 6B; d sequential FISH using (CTT)<sub>10</sub> probes on the silver-stained preparation in ‘c’ to identify the chromosomes. Scale bar 5 μm](https://example.com)
directions) (Fig. 3; Supplementary Fig. 4). FISH using CTT oligonucleotide and pTa71 probes enabled us to identify chromosomes of wheat and Aegilops in the amphiploids (Cuadrado et al. 2008). The pTa71 probe revealed the presence of 13 rDNA signals in the amphiploids, whereas Ag-NOR staining showed 4 signals that were located on the 1C t and 5C t chromosome pairs (Fig. 3; Supplementary Fig. 4). These active NORs gave rise to secondary constrictions on metaphase chromosomes (Fig. 3c). Although the wheat NORs showed weak activity in F1 plants, they were silenced in amphiploids of both (reciprocal) crosses, indicating a dominant role of the C t subgenome in wheat interspecific hybrids unaffected by maternal parent. In some F3 cells especially those with longer chromosomes, a very faint Ag-NOR signal was also observed on 1B chromosomes of wheat but the most frequent observation was no activation. During Ag-NOR and FISH analysis, we counted the chromosome number in 39 metaphase chromosome spreads from seven seeds of F3 generation from Ae. triuncialis (♂) × Pishgam (♀) crosses. More than half of cells (51%) had a complete amphiploid chromosome complement, and chromosome numbers ranged from 65 (2%) to 70. Mode of the chromosome number in 6 seeds were 70. Chromosome number in one seed ranged from 65 to 68 with the mode of 68.

Discussion

Nucleolar dominance is a phenomenon in interspecific hybrids and allopolyploids by which cell machinery regulates nucleolus formation by deactivating nucleolar organizing regions (NORs) from one or more parental species. We studied rDNA patterns in Aegilops and wheat species, interspecific hybrids and synthetic allopolyploids with genome compositions CC, UU, C t-C t, C t-C t-U t-U t, ABDD-C t-C c-D c-D c, AABBDD-C t-U t-U t using Ag-NOR staining and in situ hybridization. NOR activity of AABBDD-C t-U t-U t amphiploids was assessed in the F3 generation because in synthetic hybrids, NOR silencing is variable, and needs at least two generations to be fully established (Chen et al. 1998; McStay 2006). We found that Ae. markgrafii (C genome) NOR activity was the most dominant relative to T. aestivum (A, B and D genomes) and Ae. umbellulata (U genome). Our data excluded the involvement of any maternal or paternal effects on nucleolar dominance in studied amphiploids, but intraspecific variation in NOR activity patterns was observed between Ae. cylindrica accessions, and this variation was inherited by the resulting F1 hybrids with T. aestivum. This intraspecific variation in NOR activity could be due to chromosomal translocations involving the 1C c and/or 5C c chromosomes pairs between the two accessions studied, as...
karyotype variation has previously been identified in this species (Mirzaghaderi et al. 2014). Our results should be taken into consideration when utilizing these *Aegilops* species for wheat improvement via interspecific hybridization and targeted introgression.

In *Ae. triuncialis* (C′C′UtUt), weaker signals of rDNA loci in the Ut subgenome relative to the C′ subgenome suggests that most of the rRNA gene copies have been eliminated from Ut subgenome (Fig. 1h), the NORs of which were also transcriptionally recessive based on silver staining results (Fig. 1i). Similar biased elimination of rDNA has been reported in synthetic allohexaploid wheat, where A-genome NORs were rapidly silenced in early generations and lost completely in later generations (Guo and Han 2014). Directed loss of silenced genes may be associated with epigenetic alterations rather than maternal effects (Guo and Han 2014; Książczyk et al. 2011), and may facilitate diploidization over evolutionary time periods (Soltis et al. 2010).

The chromosome numbers in F2 seeds from *Ae. triuncialis* (♀) *×* ‘Pishgam’ wheat (♂) crosses ranged from 65 (2%) to 70 (51%): although more than half were complete amphidiploids, aneuploidy was very common. We have reported variation in chromosome number for the reciprocal crosses as well i.e. the F2 generation of bread wheat (♀) *×* *Ae. triuncialis* (♂). Surprisingly, a proportion of seeds in that study had 35 chromosomes (similar to the parental hybrid seeds) such that the number of chromosomes ranged from 35 (26%, mainly when ‘Omid’ and ‘Navid’ bread wheat cultivars were used as male parents) to 70 (22% mainly when the ‘Pishgam’ cultivar was the male parent) (Mirzaghaderi and Fathi 2015). Collectively, these results imply that the ‘Pishgam’ wheat cultivar may harbor genes for both male and female unreduced gamete formation.

Using sequential silver staining and FISH on *T. aestivum* *×* *Ae. triuncialis* amphidiploids, we showed that out of 13 NOR loci, only those from the C-subgenome (on the 1C′ and 5C′ chromosomes pairs) were active in complete or nearly complete amphidiploids. Silver staining of the NORs in these amphidiploids showed obvious secondary constrictions on metaphase chromosomes of the C′ subgenome, which are believed to be due to rRNA transcription (Pikaard 2000a). NORs of the C′ subgenome were also consistently active in amphidiploids resulting from both reciprocal cross directions, suggesting that nucleolar dominance occurs independently of parental effects, with no effect of gametic imprinting involved in this phenomenon. This finding is in agreement with the generally accepted concept that nucleolar dominance usually occurs independently of maternal or paternal effects in interspecific hybrids of both plants and animals (Idziak and Hasterok 2008; Pikaard 2000b; Pontes et al. 2007). Nucleolar dominance is developmentally regulated following embryogenesis, so imprinting at the gamete stage does not appear to be involved (Tucker et al. 2010). However, Michalak (2014) reported that the pattern of 45S rRNA transcription in F1 *Xenopus* interspecific hybrids is maternally imprinted rather than species-specific, so exceptions do exist.

In wheat-*Ae. cylindrica* F1 hybrids (2n = 5x = 35, ABDD′C′C′), NOR expression patterns were affected by the parental *Ae. cylindrica* genotype. While strong active NORs were observed on 5C′ and 6B and a very weak signal on 1B in *T. aestivum* var. Pishgam × *Ae. cylindrica* accession 387 (Fig. 2c), only a single very strong active NOR was observed on 1C′ chromosome in *T. aestivum* var. Pishgam × *Ae. cylindrica* accession 236 (Supplementary Fig. 3). The difference in NOR activity patterns of the studied F1 hybrids of wheat-*Ae. cylindrica* is likely to be due to the variation within parental species, as the parental *Ae. cylindrica* accessions of the studied F1 hybrids varied themselves in their active NOR patterns. In one accession NORs on 1C′ were active, and in the other accession NORs on 5C′ were active. An intriguing although as yet unsubstantiated hypothesis is that this intraspecific variation in NOR activity might be due to chromosomal translocations involving these chromosome pairs, as inter-varietal chromosomal translocations have been previously observed in this species (Mirzaghaderi et al. 2014), and are known to cause variation in NOR activity patterns in allopolyploids. For instance, in triticale, substitution of rye chromosome 2R by chromosome 2D from hexaploid wheat activated the rye-origin rDNA (Neves et al. 1997). Inter-varietal variation of nucleolar activity has also been observed in allopolyploid *Brassica napus* (AAC′C′ genome). Although most cultivars exhibit stable A-genome nucleolar dominance, some show co-dominant expression of A- and C-genome NORs (Sochorová et al. 2016).

FISH and silver staining in the studied amphidiploids and hybrids implied the dominance of C-genome NOR activity from *Ae. markgrafii* (CC genome) over that of *T. aestivum* (A, B and D genomes) and *Ae. umbellulata* (UU genome). These results are contrary to the reports of Cermeno et al. (1984), who suggested NORs of the U subgenome are dominant in *Ae. triuncialis*. Considering that the *Ae. umbellulata* (U genome) NORs are dominant over those of wheat (Martini et al. 1982), one can deduce that nucleolar dominance in the studied subgenomes follows a hierarchical order of C > U > B > A or D. Nucleolar dominance has also been reported in other synthetic amphidiploids in the Triticeae tribe. In both *Ae. ventricosa* (DDNN genome) × rye (RR genome) and wheat × rye amphidiploids, rye NOR activity is suppressed (Lacadena et al. 1984; Thomas and Kaltsikes 1983). However, there may also be some exceptions to nucleolar dominance in the wheat
group. For example, Orellana et al. (1984) reported that both parental NORs remain active in tetraploid whea-
t × Ae. ventricosa amphiploids. In Thinopyrum ponticum
(2n = 10x = 70, JJJJJ*) 17 distal NOR loci were identified on the short arms of chromosomes, of which 14–17 were active during metaphase (Brasileiro-Vidal et al. 2003). Fradkin et al. (2016) reported 8–10 active NOR loci in allo-octaploid trigo
tiro (2n = 8x = 56; AABBDJJJ). However, the genome and homoelogous group of these NOR-bearing chromosomes were not identified, and hence no implication could be inferred as to nucleolar dominance subgenome in these studies.

Author contribution statement GM conceived and designed research. GM, ZA, MZ and ZM conducted experiments. GM analyzed data and wrote the manuscript. ASM provided critical discussions and corrected the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical standards The manuscript has not been submitted to more than one journal for simultaneous consideration. No data have been fabricated or manipulated to support our conclusions. We have acknowledged and cited the work of others to the best of our knowledge. All authors read and approved the final manuscript.

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