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Isolating and location of Tandem Repetitive Afa-family Sequences from Mongolian Wheatgrass (*Agropyron mongolicum* Keng)

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Abstract: Tandem repetitive Afa-family sequences are known to occur in the wheat and related species of Triticeae. A member of the Afa-family sequences isolated from Mongolian Wheatgrass (*Agropyron mongolicum* Keng) (2n=2x=14, PP) is 233 bp, named as *pAmAfa*1, which is most similar with other Afa-family sequences in *Triticeae* species. The phylogenic analysis results show that *pAmAfa*1 is clustered with *pLrAfa*3 and *pLrAfa*5, and the findings indicate that P genome has homology with N and X genomes. To know which the genome of Mongolian wheatgrass carries the Afa-family sequence; FISH is carried out using *pAmAfa*1 as the probe. The signals appear in the telomeric regions and subtelomeric regions of all chromosomes. This finding indicates that P genomes contain Afa-family repeats.

Key words: Mongolian Wheatgrass; Tandem repetitive Afa-family sequences; Molecular Cloning; FISH

蒙古冰草 Afa 家族串联重复序列克隆及染色体定位

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摘要: Afa 家族串联重复序列因只出现在小麦及小麦族近缘属物种而得名,本研究从蒙古冰草中克隆得到一个 Afa 家族序列,长度为 233 bp,命名为 *pAmAfa*1,该序列在 GENBANK 中进行同源序列比对,结果表明该序列与大多数小麦族其他物种的 Afa 家族串联重复序列存在较高的相似性;系统进化分析表明,蒙古冰草 *pAmAfa*1 序列与大赖草 *pLrAfa*3, *pLrAfa*5 序列聚在一起,表明蒙古冰草 P 染色体组与大赖草的 N、X 染色体亲源关系较近。为了明确 Afa 家族串联重复序列在蒙古冰草染色体上的位置,双色荧光原位杂交技术被采用,以 *pAmAfa*1 为探针检测到杂交信号出现在染色体的末端或近端部的区域,每条染色体上都有杂交信号,表明 Afa 家族串联重复序列普遍存在于 P 染色体组中。

关键词: 蒙古冰草; Afa 家族串联重复序列; 克隆; 荧光原位杂交
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The first clone of tandem repetitive Afa-family sequences, pAs1, is cloned from *Aegilops squarrosa* L. (2N=14, genome DD), and described as a D-genome species repetitive sequence [1], and the sequences homologous to pAs1 exist in many genomes of the tribe Triticeae [2-4]. The repeat units are about 340 bp long. The tandem arrays of Afa-family repeats are dispersed in several subtelomeric and interstitial chromosomal regions, and, therefore, have been used as chromosome markers [5-8].

Tribe Triticeae (Gramineae) include many wild species, all the genomes recognized in this tribe contain seven chromosomes. Mongolian wheatgrass (*Agropyron mongolicum* Keng) (2n=

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2x=14, PP) is one of perennial wild relative species of wheat, the species is a narrow-spiked diploid (*A. fragile* ssp. *mongolicum*) distributing in desert grassland and typical grassland of China. Long-term evolution and adaptation to harsh conditions make Mongolian wheatgrass rich in tolerance genes for a range of biotic and abiotic stresses such as pest and fungal attacks, drought, cold, barren and high salinity [9-11]. In view of all these attributes, Mongolian wheatgrass has been proposed to be a valuable genetic resource in forge grass and crop improvement for resistances or tolerances. There are abundant germplasm resources of Mongolian wheatgrass in Northwest China. However, up to now, the evolutional lineages of the species and relationship with wheat have not been revealed.

In this study, Afa-family sequences were isolated from Mongolian wheatgrass, and the characters were examined in order to provide valuable evidence for the genetic evolution of Mongolian wheatgrass.

1 Materials and Methods

1.1 Plant materials

Wild Mongolian wheatgrass species (2n=2x=14, PP) were collected from its natural habitat in Xilingol prairier, Inner Mongolian, China.

1.2 Cloning and sequencing

Genomic DNA was extracted from Mongolian wheatgrass using CTAB method. Genomic DNA as template was amplified by PCR with pAs1 specific primers (AS-A 5′-GATGATGTGGCTTGAATGG and AS-B 5′-GCATTTCAAATGAACTCTGA) [2]. The fragments were cloned into pUC19 in Escherichia coli strain DH5α and sequenced by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China).

1.3 Sequence and phylogenic analysis

Sequences were analyzed with DNAman and DNAuser software. Sequence homology with the nucleotide database of GenBank was analyzed using BLAST tools. The phylogeny of the sequences was analyzed by computer software, DNAMAN. full. version. v5. 2. 2.

1.4 FISH analyses

Slides for fluorescence in situ hybridization (FISH) were prepared by the acetocarmine squash method using root-tip meristem cells. The pAmAfa1 was labeled with biotin-14-dATP and detected with avidin-FITC according to Mukai [12]. Images were taken with a cooled CCD camera and analyzed with IPLAB SPECTRUM computer software (Signal Analytics).

2 Results

2.1 Molecular cloning and Sequence analysis of pAmAfa1

An Afa-family gene from Mongolian wheatgrass was 233 bp and AT rich (61.2%), named as *pAmAfa1* (GenBank accession no. KC990463), which was similar to the Afa-family sequences of other Triticeae species [2].

NCBI Blastn results (Fig. 1) showed that the max identical degree was 95% between the sequence of *pAmAfa1* and *pPjAfa2* (GenBank accession no. AB022724. 1), between the sequence of *pAmAfa1* and contig ctg447 from *Triticum aestivum* chromosome arm 3DS-specific BAC library (GenBank accession no. HE774676. 1), and was 94% between the sequence of *pAmAfa1* and Aegilops tauschii chromosome 1Ds prolamin gene locus, complete sequence (GenBank accession no. JX295577. 2). Blast analysis showed that the sequence of *pAmAfa1* had higher homology with the Afa-family sequences of other Triticeae species.

2.2 Phylogenic analysis

To know the relation of the Afa-family sequences from Mongolian wheatgrass with those of other Triticeae species, 27 Afa-family sequences from the seven diploid and two tetraploid species were chosen at random from GENBANK (Table 1). These sequences

were analyzed by the NJ method, and clustered by DNAman (Fig. 2). The Afa-family sequences from Mongolian wheatgrass was clustered with *pLrAfa*3 and *pLrAfa*5 (Fig. 1), *Afa-mon*2 was clustered with *ptuafa*1 and *ptuafa*3, they had the same genome (AA). Since *Afa*-4*DCSL*5, *Afa*-4*DCSL*7, *Afa*-4*DCSL*8, *Afa*-4*DCSL*9, *Afa*-5*DCSL*1, *Afa*-6*DCSL*2, *Afa*-6*DCSL*2 and*Afa*-7*DCSL*1 were characterized to be AABB genome specific (Table 1), the cluster was designated as AABB. Significant clusters were indicated by bars with the name of the genome from which they were derived.

Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
Agropyron mongolicum clone Afa-18 satellite sequence	431	431	100%	2e-117	100%	KC990463.1
Psathyrostachys juncea DNA, tandem repetitive Afa-family sequence, clone:pPjAfa2	372	372	100%	1e-99	95%	AB022724.1
Triticum aestivum chromosome arm 3DS-specific BAC library, contig ctg447	361	2030	100%	2e-96	95%	HE774676.1
Aegilops tauschii chromosome 1Ds prolamin gene locus, complete sequence	350	6585	100%	5e-93	94%	JX295577.2
Triticum aestivum cultivar Glenlea clone BAC 1648_464 disease resistance protein (Lr1) genomic region	348	665	100%	2e-92	94%	EF567062.1
Triticum urartu DNA, tandem repetitive Afa-family sequence, clone pTuAfa1	348	348	100%	2e-92	94%	AB003259.1
Triticum aestivum, storage protein activator (spa) locus region, D genome, clone BAC Ren2409K09	333	561	100%	5e-88	93%	FM242578.1
Triticum aestivum chromosome arm 3DS-specific BAC library, contig ctg1484	331	1218	100%	2e-87	92%	HE774675.1
Triticum aestivum clone 1144N5 genomic sequence	331	1239	100%	2e-87	92%	JF758493.1
Leymus racemosus DNA, tandem repetitive Afa-family sequence, clone:pLrAfa2	331	331	100%	2e-87	92%	AB022727.1
Triticum urartu DNA, tandem repetitive Afa-family sequence, clone pTuAfa3	331	331	100%	2e-87	92%	AB003261.1
Triticum aestivum gene for TaAP2-D, complete cds, cultivar: Chinese Spring, clone: BAC:WCS0049K23	326	478	100%	8e-86	92%	AB749310.1
Psathyrostachys juncea DNA, tandem repetitive Afa-family sequence, clone:pPjAfa3	326	326	100%	8e-86	92%	AB022725.1

Fig. 1 The Blastn results of *pAmAfa*1 sequences

Table 1 Detail information of Afa-family sequences

Afa sequence name	Origin	Genomes	GenBank accession No.
Afa-4*DCSL*5	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003212
Afa-4*DCSL*7	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003214
Afa-4*DCSL*8	*Triticum turgidum* subsp. *durum* DNA	AABB	AB00325
Afa-4*DCSL*9	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003216
Afa-5*DCSL*1	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003217
Afa-6*DCSL*2	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003221
Afa-6*DCSL*2	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003221
Afa-7*DCSL*1	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003223
*Afa-dur*1	*Triticum turgidum* subsp. *durum* DNA	AABB	AB003235
*Afa-cer*3	*Secale cereale* DNA	RR	AB003228
*Afa-cer*4	*Secale cereale* DNA	RR	AB003229
*Afa-vur*2	*Hordeum vulgare* DNA	HH	AB003252
*Afa-vur*4	*Hordeum vulgare* DNA	HH	AB003254
*Afa-spe*3	*Aegilops speltoides* DNA	DD	AB003242
*pAsAfa*2	*Aegilops triuncialis* var. *triuncialis* DNA	DD	AB3256
*pTuAfa*1	*Triticum urartu* DNA	AA	AB003259
*pTuAfa*2	*Triticum urartu* DNA	AA	AB003260
*pTuAfa*3	*Triticum urartu* DNA	AA	AB003261
*pTuAfa*4	*Triticum urartu* DNA	AA	AB003262
*pPjAfa*2	*Psathyrostachys juncea* DNA	NN	AB022724
*pPjAfa*3	*Psathyrostachys juncea* DNA	NN	AB022725
*pLrAfa*2	*Leymus racemosus* DNA	NNXX	AB022727
*pLrAfa*3	*Leymus racemosus* DNA	NNXX	AB022728
*pLrAfa*5	*Leymus racemosus* DNA	NNXX	AB022730
*Afa-mon*3	*Triticum monococcum* DNA	AA	D82989
*Afa-mon*1	*Triticum monococcum* DNA	AA	D82987
*Afa-mon*2	*Triticum monococcum* DNA	AA	D82988
*pAmAfa*1	*Agropyron mongolicum* DNA	PP	KC990463

2.3 FISH analyses

To know if both or only one of the genomes of Mongolian wheatgrass carried the Afa-family sequences, FISH was carried out using *pAmAfa*1 as the probe. The signals appeared and dispersed in the telomeric regions and subtelomeric regions of all chromosomes (Fig. 3), and were very strong. This finding indicated that P genomes contained Afa-family repeats.

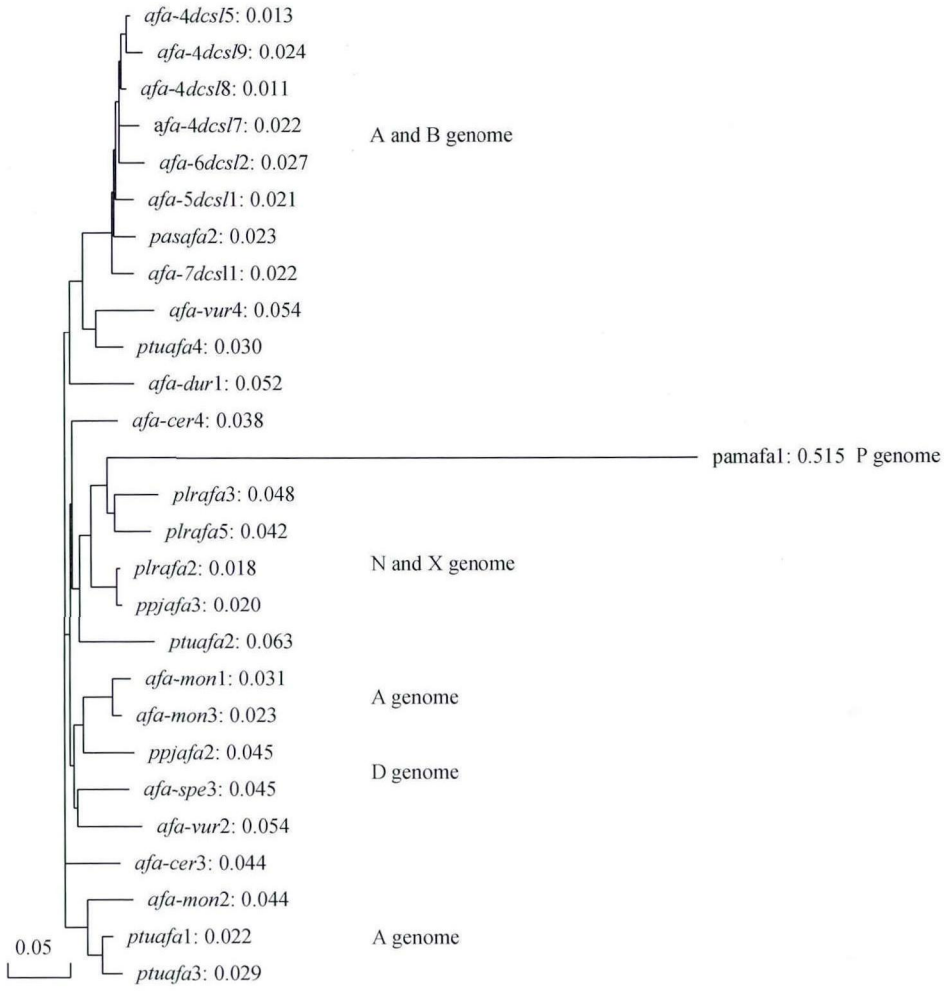

Fig. 2　Phylogenic tree of the Afa-family sequences from Mongolian wheatgrass

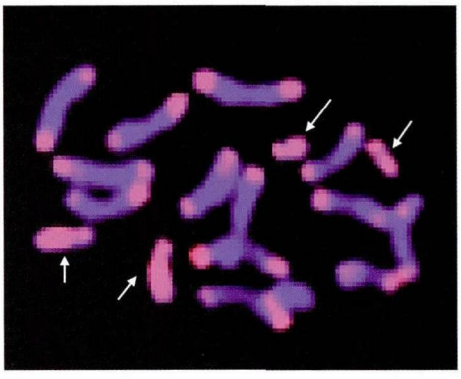

Fig. 3　FISH of Mongolian wheatgrass metaphase chromosomes (2n=2x=14) probed with *pAmAfa*1

Note: The arrows show B chromosomes. Scale bar=10 μm.

3　Discussion

3.1　Genetical property and phylogenic analysis of Afa-family sequence

A previous investigation of Nagaki [2] demonstrated the important properties of the Afa-family sequences. There was highly variable copy number per genome among species; the sequences in the genomes including more copies were more uniform; there was no chromosome specificity in the sequences within genomes; No large-scale transposition or conversion took place between genomes during the past 7000 years; the neighboring sequences were the most similar with each other.

*pAmAfa*1 sequence cloned from *A. mongolicum* Keng was the most similar to other Afa-family sequences in Triticeae species. Our findings were consistent with the above. The DNA sequences of the Afa-family clones chosen at random from NCBI database were analyzed. To know the relation of the Afa-family sequences from *A. mongolicum* Keng with those of other Triticeae species, 28 repeated units amplified by PCR with AS-A and AS-B primers were analyzed by the NJ method (Fig. 2). The finding indicated that P genome had homology with N and X genome, and *A. mongolicum* Keng existed earlier than *L. racemosus* and *P. juncea*. So it was supported that P genome was a

donor of N and X genomes, or there was a transposition or conversion among P, N and X genomes.

3.2 Chromosomal localization of Afa-family sequence

In repetitive sequences located in specific locations, such as ribosomal RNA genes and subtelomeric repetitive sequences, the gene conversion probably played an important role for the homogenization of repetitive sequences because such sequences were closely positioned by telomere association on nuclear membrane in interphase nuclei [7,13-16]. However, Afa-family sequences were widely distributed not only in subtelomeric but also in interstitial regions. *pHvA*14, a Afa-family repetitive sequences isolated from barley distinguished each barley chromosome by in situ hybridization [8].

To know if both or only one of the genomes of *A. mongolicum* Keng carried the Afa-family sequences, FISH was carried out using *pAmAfa*1 as the probe, and the sequence hybridized with all telomeric regions and subtelomeric regions of the chromosomes. The signals appeared mostly at two ends of the chromosomes. This finding indicated that P genome contained Afa-family repeat sequences, and there was no chromosome specificity in the sequences within genomes.

4 Conclusion

*pAmAfa*1 sequence cloned from *A. mongolicum* Keng is most similar with other Afa-family sequences in Triticeae species. This sequence can be used for the phylogenic analysis of Triticeae species. P genome must have homology with N and X genome; *pAmAfa*1 sequences are located in the telomeric regions and subtelomeric region of the every chromosomes of *A. mongolicum* Keng. P genome contains Afa-family repeat sequences, and there is no chromosome specificity in the sequences within genomes.

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