УДК 631.523:577.21:635.25

A NEW CHROMOSOME SPECIFIC SUBTELOMERIC TANDEM REPEAT IN *ALLIUM FISTULOSUM* (L.)

M.A. SHEIKH BEIG GOHARRIZI, I.V. KIROV, L.I. KHRUSTALEVA

(Russian Timiryazev State Agrarian University)

Evaluation and function of tandem repeats (TRs) are poorly understood. TRs are associated with important chromosomal landmarks such as centromeres, telomeres, subtelomeric and other heterochromatic regions. The genomes of Alliums remain poorly investigated because of their large size, the high frequency of duplications, and increased heterozygosity. Using the next generation sequencing data we found a new tandem repeat (CL26) that occupied 0.2% of A. fistulosum genome. Polymerase chain reaction (PCR) with designed primers on known CL26 sequence and genomic DNA of A. fistulosum as a matrix DNA resulted in a 400 bp band. Fluorescent in situ hybridization (FISH) with the 400 bp PCR product showed that CL26 was located in the subtelomeric region on the short arm of chromosome 6. FISH with CL26 on close related species showed hybridization signal also in the subtelomeric region on the short arm of chromosome 6 of A. cepa and no hybridization signals were observed on chromosomes of A. porrum and A. schoenoprasum. Using FISH in combination with BAC (Bacterial Artificial Chromosome) clones has been an effective approach for genome study, physical mapping, identification repeats elements and their organization in genome. Previously in our group, a total of 1100 BAC clones were constructed from the A. fistulosum genomic DNA. Screening of the A. fistulosum genomic DNA BAC library revealed a single BAC clone possessing the CL26 TRs. We proved that the BAC clone consists, along with CL26 TRs, a 378 bp subtelomeric tandem repeat. Multi-color FISH showed that CL26 was located more proximal on the chromosome 6 as compared to the 378 bp subtelomeric repeat location. The structural organization of the CL26 as well as their application as chromosome marker is discussed.

Key words: *tandem repeat, Allium fistulosum, fluorescence in situ hybridization, BAC clone.*

Tandem repeats are identical DNA sequences laying one after other and reiterating many times in a row. Usually TRs are associated with important chromosomal landmarks such as centromeres, telomeres, subtelomeric and other heterochromatic regions [17, 24, 46). A common feature of a tandem repetitive DNA is the rapid divergences which lead to changes in sequence composition, distribution among species and abundance [42], and results in species-specific repeat variants and/or novel sequence families. On the other hand, members of many repetitive families show a remarkably high conservation [34]. This ambivalence is a key feature of repeats in genome evolution [13]. Knowledge of the genomic organization, chromosomal location and evolutionary origin of repetitive DNA sequences is important for insight into the organization, evolution, behavior and functional potential of repetitive sequences in plant genomes [36].

The *Allium* genus is including many important agricultural vegetable crops such as onion (*A. cepa*), garlic (*A. sativum*), rakkyo (*A. chinense*), shallot (*A. cepa* Aggregatum

group) and Japanese bunching onion (*Allium fistulosum*) that belongs to the order Asparagales [5, 40]. The *Allium fistulosum* (2n = 16) is the major crop of the Alliaceae family following *A. cepa*. The genomes of *Alliums* remain poorly investigated because of their large size, the high frequency of duplications, and increased heterozygosity [20]. *Alliums* have large mitotic chromosomes, making them good cytological model. Multiple genome duplication events and amplification of repetitive DNA have a major influence on the *Allium* genome sizes [20, 37]. Numbers of repetitive DNA families were founded in *Allium* including retrotransposons [19, 23, 31], tandem repeats [3, 7, 9, 17, 43] and non-tandem repeats [27, 44].

BAC clones in combination with fluorescence *in situ* hybridization (FISH) has been an effective approach for genome study, physical mapping, identification repeats elements and their organization in genome. Genomic DNA library and BAC-FISH has been achieved for a number of plant species including rice [18], cotton [14], Arabidopsis [10], potato [6] and etc.

Until recently a BAC library of *A. cepa* with relatively low (0.3x) coverage has been published [45]. In our group a total of 1100 BAC clones were constructed from the *A. fistulosum* genomic DNA [22].

The aims of this study were: 1) the identification of novel tandem repeats (TRs) using a bioinformatics search in Next generation sequencing data; 2) FISH location of the TRs on chromosomes of *A. fistulosum*; 3) screening of *A. fistulosum* BAC library with the designed TRs primers; 4) analysis of genomic organization of TRs using BAC clone possessing TRs. In this study we report a novel chromosome specific 400 bp TR CL26 that is occupied 0.2% of *A. fistulosum* genome. CL26 was located by FISH in the subtelomeric region on the short arm of chromosome 6. A BAC clone 5.3.1 bearing CL26 was screened from totally 1100 BAC clones of the *A. fistulosum* genomic DNA. PCR analysis of BAC clone 5.3.1 revealed the presence of a 378 bp subtelomeric repeat along with TR CL26.

Materials and Methods

Plant material

The bunching onion (*A. fistulosum*) 2n = 16, Russkiy Zimniy cultivar produced by Gavrish breeding agricultural company was used in all our experiments. Genomic DNA was isolated from 5-days-old seedling of *A. fistulosum* according to the protocol of Rogers and Bendich [39].

Bioinformatic identification of tandemly repeat

A total of 5,101,906 reads were derived from Illumina sequencing (Next Generation Sequence) of genomics DNAs of *A. fistulosum* (accession Number SRX268217 in the NCBI server). These NGS reads from genome were subjected to graph-based clustering the similar repeat in Repeat Explorer [29] to identify the group of repetitive elements in *A. fistulosum* genome. Clusters containing tandem repeats were identified based on the shape of cluster that is a ring-like structure corresponding to tandemly organized repeats [28]. The contigs were assembled from reads belonging to the cluster. Assembled contigs of each cluster were characterized by comparing to each other using BLASTN.

Screening of BAC library

The total 1100 BAC clones from the BAC Library derived from *A. fistulosum* genomic DNA [22], were used in this study. BAC DNA was isolated following a modified

alkaline lyses protocol [32]. BAC Library screening was performed with column pools (each pool 8 clones). The BAC DNAs were used as a template DNA in PCR reaction. Primers were designed by Primer 3.0 plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) according to the monomers sequences of CL26 tandem repeat and subtelomeric satellite (Table 1). The PCR amplification was performed in 20 µl reaction of PCR mixture containing 1xTaq buffer, 3mM $MgCl_2$, 0.2 dNTPs, 0.2 mM primers, 0.5 U Taq polymerase and 10 ng DNA temple for 35 cycles of 45 second at 58°C, 90 second at 72°C and annealing temperature was 57°C in 30 second for both primers. The PCR amplification was carried out in thermal cycler (Bio_Rad, United States).

Table 1

Primers used for the repeat amplification and the expected length of the PCR products

Tandem repeats	Primers, 5'-3'	Expected length of PCR product (bp)
CL26	F: GAAGCAAGCCCGAGGAAG R: GTCGACCTGGAGCACGAT	400
Subtelomeric satellite	F: ATCGATTCTTCGGACGGCCT R: AGATGTTGCACCCTTCGGAT	378

Mitotic chromosome preparation

Young root tips pretreated by 0.75 mM hydroxyl urea for 20 hours, and also treated directly in pressurized N_2O gas for 1.5–2 hours, finally the root tips were fixed in a 3:1 fixative solution of ethanol: acetic acid (3:1) for 30 minutes. Slide preparation for fluorescence *in situ* hybridization was performed by the SteamDrop method [21].

Probe preparation and hybridization

Probes were prepared by BAC-DNA (complete plasmid), PCR products amplification of BAC-DNA with CL26 and subtelomeric satellite DNA primers. Probes were labeled either Digoxigenin (Dig)-11-dUTP or Biotin (Biot)-11dUTP (Roche Diagnostics Gmbh, Mannheim, Germany) using a standard Nick-translation protocol.

Fluorescence in situ hybridization (FISH)

The amount of each probe was 100–200 ng per slide in a hybridization mixture containing 50% deionized formamide; 10% dextran sulphate; 2x SSC; 0.25% SDS. Slides were denatured with the probe at 75°C and hybridized in a moist chamber at 37°C for overnight. The Biotin-labeled probe was detected by streptavidin-Cy3 followed by anti-streptavidin-biotin and finally with streptavidin-Cy3 (vector Laboratories, Ca, US). Digoxigenin-labeled probe was detected by anti-digoxigenin-FITC (Fluorescein isothiocyanate) risen in sheep and amplified with anti-sheep-FITC risen in rabbit followed by anti-rabbit-FITC risen in goat (Vector Laboratories, Ca, US). Chromosomes were counterstained with DAPI (4',6-diamidino-2-phenylindole) 1 µg/ml in Vectashield (Vector laboratories, Ca, US). Digital imaging analysis was carried out with Zeiss Axio Imager M1 fluorescence microscope (Carl Zeiss Micro Imaging, Germany), equipped with the Axio Cam MRm digital camera And Axio Vision, version 4.6.3 software program (Carl Zeiss Micro Imaging, Germany). Image adjustments were performed using Photoshop software (Adobe Inc., Ca, US).

Results and Discussion

Identification of the chromosome specific CL26 tandem repeat

The CL26 tandem repeat of *A. fistulosum* genomic reads was characterized by annotation data of cluster, which generated by Repeat Explorer on the NGS reads from *A. fistulosum*. CL26 repeat cluster was identified with ring-like structure (Figure 1B), which is typical for tandemly organized repetitive sequences [25, 28, 38] this cluster cover up 0.2% of *A. fistulosum* genome. Analysis in Tandem Repeat finder [4] showed that the length of the monomer for CL26 was 400 bp. Moreover, the CL26 coting was investigated using the alignment approach BLASTN GenBank database. The BLASTN analysis revealed high homology within IGC spacer of 45S rDNA from A. *cepa* (in GSS database of NCBI).

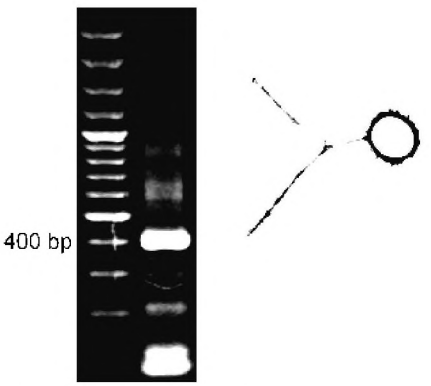

Fig. 1. PCR amplification with primer specific CL26 repeats and *A. fistulosum* genomic DNA (A). The DNA ladder is 100bp Plus. The shape of the CL26 cluster (B)

PCR with CL26 primers and genomic DNA of *A. fistulosum* resulted in ladder-like PCR product (Figure 1A). Thus the result of BLASTN search and PCR confirmed the tandem organization of this repeat. FISH probing with the PCR product of CL26 revealed the hybridization signal in the subtelomeric region on the short arm of chromosome 6 of *A. fistulosum*. FISH with CL26 on closely related species showed hybridization signal also in the subtelomeric region on the short arm of chromosome 6 and 8 of *A. cepa* (Figure 2), and no hybridization signals were on chromosomes of *A. porrum* and *A. schoenoprasum* (data not shown).

PCR screening of BAC library

PCR screening was performed by Column pools method, 136-column pools were screened in this study. Plasmid DNA from each pool was isolated and screened with the specific CL26 primers for identifying BAC clones containing the CL26 repeat. One pool initially was screened. The each BAC clone from positive BAC pool was analyzed for identifying individual clone that possessed the CL26 repeat. Finally, one positive BAC clone (5.3.1) possessing CL26 was identified from total of 1100 clones. The expected 400 bp PCR product was obtained (Figure 3A).

BAC-FISH and FISH on mitotic chromosomes

BAC-FISH with clone 5.3.1 that possessed CL26 tandem repeat revealed signal hybridization at distal end on all 16 chromosomes of *A. fistulosum* (Figure 4A). Previously it was shown that *A. fistulosum* contains 378 bp tandem repeats in subtelomeric region of all chromosomes [9, 15]. To answer the question whether BAC clone 5.3.1 possesses the subtelomeric repeats along with CL26 TR we carried out the PCR with primers on the subtelomeric repeat. PCR with BAC clone 5.3.1 and the subtelomeric repeat primers resulted in a 378 bp band (Figure 3B).

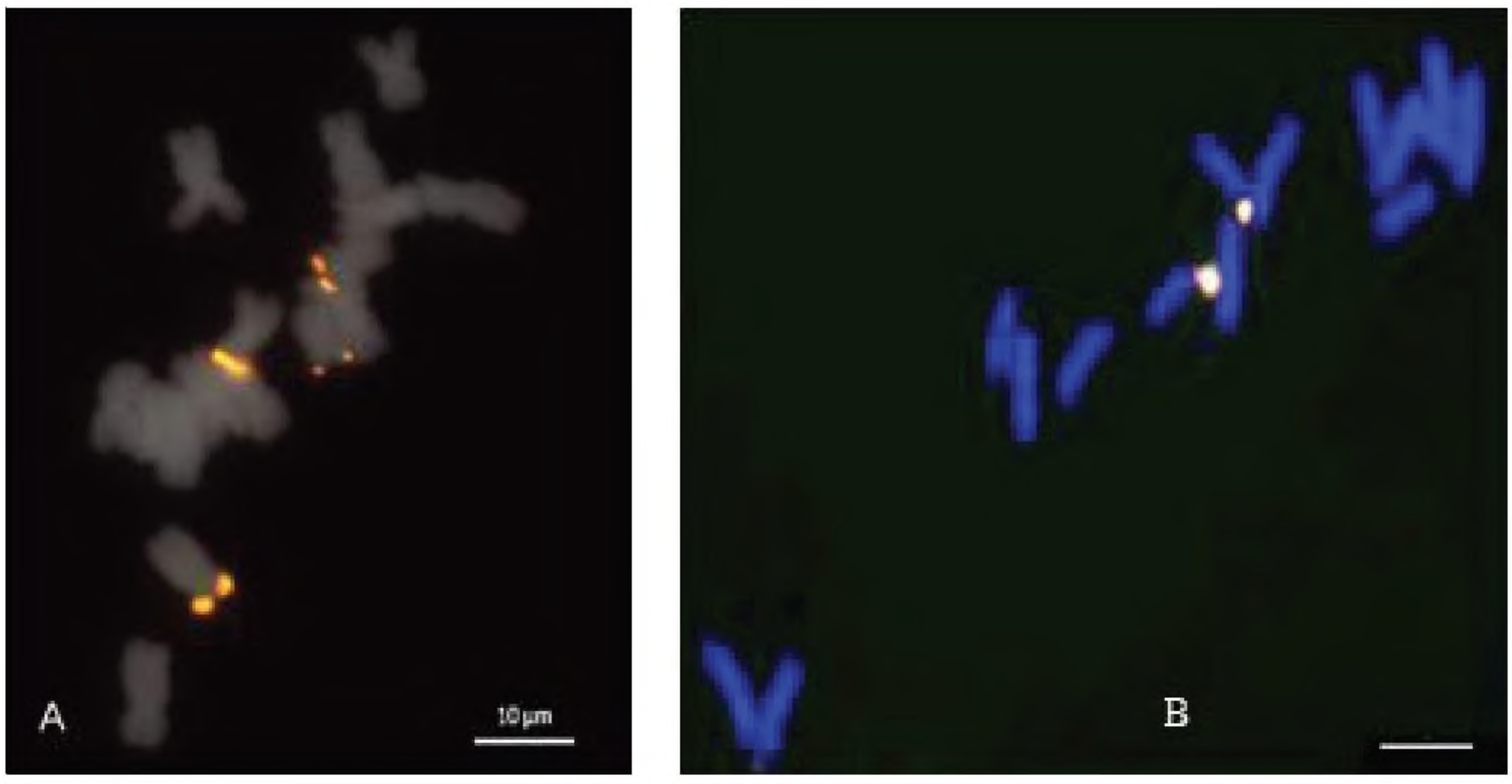

Fig. 2. Fluorescence *in situ* hybridization (FISH): A) probing with PCR product of CL26 primers and genomic DNA of *A. cepa* on mitotic metaphase chromosomes of *A. cepa*; B) probing with PCR product of CL26 primers and genomic DNA of *A. fistulosum* on mitotic metaphase chromosomes of *A. fistulosum*. CL26 PCR product was labeled with Biotin-11-dUTP and detected with streptavidin-Cy3 (red); chromosomes were counterstained with DAPI (blue/grey). Scale bars = 10 µm.

FISH probing with the PCR product of the 378 bp revealed a fluorescence signal in subtelomeric region of all chromosomes of *A. fistulosum* (Figure 4B). In contrast, FISH probing with PCR product of CL26 primers showed the fluorescence signal only in subtelomeric region of the short arm of chromosome 6 (Figure 4C). To clarify whether CL26 and a 378 bp subtelomeric repeat are co-localized on chromosome 6, multi-color FISH was performed simultaneously with both PCR products: CL26 TR and a 378 bp subtelomeric repeat. FISH showed that CL26 was located more proximal as compared to the 378 bp subtelomeric repeat location (Figure 4D, D').

A novel chromosome specific tandem repeat (Cl26) in *A. fistulosum* was described in this study. Subtelomeric specific tandem re-

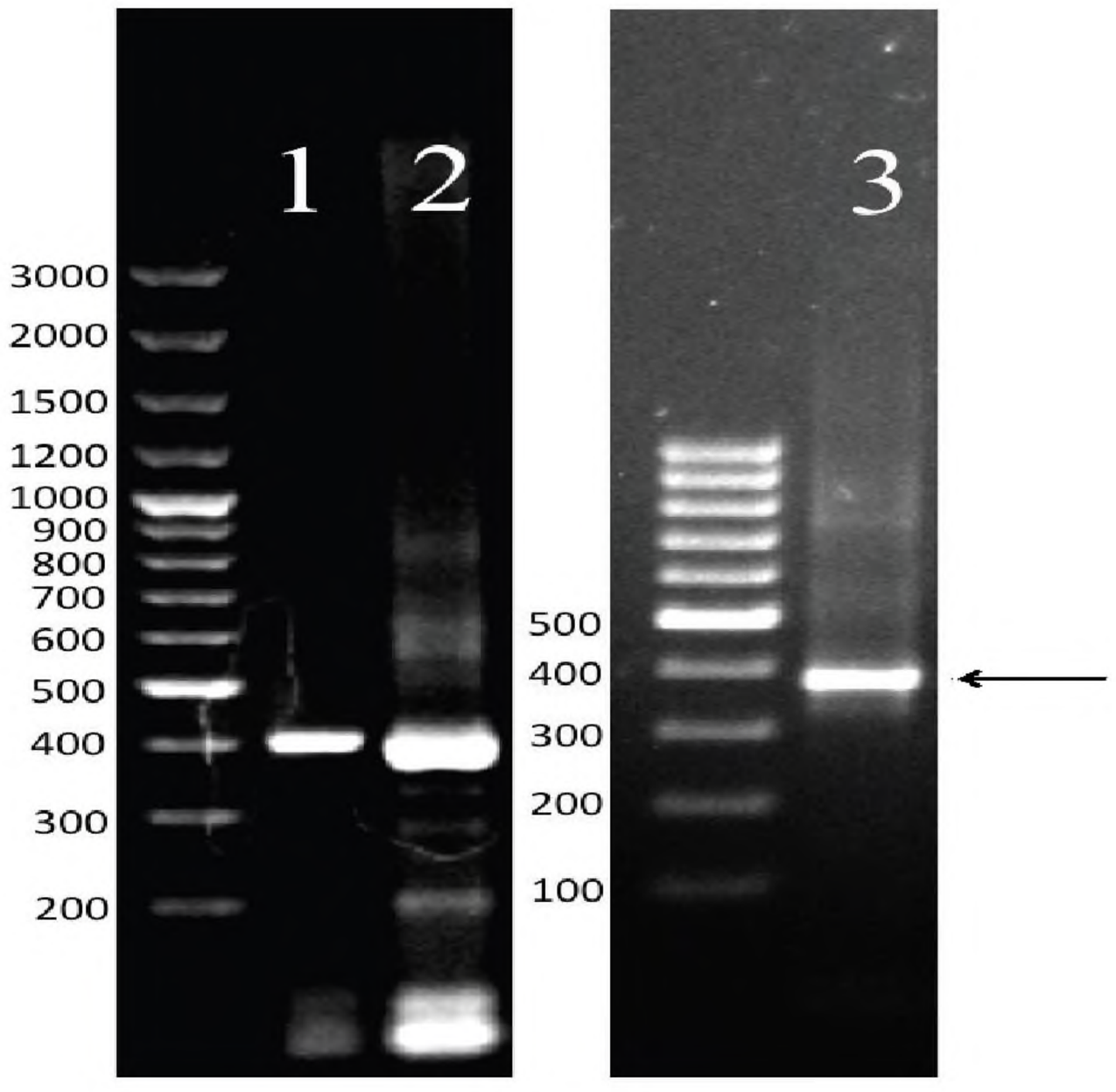

Fig. 3. The results of PCR:(A) with specific primers on CL26 repeat sequence: lane 1 — BAC clone 5.3.1 as a matrix DNA, lane 2 — genomic DNA of A. *fistulosum* as a matrix DNA; (B) with primers on 378bp subtelomeric repeat and BAC clone 5.3.1 as a matrix DNA

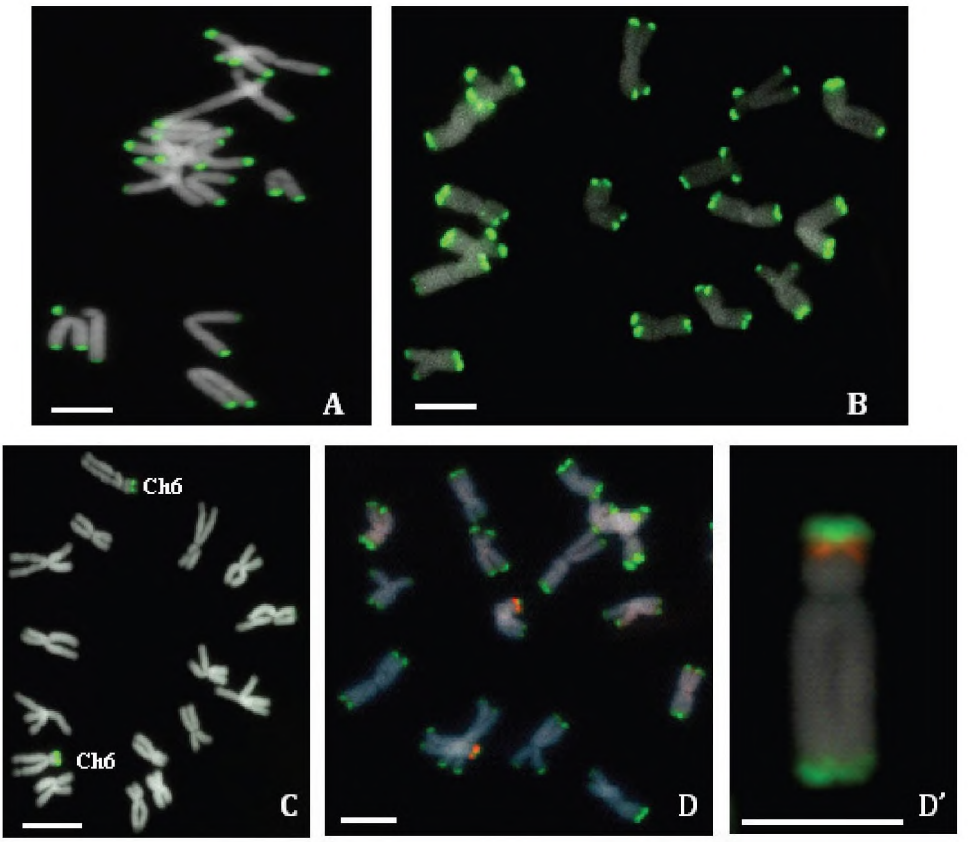

Fig. 4. Fluoresence *in situ* hybridization (FISH) on the mitotic metaphase chromosomes of *A. fistulosum* probing with: A)BAC clone 5.3.1 plasmide DNA labeled with Dig-11-dUTP (green fluorescence), yielded signals at the telomeric region of all chromosomes; B) PCR product from BAC DNA 5.3.1 with primers on a 378 bp subtelomeric repeat DNA labeled with Dig-11-dUTP, yielded signals at the telomeric region of all choromosomes;C) PCR product from BAC DNA 5.3.1 with primers on CI26 repeat labeled with Dig-11-dUTP, yielded signals exclusively in the telomeric region of the short arm of homologous choromosomes 6. D) Multi-colour FISH with PCR products from BAC DNA 5.3.1 with primers on a 378 bp subtelomeric repeat DNA labeled with Dig-11-dUTP (green fluorescence) and PCR product from BAC DNA 5.3.1 with primers on CI26 repeat labeled with Biot-11-dUTP (red fluorescence); D') Multi-colour FISH exstracted chromosome 6. Scale bars = 10 μm

peats have been reported in Triticeae species [2], *Ae. speltoides* [41] and rice [30] chromosomes.

To analyses genomic organization of CL26 tandem repeat we used a BAC library of *A. fistulosum*. Screening of the BAC library revealed a single BAC clone (5.3.1) that possessed CL26 tandem repeat. Surprisingly BAC-FISH with clone 5.3.1 produced hybridization signal in subtelomeric region of all chromosomes of the compliment. We suggested the presence within the BAC clone 5.3.1 along with CL26 a 378 bp subtelomeric repeat that was previously revealed in the *A. fistulosum* genome [9, 16]. PCR with primers on the 378 bp subtelomeric repeat and the BAC clone 5.3.1 supported our hypothesis. The question was put forward: how these two tandem repeats are organized relative to

each other on a physical chromosome. Simultaneous use of both repeats as a probe in multi-color FISH revealed that CL26 tandem repeats are located more proximal than a 378 bp subtelomeric repeat. We may suggest that the BAC clone 5.3.1 possesses genomic DNA from a boundary region between CL26 and the 378 bp tandem repeats. Another hypothesis is that the 378 bp repeat was embedded into sequence of CL26 tandem repeats on chromosome 6 by retrotransposons. Fesenko et al. [9] reported that the subtelomeric heterochromatin of *A. fistulosum* is enriched with the reverse transcriptase fragments of Ty1-copia retrotransposon. So the retrotransposons may transpose some sequence of the 378 bp repeat into CL26 tandem repeat.

Conclusions

In the present study, a novel repetitive DNA was identified and mapped that can be used as cytogenetic markers for chromosome identification. Our approach based on the use of bioinformatic tools, BAC library resource and molecular-cytogenetic techniques allowed us to identify rapidly repeated sequences from data of NGS and study their characteristics in the *A. fistulosum* genome.

Acknowledgement

We gratefully acknowledge the financial support of a PhD fellowship to M.A. Sheikh Beig Goharrizim (VN 258163) from the Ministry of Education and Science of the Russian Federation.

References

1. *Adams S., Hartman T., Lim K.Y., Ghase M.W.,Bennett M.D., Leitch I.J. and Leitch A.R.* Loss and recovery of Arabidopsis-type telomere repeat sequences 5'-(tttaggg)n-3' in the evalution of a majore radiation of flowering plants. Proc. of the royal society of London. 2001. № 268. P. 1541–1546.

2. *Anamthawat-Jonsson K., Heslop-Harrison J.S.* Isolation and characterization of genome-specific DNA sequences in Triticeae species. Molecular and General Genetics MGG. 1993. № 240.2. P. 151–158.

3. *Barnes S.R., James A.M., Jamiesson G.* The organization, nucleotide sequence, and chromosomal distribution of a satellite DNA from A. cepa. Chromosom. 1985. № 92. P. 185–192.

4. *Benson G.* Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 1999. № 27(2). P. 573–580.

5. *Damaris A., Odeny and staya S. Narina.* Genomic and Breeding Resources, Vegetables. Springer-Verlag Berlin Heidelberg. 2011. P. 1–10.

6. *Dong F., Song J., Naess S.K., Helgeson J.P., Gebhardt C., and Jiang J.* Development and application of a set of chromosome-specific cytogenetic markers in potato. Theor. Appl. Gen. 2000. № 101. P. 1001–1007.

7. *Fajkus P. et al.* Allium telomeres unmasked: the unusual telomeric sequence (CTCGGTTATGGG) n is synthesized by telomerase. The Plant Journal. 2015.

8. *Fajkus J., Kovarik A., Kralovics R. and Bezdek M.* Organization of telomeric and subtelomeric chromatin in the higher plant nicotianatabacum. Molecular and General genetics. 1995. № 247 (5). P. 633–638.

9. *Fesenko I.A., Khrustaleva L.I., Karlov G.I.* Organization of the 378-bp satellite repeat in terminal heterochromatin of Allium fistulosum. Russian Journal of Genetics. 2002. Vol. 38. № 7. P. 745–753.

10. *Fransz P.F., Armstrong S., de Jong J.H., Parnell L.D., van Drunen C., Dean C., Zabel P., Bisseling T., Jones G.H.* Integrated cytogenetic map of chromosome arm 4S of A. thaliana: structural organization of heterochromatic knob and centromere region. Cell. 1995. № 100. P. 367–376.

11. *Fuchs J., Brandes A., and Schubert I.* Telomere Sequence Localization and Karyotype Evolution in Higher Plants. Plant Syst. Evol. 1995. № 196. P. 227–241.

12. *Grunstein M.* Molecular model for telomeric heterochromatin in yeast. Current opinion in cell biology. 1997. № 9 (3). P. 383–387.

13. *Hall S.E., Kettler G. and Preuss D.* Centromere satellites from Arabidopsis populations: Maintenance of conserved and variable domains. Genome research. 1997. № 13 (2). P. 195–205.

14. *Hanson R.E., Zwick M.S., Choi S.D., Islam-Faridi M.N., McKnight T.D., Wing R.A., Price H.J., Stelly D.M.* Fluorescent in situ hybridization of a bacterial artificial chromosome. Genome. 1995. № 38. P. 646–651.

15. *Hizume M.* Allodiploid nature of Allium wakegi Araki revealed by genomic in situ hybridization and localization of 5S and 18S rDNAs. Jpn J Genet. 1994. № 69(4). P. 407–415.

16. *Irifune K., Hirai K., Zheng J., Tanaka R., Morikawa H.* Nucleotide sequences of a highly repeated DNA sequences and its chromosomal localization in Allium fistulosum. Theor. Appl. Genet. 1995. № 90(3–4). P. 312–316.

17. *Jiang J., Birchler J.A., Parrott W.A., Dawe R.K.* A molecular view of plant centromeres. Trends Plant Sci. 2003. № 8(12). P. 570–575.

18. *Jiang, J., Gill B.S., Wang G.L., Ronald P.C., and Ward D.C.* Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes. Proc. of the National Academy of Sciences of the United States of America. 1995. № 92. P. 4487–4491.

19. *Kim S., Park J.Y., Yang T.J.* Characterization of three active transposable elements recently inserted in three independent DFR-A alleles and one high-copy DNA transposon isolated from the Pink allele of the ANS gene in onion (Allium cepa L.). Molecular Genetics and Genomics. 2014. P. 1–11.

20. *King J.J., Bradeen J.M., Bark O., McCallum J.A., M.J. Havey M.J.* A low-density genetic map of onion reveals a role for tandem duplication in the evolution of an extremely large diploid genome. Theor. Appl. Genet. 1998. № 96. P. 52–62.

21. *Kirov I., Divashuk M., Van Laere K., Soloviev A., Khrustaleva L.* An easy — Steam Drop method for high quality plant chromosome preparation. Molecular cytogenetics. 2014. № 1. 21 p.

22. *Kiseleva A.V., Fesenko L.I., Khrustaleva L.I.* construction of a genomic BAC library for development of cytogenetic markers in Allium fistulosum L. Izvestiya of Timiryazev Agricultural Academy. 2012. № 6. P. 31–39.

23. *Kiseleva A.V., Kirov I.V., Khrustaleva L.I.* Chromosomal Organization of Centromeric Ty3/gypsy Retrotransposons in Allium cepa L. and Allium fistulosum L. RUSS J GENET. 2014. № 50 (6). P. 586–592.

24. *Koo D.-H., Hong C.P., Batley J., Chung Y.S., Edwards D., Bang J.-W., Hur Y., Lim Y.P.* Rapid divergence of repetitive DNAs in Brassica relatives. Genomics. 2011. № 97(3). P. 173–185.

25. *Macas J., Kejnovský E., Neumann P., Novák P., Koblížková A., Vyskot B.* Next generation sequencing-based analysis of repetitive DNA in the model dioecious plant Silenelatifolia. PLoS One. 2011. № 6(11)

26. *Muller H.* The remaking of chromosomes. *The collecting Net.* 1938. № 13. P. 183–195.

27. *Nagaki K.1., Yamamoto M., Yamaji N., Mukai Y., Murata M.* Chromosome dynamics visualized with an anti-centromeric histone H3 antibody in Allium. PLoS One. 2012. № 7(12).

28. *Novák P., Neumann P., Macas J.* Graph-based clustering and characterization of repetitive sequences in next-generation sequencing data. BMC Bioinformatics. 2010. № 11. 378 p.

29. *Novák P., Neumann P., Pech J., Steinhaisl J., Macas J.* RepeatExplorer: a Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. Bioinformatics. 2013. № 29(6). P. 792–793.

30. *Ohmido, Nobuko, et al.* Visualization of the terminal structure of rice chromosomes 6 and 12 with multicolor FISH to chromosomes and extended DNA fibers. Plant molecular biology. 2001. № 47.3. P. 413–421.

31. *Pearce S.R., Pich U., Harrison G., Flavell A.J., Heslop-Harrison J.S., Schubert I., Kumar A.* The Ty1-copia group retrotransposons of Allium cepa are distributed throughout the

chromosomes but are enriched in the terminal heterochromatin. Chromosome. 1996. № 4(5). P. 357–364.

32. *Peterson D.G., Tomkins J.P., Frisch D.A.* Construction of plant bacterial artificial chromosome (BAC) libraries: an illustrated guide. Journal of Agricultural Genomics. 1996. № 5. P. 1–100.

33. *Pich U., Schubert I.* Terminal heterochromatin and alternative telomeric sequences in Allium cepa. Chromosome Res. 1998. № 6. P. 315–321

34. *Pich U., Fritsch R., and Schubert I.* Closely related Allium species (Alliaceae) share a very similar satellite sequence. Plant Sys. 1996. № 202. P. 255–264.

35. *Pich U., Fuchs J., and Schubert I.* How do Alliaceae stabilize their chromosome ends in the absence of TTTAGGG sequences? Chromosome. 1996. № 4. P. 207–213.

36. *Plohl M.* Those mysterious sequences of satellite DNAs. Period Biol. 2010. № 112. P. 403–410.

37. *Ranjekar P.K., Pallotta D., Lafontaine J.G.* Analysis of plant genomes. V. Comparative study of molecular properties of DNAs of seven Allium species. Biochem Hall Genet. 1978. № 16(9–10). P. 957–970.

38. *Renny-Byfield S., Kovařík A., Chester M., Nichols R.A., Macas J., Novák P., Leitch A.R.* Independent, Rapid and Targeted Loss of Highly Repetitive DNA in Natural and Synthetic Allopolyploids of Nicotiana. PLoS One. 2012. № 7(5)

39. *Rogers SO, Bendich AJ.* Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant MolBiol.1985 № 5(2). P. 69–76.

40. *Rudall P, Furness C, Chase M, Fay M.* Microsporogeneisis and pollen sulcus type in Asparagales (Lilianae). Canadian Journal of Botani. 1997. № 75. P. 408–430.

41. *Salina, E. A., et al.* Identification of a new family of tandem repeats in Triticeae genomes. Euphytica. 1998. № 100.1–3. P. 231–237.

42. *Schmidt T., Heslop-Harrison J.S.* Genomes, genes and junk: the large-scale organization of plant chromosomes. Trends Plant Sci. 1998. № 3(5). P. 195–199.

43. *Seo BB, Do GS, Lee SH.* Identification of a tandemly repeated DNA sequence using combined RAPD and FISH in welsh onion (Allium fistulosum). Kor J BiolSci. 1999. № 3(1). P. 69–72.

44. *Shibata F, Hizume M.* The identification and analysis of the sequences that allow the detection of Allium cepa chromosomes by GISH in the allodiploid A. wakegi. Chromosoma. 2002. № 111(3). P. 184–191.

45. *Suzuki Go, AikoUra, Naoko Saito, GeumSook Do, Bong Bo Seo, Maki Yamamoto and Yasuhiko Mukai.* BAC FISH analysis in Allium cepa. Genes Genet. 2001. № 76. P. 251–255.

46. *Zakrzewski F, Schubert V, Viehoever P et al.* The CHH motif in sugar beet satellite DNA: a modulator for cytosine methylation. Plant J. 2014. № 78(6). P. 937–950.

47. *Zhang H, KoblížkováA, Wang K et al.* Boom-bust turnovers of megabase-sized centromeric DNA in Solanum species: Rapid evolution of DNA sequences associated with centromeres. Plant Cell. 2014. № 26(4). P. 1436–1447.

НОВЫЙ ХРОМОСОМ-СПЕЦИФИЧНЫЙ СУБТЕЛОМЕРНЫЙ ТАНДЕМНЫЙ ПОВТОР У *ALLIUM FISTULOSUM* (L.)

М.А. ШЕЙХ БЕЙГ ГОХАРРИЗИ, И.В. КИРОВ, Л.И. ХРУСТАЛЕВА

(РГАУ-МСХА имени К.А. Тимирязева)

Эволюция и функция тандемных повторов (ТП) остаются слабо изученными. ТП чаще расположены в важных хромосомных регионах, таких как центромера, теломера и субтеломера, а также формируют другие гетерохроматиновые регионы. Геном луковых плохо изучен

из-за его огромного размера, высокой степени дупликаций и повышенной гетерозиготности. С помощью NCBI базы данных и биоинформатических методов мы нашли новый тандемный повтор (CL26), который занимает 0,2% генома A. fistulosum. В результате проведения полимеразной цепной реакции (ПЦР) с праймерами на CL26 и геномной ДНК A. fistulosum был получен ПЦР продукт размером 400 п.н. Используя флуоресцентную in situ гибридизацию (FISH) с этим ПЦР продуктом показали, что CL26 повтор локализован в субтеломерном регионе на коротком плече хромосомы 6. FISH с CL26 ПЦР продуктом на хромосомах A. cepa показал наличие сигнала в субтеломерном регионе на коротком плече хромосомы 6 и 8. Однако на двух других близкородственных видах A. porrum и A. schoenoprasum сигнала гибридизации не было выявлено. Сочетание использования FISH и BAC (бактериальная искусственная хромосома) клонов является эффективным подходом в изучении геномов, физическом картировании, идентификации повторяющихся последовательностей и изучении их организации в геноме. Ранее в нашей группе была создана библиотека геномной ДНК A. fistulosum, всего 1100 BAC клонов. Скрининг BAC библиотеки выявил один клон, содержащий CL26. Этот клон был использован для изучения геномной организации найденного нами тандемного повтора. С помощью ПЦР было установлено, что BAC клон содержит, наряду с CL26, 378 п.н. субтеломерный тандемный повтор. Многоцветная FISH продемонстрировала, что CL26 повтор локализован на хромосоме более проксимально по сравнению с тандемным повтором 378 п.н. В работе обсуждается структурная организация CL26 и возможность использования этого повтора в качестве молекулярно-цитогенетического маркера.

Ключевые слова: *тандемный повтор, Allium fistulosum, флоресцентная in situ гибридизация, BAC клоню.*

Хрусталева Людмила Ивановна — д. б. н., гл. науч. сотр. Центра молекулярной биотехнологии, проф. кафедры генетики, биотехнологии, селекции и семеноводства РГАУ-МСХА имени К.А. Тимирязева (127550, г. Москва, ул. Тимирязевская, 5а; тел.: (499) 977-70-01; e-mail: khrustaleva@timacad.ru; ludmila.khrustaleva19@gmail.com).

Мохаммад Али Шейх Бейг Гохарризи — асп. кафедры генетики, биотехнологии, селекции и семеноводства РГАУ-МСХА имени К.А. Тимирязева (127550, г. Москва, ул. Тимирязевская, 5а; тел.: (499) 977-70-01; e-mail: sheikhbeig@gmail.com).

Киров Илья Владимирович — асс. кафедры генетики, биотехнологии, селекции и семеноводства РГАУ-МСХА имени К.А. Тимирязева (127550, г. Москва, ул. Тимирязевская, 5а; тел.: (499) 977-70-01; e-mail:kirovez@gmail.com).

Khrustaleva Ludmila Ivanovna — Doctor of Biological Sciences, principle researcher of the Centre of Molecular Biotechnology, Professor of the Department of Genetics, Biotechnology, Plant Breeding and Seed Science, Russian Timiryazev State Agrarian University (127550, Moscow, Timiryazevskaya str., 5a; tel.: +7 (499) 977-70-01; e-mail: khrustaleva@timacad.ru; ludmila.khrustaleva19@gmail.com).

Mokhammad Ali Sheikh Beig Goharrizi — PhD-student of the Department of Genetics, Biotechnology, Plant Breeding and Seed Science, Russian Timiryazev State Agrarian University (127550, Moscow, Timiryazevskaya str., 5a; tel.: +7 (499) 977-70-01; e-mail: sheikhbeig@gmail.com).

Kirov Il'ya Vladimirovich — teaching assistant of the Department of Genetics, Biotechnology, Plant Breeding and Seed Science, Russian Timiryazev State Agrarian University (127550, Moscow, Timiryazevskaya str., 5a; tel.: +7 (499) 977-70-01; e-mail: kirovez@gmail.com).