Karyotype analysis in *Agropyron cristatum*

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BACKGROUND

The genus *Agropyron* is related to genus *Triticum*, and a number of *Agropyron* species are potential sources of genes for wheat improvement. Crested wheatgrass (*A. cristatum* L.) is a perennial species of economic importance as forage; it is facultatively allogamic, auto-compatible, and shows high crossability with wheat and other members of Triticeae. In addition to diploid *A. cristatum* (2n=2x=14, PP), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) forms exist.

A number of genes that control traits of agronomic interest were identified in this species, including genes controlling resistance to barley yellow dwarf virus, wheat streak mosaic virus, yellow rust, leaf rust and stem rust, cold tolerance, salinity tolerance, drought tolerance and genes affecting yield. Hence, a detailed knowledge of the *A. cristatum* karyotype is needed to identify its chromosomes introgressed into wheat.

AIMS

- Characterize the genome composition of a tetraploid accession of *A. cristatum.*
- Identify individual chromosomes.
- Understand the chromosome structure.

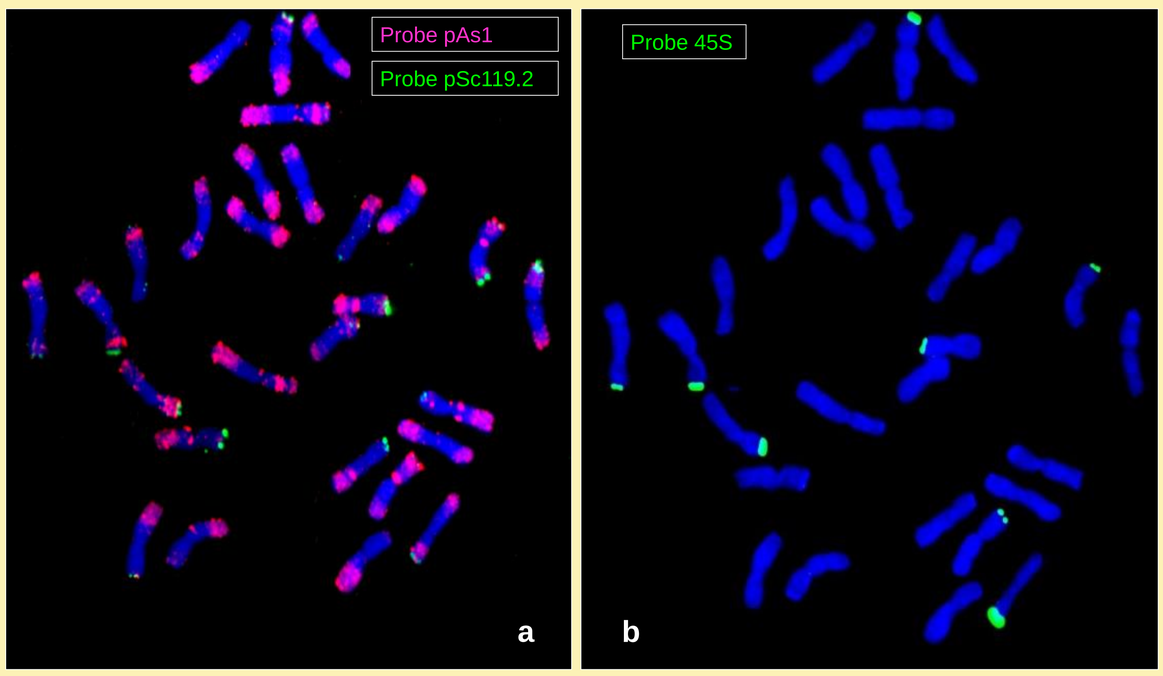

Fig 1. FISH on tetraploid *A. cristatum* (a) pSc119.2 (green) and pAs1 (red); (b) 45S rDNA (green). The chromosomes were counterstained with DAPI (blue).

MATERIAL AND METHODS

The tetraploid *A. cristatum* accession PI222957 from Iran was obtained from USDA and the diploid *A. cristatum* accession W6 19302 was kindly provided by Dr. B. Kilian (IPK, Gatersleben, Germany). A set of wheat-*A. cristatum* disomic addition lines was provided by Prof. A. Cabrera (Genetics Department, University of Cordoba, Spain).

The somatic metaphase chromosome preparation and Fluorescence *in situ* hybridization (FISH) protocols were as described by Cabrera *et al.* (2002). The pAs1 sequence of *Aegilops tauschii* in addition to a set of DNA sequences (5S rDNA, 45S rDNA and pSc119.2) were used as probes. The chromosomes were counterstained with DAPI. Chromosomes 1P and 5P were sorted from wheat-*A. cristatum* addition lines following a protocol of (Suchánková *et al.* 2006).

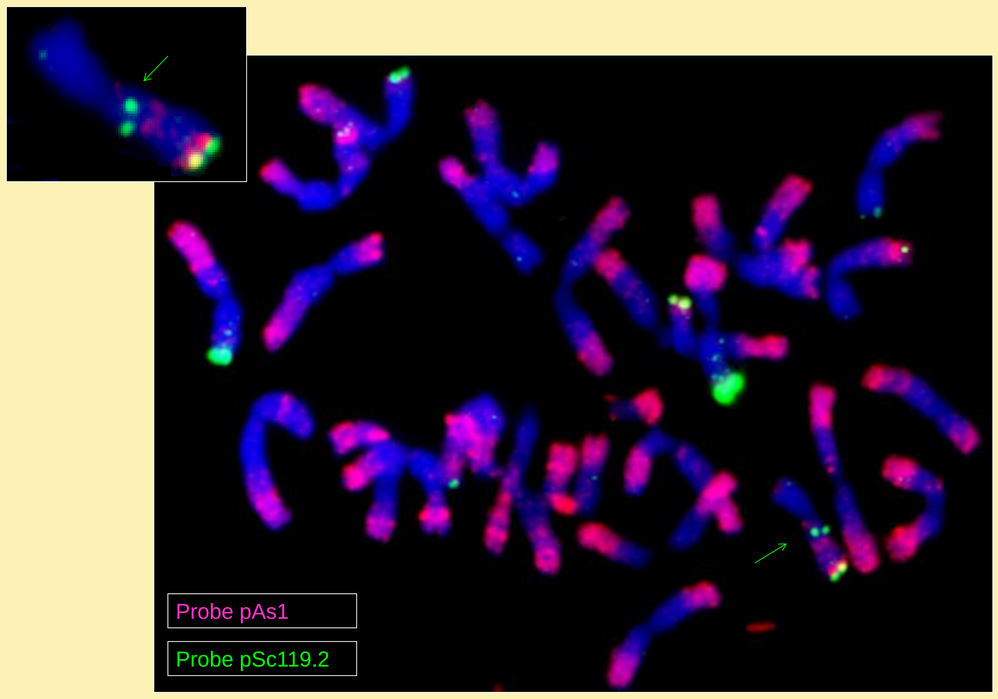

Fig 2. FISH on mitotic metaphase of tetraploid *A. cristatum* with pSc119.2 (green) and pAs1 (red) probes. The chromosomes were counterstained with DAPI (blue). A monosomic translocation involving the pSc119.2 probe were observed (green arrows).

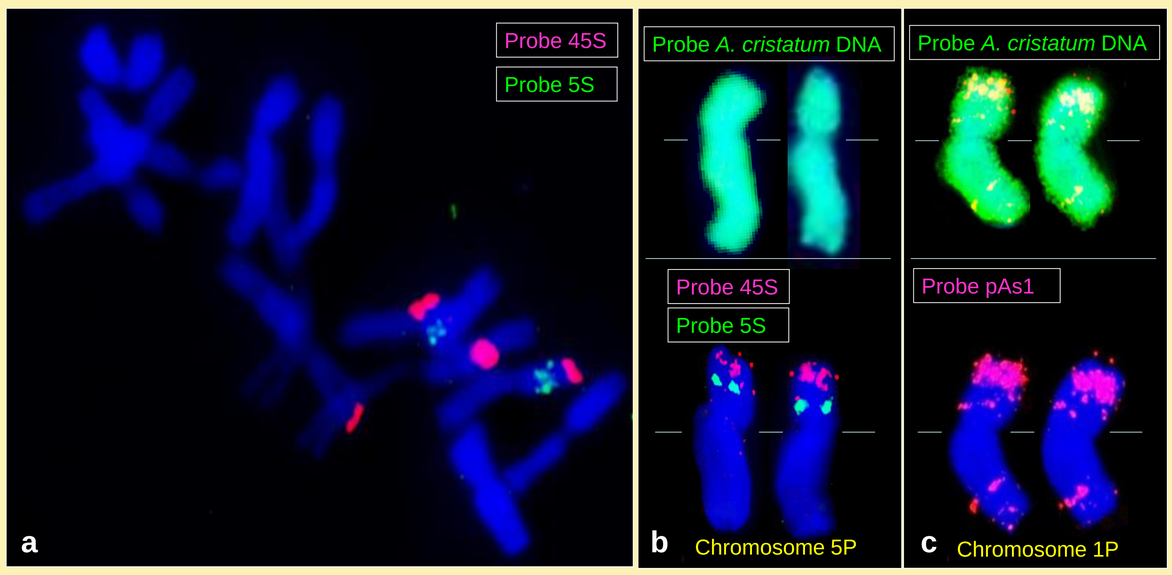

Fig 3. In situ hybridization on mitotic metaphase chromosomes of (a) diploid *A. cristatum;* (b) flow-sorted chromosome 5P with GISH (above) and 45S rDNA (red) and 5S rDNA (green) probes (below); (c) flow-sorted chromosome 1P with GISH (above) and pAs1 (red) probe (below). The chromosomes were counterstained with DAPI. (blue).

RESULTS

- *In situ* hybridization with a set of probes on tetraploid *A. cristatum* chromosomes showed specific patterns for the majority of homologous chromosome groups. However, variability in the number and position of FISH signals were observed for some homologues in different individuals.
- FISH with the pAs1 probe resulted in signals in terminal regions of all chromosomes and enabled identification of individual homologous pairs (Figures 1a and 2).
- Terminal location of the pSc119.2 sequence was detected on ten to fourteen chromosomes (Figure 1a). Differences in signals position using pSc119.2 probe were observed among individuals of the same accession (Figure 2).
- *In situ* hybridization with the 45S rDNA probe revealed eight hybridization sites located at terminal position of the short arms of four chromosome pairs (Figure1b). The 5S rDNA probe showed two to four sites of hybridization.
- FISH on metaphase spreads of diploid *A. cristatum* with 45S rDNA probe showed four hybridization sites on the short arm of two chromosome pairs corresponding to two pair of NOR per genome.
- The 5S rDNA probe showed signals in the subterminal position of the short arms of one of the chromosome pair carrying 45S (Figure 3a). This pair of chromosomes carrying both 45S and 5S rDNA genes was identified as chromosome 5P as demonstrated by FISH (Figure 3b) on flowed-sorted chromosome 5P from the wheat-*A. cristatum* disomic addition line for this chromosome (Figure 4a).
- Chromosome 1P was also identified on the basis of hybridization pattern with the probe pAs1 on 1P flow-sorted chromosome (Figure 3c) from its respective wheat-*A. cristatum* disomic addition line (Figure 4b).

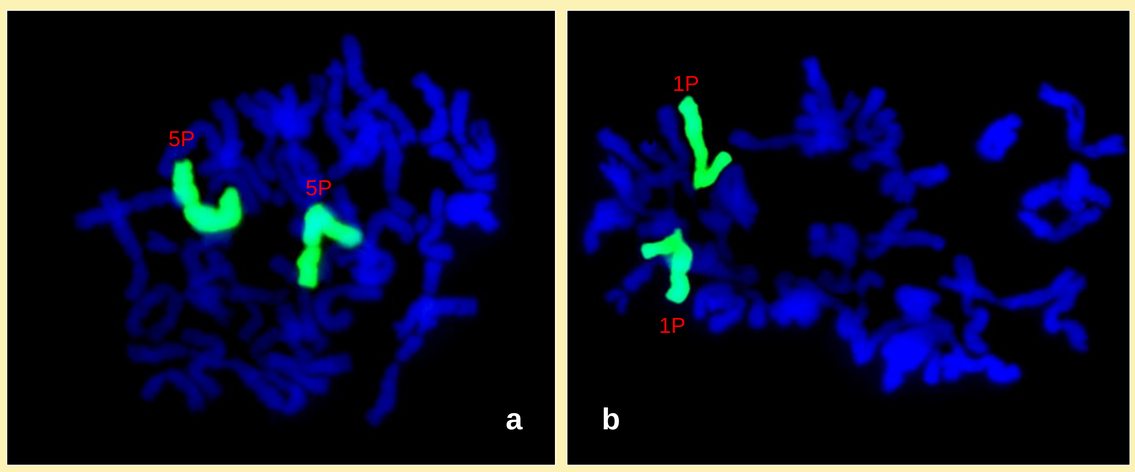

Fig 4. GISH identifies *A. cristatum* chromosomes (green) in wheat-*A. cristatum* addition lines. The chromosomes were counterstained by DAPI (blue color).

CONCLUSIONS

- Our results support the view that the tetraploid *A. cristatum* accession has a non-autoploid nature and that the four P genomes have diverged by structural changes. Hence this accession should be considered a segmental allopolyploid.
- Variability in the number and position of pSc119.2, 5S signals and pAs1 patterns can help to identify chromosomes involved in the structural changes.
- The work in progress to characterize the four P genomes in the tetrapod accession in more detail after establishing the karyotype of a diploid accession of *A. cristatum.*

REFERENCES

Cabrera *et al.* 2002. Chromosome Res 10: 49-54.
Suchánková *et al.* 2006. Theor Appl Genet 113: 651-659.

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