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IN SUSPENSION FISH LABELLED CHROMOSOMES AS A NEW ANALYTICAL TOOL FOR WHEAT GENOME MANIPULATIONS

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Many crops are of polyploid origin and their chromosome complements are often made of similar size chromosomes which can be recognized by banding techniques only. Both conditions apply to the wheat genome where its genetic analysis is hampered by the large size and the presence of homeologous genomes (A+B and A+B and D, for pasta and bread wheat, respectively). One of the ways to simplify genome analysis in wheat is to create specific resources from the three genomes on the basis of a single chromosome approach combined with flow sorting. The creation of BAC libraries from flow sorted individual chromosomes or their harms is an attractive possibility to reduce the complexity of the huge genome of polyploid wheat. However, due to the lack of sufficient differences in size between individual chromosomes in durum and bread wheat, only chromosome 3B could be discriminated and sorted into a high-purity fraction from standard lines. Up till now, the possibility to create a specific chromosome BAC library is linked to the use of mutants such as aneuploid lines with a modified kariotype.

To overcome such a limitation, for the first time we have developed a technique which allow the single chromosome characterization (banding) via chromosomes In suspension FISH labelling (InFISH) and isolation via fluorescence-activated sorting of the single chromosome fractions. Banding in suspension was achieved with a biotin labelled GAA microsatellite probe after alkali chromosome DNA denaturation and hybridization. Banding was detected after double labelling with a mouse anti biotin antibody and an anti mouse antibody fluorescein coniugated for fluorescent tagging. Biparametric flow cytogenetic analysis of durum wheat chromosomes cv Varano counterstained with DAPI and FISH labelled with the fluorescein GAA microsatellite probe allowed the discrimination of A and B genome and satellite chromosomes 1B and 6B. Our results clearly demonstrate the great potentialities of InFISH to identify cytogenetic changes for chromosome flow karyotyping and sorting of single type chromosome suspensions from standard lines in plants and potentially also for animals.