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Localization of different microsatellites and a minisatellite-like sequence on polytene chromosomes of *Phaseolus coccineus*

Nenno, M., Zink, D., Nagl, W.

Division of Cell Biology, The University of Kaiserslautern, P.O. Box 30 49, D-67653 Kaiserslautern, Germany
email: nenno@rhrk.uni-kl.de, dozink@rhrk.uni-kl.de, nagl@rhrk.uni-kl.de

Introduction

Identification of polytene chromosomes from the embryo suspensor of *Phaseolus* is hindered by great variation in chromosome morphology and because they are lacking a clear banding pattern. To overcome these problems, each chromosome should be tagged individually with molecular markers which can be detected by fluorescence *in situ* hybridization (FISH). Here we investigated four different microsatellites or simple sequence repeats (SSR) and one minisatellite-like sequence. These sequences have already been used for DNA fingerprinting in *Phaseolus* (Hamann et al., 1995; Sonnante et al., 1994).

Material and Methods

Embryo suspensors were taken from seeds of unripe pods of *Phaseolus coccineus* cv. Preisgewinner grown in the Botanical Garden at the University of Kaiserslautern. Preparations of polytene chromosomes were made from the biggest basal suspensor cells. Before squashing, the nuclei were gently squeezed out from the cell to get rid of the cell wall. Furthermore this technique resulted in a better spreading of the 22 polytene chromosomes, which correspond with the diploid complement. Prior to *in situ* hybridization, chromosome preparations were digested with proteinase K to degrade chromosomal proteins and increase accessibility.

Digoxigenin-labeled synthetic oligonucleotides (Biometra, Germany) of the microsatellite sequences $(GATA)_4$, $(GACA)_4$, $(CAC)_5$ and $(CA)_8$ were used as probes. The minisatellite probe was a fluorochrome-labeled synthetic oligonucleotide of the 15 bp consensus sequence of the protein III gene of the bacteriophage M13. Hybridization and stringency washes were performed according to a method described by Zischler et al. (1989) at T_m-10°C or T_m-5°C, respectively.

The probes were detected by a hybrid-detection system combining digoxigenin and avidin/biotin. Briefly, the probe was incubated subsequently with anti-digoxigenin from mouse, biotinylated anti-mouse, and avidin-FITC. If necessary, one layer of signal amplification by biotinylated anti-avidin and avidin-FITC was applied.

Results and Discussion

The hybridization sites of different microsatellites and minisatellite sequences were found in the euchromatin as well as in the centromeric heterochromatin of the polytene chromosomes. The most intensive signals were obtained after hybridization with the simple sequence repeats $(GATA)_4$ followed by $(GACA)_4$, $(CAC)_5$, and $(CA)_8$ in decreasing order.

Detection of $(GATA)_4$ showed a maximum number of 8 sites on six chromosomes. The two most intensive signals of $(GATA)_4$ were located in the centromeric heterochromatin of two medium-sized chromosomes. Four distinct loci could be found in the long euchromatic part of two nucleolus organizing (NO)-chromosomes. Another two sites, with low intensive signals, were located subtelomerically between the euchromatin and the centromeric heterochromatin. The observation that $(GATA)_4$ gave the strongest hybridization signals is consistent with the results of Hamann et al. (1995) where $(GATA)_4$ also produced the strongest hybridization patterns and the most informative fingerprints.

Hybridization with $(GACA)_4$ resulted in 4 sites on four chromosomes which are most likely to coincide with 4 of the 8 $(GATA)_4$-hybridization sites. However, the intensity of $(GACA)_4$ signals in the centromeric heterochromatin were lower than these of $(GATA)_4$, and there were only two $(GACA)_4$-loci within the euchromatin of the two NO-chromosomes. These results are consistent with the observation of Hamann et al. (1995) that almost all fragments containing the $(GACA)_4$-elment hybridized with $(GATA)_4$, but only about 20% of $(GATA)_4$-bands also hybridized with $(GACA)_4$ *and support their conclusion that both elements are arranged in cluster*.

$(CAC)_5$-sites were preferentially located in the centromeric heterochromatin of NO-chromosomes. Two further loci could be detected in the euchromatic part of two NO-chromosomes which were, however, clearly different from the NO-chromosomes hybridizing with $(GATA)_4$ and $(GACA)_4$.

Signals of the consensus sequence of the M13-probe were located on at least 8 chromosomes in centromeric heterochromatin. In contrast, Sonnante et al. (1994) mapped the M13 sequence to the end of two linkage groups and therefore suggested that the M13-sequence is located at the end of chromosomes. However, this contradiction could easily be reconciled by the assumption that both linkage groups have to be combined.

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