Mapping Phaseolin Genes to Polytenic Chromosomes by Fluorescence in situ Hybridization (FISH)

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INTRODUCTION

The karyotype of Phaseolus (2n = 2x = 22) is still under investigations, not at least due to the small size of mitotic chromosomes and the low frequency of cells in mitotic divisions.

To avoid these difficulties an alternative lies in the study of the polytenic chromosomes of the embryo suspensor of Phaseolus coccineus and Phaseolus vulgaris (Nagl, 1974). The major advantage is that they are polyploid due to endoreduplication cycles, which lead to lateral amplification of DNA sequences. Furthermore, polytenic chromosome become 30 times longer than the metaphase chromosomes. Although their length and distribution of heterochromatin can be used for the establishment of a preliminary karyotype, these characteristics show some variability and do not allow the identification of individual chromosomes in any case.

In a new approach we try to establish a karyotype of Phaseolus not based on morphological features but on molecular markers, and therefore called molecular karyotype. Molecular markers in this sense are genes or other DNA sequences which are mapped to chromosomes. To localize these markers they were labeled, hybridized to the chromosomal DNA and detected directly on the chromosome. When fluorescence dyes are used for detection this technique is called fluorescence in situ hybridization (FISH). A disadvantage of FISH was so far, that it could be well used for detection of repetitive genes and DNA sequences, but not for low copy and single copy genes.

In this work we report for the first time the mapping of phaseolin genes (low copy genes) to polytenic chromosomes of Phaseolus coccineus by fluorescence in situ hybridization.

MATERIALS AND METHODS

The embryo suspensors were taken from seeds of unripe pods of Phaseolus coccineus cv. Hammond's Dwarf Scarlet. Preparation of polytenic chromosomes was made from the big basal suspensor cells. Prior to in situ hybridization chromosome preparations were digested with pepsin/HCl to degrade cytoplasmatic and chromosomal proteins.

The clone pTA250 carrying a 8.8kb repeat unit of ribosomal RNA genes (rDNA) from Triticum aestivum var. Chinese Spring (Gerlach and Bedbrook, 1979) was used for test hybridization to monitor the effects of protein degradation.

The DNA probe for mapping phaseolin genes was the 3kb insert of the clone AG-pPVPh 3.0, which includes a 1.9kb coding sequence for β-phaseolin from Phaseolus vulgaris cv. Tendergreen (Slighetom et al., 1983). Probes were labeled with biotin and detected with avidin-FITC (fluorescein isothiocyanat) after in situ hybridization. Signals were amplified with biotinylated antibodies and avidin-FITC.
RESULTS AND DISCUSSION

Chromosome preparations were digested with pepsin/HCl to degrade proteins, in order to increase accessibility for in situ hybridization and detection. After this pretreatment the test system of the rDNA probe showed increased density and number of signals spots. When the same pretreatment was used with the phaseolin probe, we got hybridization signals on two homologous chromosomes. According to their size and the distribution of heterochromatin, they were classified as chromosome no. 7. In a previous report we identified them as no. 3, but the new data support a classification as chromosome no. 7.

This is the first time that low copy genes like those for phaseolin have been localized on polytene chromosomes of plants by fluorescence in situ hybridization (FISH). In general, radioactive in situ hybridization is used for mapping low and single copy genes, but FISH has several advantages, like higher spatial resolution, short detection time, and the possibility to detect several probes on one slide.

Now we are going to map further low copy genes. In combination with the detection of different probes with different fluorochromes, this will allow us to establish a molecular karyotype.

This technique would also be of interest for mapping RFLP markers to chromosomes, and to reduce the current number of fifteen linkage groups (Nodari et al., 1993) to the eleven pairs of chromosomes. So FISH can combine the genetic and the physical map of Phaseolus.

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REFERENCES

Request

Genes and DNA sequences for fluorescence in situ hybridization (FISH)

I am interested in establishing a molecular karyotype of Phaseolus by molecular markers. The ideal molecular markers would be DNA sequences with a length of several kilo base pairs cloned in the plasmid vector pUC19. Sequences with a high copy number in the genome are of special interest.

But also all others would be gratefully accepted.

If you like to provide me with sequences, please contact:

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