GENETICS

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PHYSICAL MAPPING OF PROTEIN-CODING GENES ON ONION CHROMOSOMES (ALLIUM CEPA L.)

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Abstract

We used onion EST clones to develop DNA probes for *in situ* hybridization. We produced DNA probes that carried out introns to reduce background fluorescence. Tyramide-FISH technique was used to increase the detection sensitivity of the small target DNA sequences. Tyramide-FISH mapping of ESTs revealed hybridization signals on onion chromosomes.

Through the integration of genetic and chromosomal maps we were able to analyze the distribution of recombination events along onion chromosomes. The highest recombination frequency was found in the interstitial region. The recombination frequency between the markers located in the proximal region was over 20

times less than between markers located in the interstitial region. Uneven distribution of recombination frequencies along the chromosome has been reported by a large number of studies, such as in wheat, barley, maize, and the Alliums.

Key words: Tyramide-FISH, EST, recombination.

Introduction

Integrated genetic maps use data from classical and molecular genetics and genomics. Integrated recombination and physical maps have been created for several important crops. The most direct way to integrate genetic and physical maps is to use FISH to localize genetically mapped markers onto chromosomes. Expressed sequence tags (ESTs) are attractive candidates for chromosomal gene mapping because they possesses protein-coding sequences and often do not contain dispersed repetitive DNA sequences that may complicate FISH signals. Numerous approaches have been tried to reliably detect shorter single-copy DNA sequences on plant chromosomes [1]. One of these approaches is based on peroxidase-mediated deposition of fluorochrome-labeled tyramides onto plant chromosomes [2]. The Tyramide-FISH technique combines the advantage of an enzymatic procedure that provides signal amplification due to the deposition of many substrate molecules, with that of fluorescence-based detection which has higher absorbency than that used in enzymatic detection [3; 4]. Tyramide-FISH method has been applied to plant cytogenetics and revealed the positions of T-DNA inserts as small as 710 bp on condensed metaphase chromosomes of transgenic shallots [2].

Materials and Methods

Plant material and chromosome preparation

Seeds of onion variety ‘Khalcedon’ were germinated on moist filter paper for 72 hours at 25° C. For cell cycle synchronization, the seedlings were incubated in 0.75 mM hydroxyurea for 24 hours at 25 ° C and then transferred to water for 4 hours at 25 ° C. For metaphase arresting, the seedlings were treated in a chamber with nitrous oxide for 2 hours. The seedlings were fixed in ethanol : acetic acid (3:1) at room temperature for 30 minutes and stored overnight at - 20 ° C. Preparation of chromosomes slides were performed as described by Kirov et al. [5].

DNA probes and labeling.

Synthesis and cloning of onion cDNAs and genetic mapping as RFLPs have been described by King et al. [6]. Plasmid DNA isolation was performed by GeneJET Plasmid Miniprep Kit

(Fermentas, USA). DNA labeling was performed by nick translation with plasmid DNA using DIG-Nick Translation Mix (Roche, Germany).

Fluorescence in situ hybridization (Tyramide-FISH).

Pre-hybridization treatment and hybridizations were performed as described by Kuipers et al. [7]. Tyramide-detection was performed as described by Khrustaleva and Kik [2] modified by the use of 0.01% HCl for 8 minutes to inactivate endogenous peroxidase.

Microscopy and image analysis.

Chromosome preparations were viewed by a Zeiss AxioImager M1 microscope with filters to detect DAPI and FITC, ultraviolet lamp HBO 100 W, and digital camera AxioCam. Multichannel fluorescence recording, images processing for brightness/contrast and color settings were performed by the program AxioVision v.4.6. The chromosome morphometric analysis was performed by the program MicroMeasure version 3.2.

Results

EST-clone mapping

The insert size of EST-clones was from 750 bp to 3000 bp. Tyramide-FISH of the EST clones revealed hybridization signals on different onion chromosomes.

Relationship between genetic and physical distances on onion chromosome 5

Through the integration of physical positions of genetic markers we were able to estimate the relationship between genetic and physical distances along onion chromosomes. The average degree of compaction of an onion chromosome has been estimated at approximately 250 Mb/μm [2]. Different regions corresponds from high to low frequency of recombination.

Discussion

We demonstrated the efficacy of chromosomal *in situ* mapping of ESTs for the extremely large genome of onion. We were able to visualize relatively small target DNA sequences on compacted onion metaphase chromosomes. Unlike genetic mapping, chromosomal mapping reveals the physical location of markers on chromosomes. However with chromosomal mapping it is not possible to distinguish between two sequences that have more than 80% homology; while genetic markers like SNPs may differ for only one nucleotide and can be genetically mapped.

The relationship between genetic and physical distance for the entire onion genome, based on a genome size of 16,415 Mb and a genetic map of 1,907 cM [8], is about 8.6 Mb/cM. This is a large average physical distance per centimorgan as compared to other species such as wheat (5.5 Mb/cM) [9], rice (244 kb/cM) [10], or tomato (750 kb/cM) [11].

Combination of bioinformatics resources and Tyramide-FISH mapping, integration of the physical and genetic maps will aid in the map-based cloning of genes conditioning important traits. Also the results of this work may assist in future sequencing of the onion genome.

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