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PLANT GENOME MAPPING

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Summary

Genome mapping is the term used to create a set of ordered fragments of a particular genome. A genome map describes the order of genes or other markers and the spacing between them on each chromosome. The genome maps are constructed on several different scales or levels of resolution. At the coarsest resolution are genetic linkage maps, which depict the relative chromosomal locations of DNA markers (genes and other identifiable DNA sequences) by their patterns of inheritance. Physical maps (chromosomal map, cDNA map, contig map, macrorestriction map, sequence map) describe the chemical characteristics of the DNA molecule itself.

Key words: genome mapping, genetic map, physical maps

Introduction

Plant cells contain genetic information within nuclei, chloroplasts and mitochondria. In all of these cell organelles the genetic information is encoded within molecules of deoxyribonucleic acid (DNA). The nuclei of plant cells contain various linear DNA molecules, the number and length of which vary between different plant species. The chloroplasts and the mitochondria also contain DNA but in the form of circular molecules. Most of the plants genetic information is contained of the nucleus. Each nucleus contains sets of chromosomes (large molecules of DNA) composed of the genes. This set of genetic information is known as the nuclear genomes. The nuclear genomes of different plant species contain different amounts of DNA.
Genome mapping is the term used to create a set of ordered fragments of a particular genome. Mapping involves dividing the chromosomes into smaller fragments that can be propagated and characterized and mapping them to correspond to their respective location on the chromosomes. The base sequence of each of the ordered DNA fragments are determined. The goals of plant genome analysis is (a) to find all the genes in the DNA sequence of particular plant species, (b) to develop tools for using this information in the study of biology and agriculture and (c) automating methods to extract the maximum useful information from maps and sequences (http://www.ort.edu.uy/REDOC/maphugen.htm, 2001).
A genome map describes the order of genes or other markers and the spacing between on each chromosome. Genome maps are divided into two general types (a) genetic linkage maps and (b) physical maps. Genetic linkage maps or phenotype maps shows the relative chromosomal location of DNA markers (genes and other identifiable sequences) by their patterns of inheritance. Physical maps describe the chemical characteristics of the DNA molecule itself (http://www.ort.edu.uy/REDOC/maphugen.htm, 2001).

Genetic mapping methods review

Genetic linkage maps

A genetic linkage map shows a relative locations of specific DNA markers along the chromosome. Any inherited physical or molecular characteristic that differs among individuals and is easily detectable in the laboratory is a potential genetic marker. Markers can be expressed DNA region (genes) or DNA segment that have no known coding function but whose inheritance pattern can be followed. DNA sequence differences are especially useful markers because they are plentiful and easy to characterize precisely (http://www.ort. edu.uy/REDOC/maphugen.htm, 2001).
On the genetic map, distances between markers are measured in the terms of centimorgans (cM), named after the American geneticist Thomas Hunt Morgan. Two markers are said to be 1 cM apart, if they are separated by recombination 1 % of time. A genetic distance of 1 cM is roughly equal to a physical distance of 1 million bp (1Mb).
Three kinds of genetic maps are available in *Arabidopsis* genetic map: (1) classical, (2) RI and (3) mi-RFLP.
The classical map was originally built using genetic distances based on recombination frequencies between visible markers. Other mutant genes were later cloned and placed on the recombinant inbred (RI) map or assigned a position relative to molecular markers on the RI and / or physical maps. The major function of the classical genetic maps is to show the relative locations of genes identified by mutation. The Recombinant Inbred (RI) lines were generated from a cross of *Arabidopsis* ecotypes Columbia and *Landsberg erecta*.
The RI map was constructed using a „framework" of markers that act as a consistent backbone for the map. 129 *Arabidopsis thaliana* RFLP (restriction fragment length polymorphisms) markers were established based upon DNA fragments cloned in the pUC119 plasmid vector and insert end sequences of P1 clones. These markers are useful for map-based gene isolation

and genome physical mapping in *Arabidopsis thaliana* as well as studies of chromosome colinearity with related species (http://www.arabidopsis.org/map Viewer/help /mapkey2a.htm, 2001).
Genetic maps may consist of actual gene coding regions, phenotypic traits, and molecular markers that do not encode genes but serve as landmarks, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers (http://bldg6.arsusda.gov/benlab/soybean_mapping.htm, 2001).
The soybean genetic linkage map contains over 200 RFLP, SSR, AFLP, RAPD and phenotypic markers. The map encompasses over 1200 cM of the soybean genome. Many RFLP markers placed on the map represent cDNAs of known function (http://bldg6.arsusda. gov/benlab/genetic_map.html, 2001).

Physical maps

Different types of physical maps vary in their degree of resolution. The lowest-resolution physical map is a **chromosomal** (cytogenetic) map. Higher resolution provides a **cDNA map**, which shows the locations of expressed DNA regions (exons) on the chromosomal map. More detailed cosmid **contig map** depicts the order of overlapping DNA fragments spanning the genome. A **macrorestriction map** describes the order and distance between enzyme cutting sites. The highest-resolution physical map is the complete elucidation of the DNA base-pair sequence (**sequence map**) of each chromosome in the genome (http://www. ort.edu.uy/REDOC/maphugen.htm, 2001).
In a **chromosomal map**, genes or other identifiable DNA fragments are assigned to their respective chromosomes. These DNA fragments can be physically associated with particular bands (identified by cytogenetic staining) primarily by *in situ* hybridization, a technique that involves tagging the DNA fragment with an observable label. The location of the labeled probe can be detect after it binds to its complementary DNA strand in an intact chromosome.
A **cDNA map** shows the position of expressed DNA regions (exons) relative to particular chromosome or bands. The cDNA molecule is synthesized in the laboratory using the mRNA molecule as a template. A cDNA map provide the chromosomal location for genes.
The result of top-down mapping is a **macrosrestriction map**. In this mapping, a single chromosome is cut (with rare-cutter restriction enzyme) into large pieces, which are ordered and subdivided. The smaller pieces are then mapped further. The resulting macrorestriction maps depict the order of a distance between sites at which rare-cutter enzyme cleave. This approach yields maps with more continuity and fewer gaps between fragments than contig maps. The map resolution is lower and may not be useful in finding particular genes. In addition, this strategy generally does not produce long stretches of mapped sites. This approach allows DNA fragments to be located in regions measuring about 100 000 bp to 1 Mb.
The result of bottom-up mapping is a **contig map**. The bottom-up approach involves cutting a chromosome into small pieces, each of which is cloned and ordered. The ordered fragments form contigous DNA block (contigs). Currently, the resulting library of clones varies in size from 10 000 bp to 1 Mb. An advantage of this approach is the accessibility of these stable clones to other researchers. Contig construction can be verified by FISH method, which localizes cosmid to specific regions within chromosomal bands (http://www.nalsuda. gov/pgdic/tutorial/lesson8.htm, 2001).
The ultimate physical map is the complete DNA sequence determination (**sequence map**) of all base pair on each chromosome. This map will be formed by assembling contigs of at least 2 Mb in length with sequence-tagged sites (STS) spaces every 100 kb. A STS is a 100 – 1000 nucleotide sequence with three characteristics: (1) unique in the genome, (2) identifies a mapped element and (3) amplifiable by the polymerase chain reaction. STS can be used to identify clones to generate overlapping inserts for a physical map. STS can also be used as a correspondence points between genetic and physical maps (http://www.npac.syr.edu/users/ houle/GeneticMaps.html, 2001).

Conclusion

Linkage maps are important for genetic analysis, breeding and population studies. By knowing the locations of a particular gene in reference to other genes and markers, the breeders can devise strategies for developing new crop varieties. Linkage analysis using polymorphic DNA markers has expanded our knowledge of genomes tremendously and has allowed quantitative traits to be linked with molecular markers (QTL – quantitative trait loci). Molecular linkages to important traits also provide footholds for the molecular biologists to clone genes by map position. For example, specific genes encoding economically important phenotypes, such as resistance to fungi, insects, bacteria etc., could be cloned by without having knowledge of the product encoded gene, but just by knowing the location of the phenotype on the map.

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THE TESTING OF EFFICACY OF CHA GENESIS® FOR PRODUCTION OF HYBRID WHEAT AND HYBRID TRITICALE

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Summary

The objectives of the joint project "Hybrid Wheat and Hybrid Triticale Research in the Czech Republic" conducted by Monsanto Company (USA) and the Agricultural Research Institute Kroměříž, Ltd. were to evaluate the efficacy of the CHA Genesis under climatic and soil conditions of the Czech Republic using potential parent components of wheat and triticale, to assess an efective Genesis dose and produce a necessary amount of experimental hybrid wheat and hybrid triticale seed for yield trials. Evaluating the yield of tested wheat parental components, significant differences were found between individual variants of treatment. If the percentages of sterility and hybrids using the technique of bagging were assessed, significant differences in all doses in relation to the untreated check and significant differences between the dose of 1.6 kg.ha^{-1} Genesis to both increased doses were calculated. Insignificant differences were found between the dose of 3.6 and 4.9 kg.ha^{-1} Genesis. If the percentage of hybrids was assessed by electrophoresis of storage proteins, significant differences were also calculated between the dose 3.6 and 4.9 kg.ha^{-1} Genesis. Based on the obtained results, it is possible to conclude the CHA Genesis dose of 4.9 kg.ha^{-1} appeared sufficient to induce nearly 100% (99.5% in wheat and 96.7% in triticale) male sterility of female plants under investigated soil and climatic conditions in the Czech Republic.

Key words: chemical hybridising agent Genesis, wheat, triticale, male sterility, hybrid purity, electrophoresis

Introduction

Chemical hybridising agents (CHA) are chemical substances that are used to induce male sterility in female plants. The possible use of CHAs has been investigated in at least 40 species, including all the major cereales of the world and several other crops of great economic importance (Pickett, 1993). CHAs prevent from normal production of pollen at keeping female fertility which enables to produce hybrid seed and to overcome a lot of problems associated with exploitation of cytoplasmic male sterility (Nesvadba et al., 1998). Great experience with utilization of CHA and production of hybrid wheat has been obtained in the USA and France. On the basis of Genesis, the first wheat hybrids of the Quantum series in hard and soft red wheats were developed and released to farmers for commercial production in the USA in 1996. In France, six hybrid wheats (Cockpit, Cabestan, Domino, Mercury, Sextant and Twin) have been registered since 1995. In Germany the first hybrid wheat Hybnos 1 from Nordsaat - Saatzucht was registred in a List of Registered Varieties in 1999 (Nesvadba, Vyhnánek, 2001).
This paper presents the results of the yield evaluation, male sterility and hybrid purity of twenty varieties and lines of wheat and five genotypes of triticale produced using Genesis compound.

Materials and methods

Genesis is a chemical agent developed by Monsanto (USA) for HybriTech for wheat hybridization. Genesis contains 244 g.l^{-1} active ingredient clofencet [2-(4-chlorphenyl)-3-ethyl-2,5-dihydro-5-oxopyridazine-4-karboxylate, potassium salt]; C13H10N2O3ClK (Fig. 1).
Clofencet is a systemic product which is translocated in wheat from leaves to flowers. It is in accordance with development of anthers when it sterilizes pollen grains (Fichet and Adams, 1996).
The experiments in both years were established in the fields of the Agricultural Research Institute Kroměříž, Ltd. which is located in the mild dry area of the warmer sugar beet-growing region with mild winter (a long-term annual sum of rainfall is 599 mm and average daily temperature is 8.7 °C). The altitude of experimental fields is 210 to 220 m. The soil is Luvi-haplic Chernozem with pH 6.1- 6.8.