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RFLP and RAPD mapping in flax (*Linum usitatissimum*)

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Abstract A map of flax (*Linum usitatissimum*) using restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs), and comprising 15 linkage groups containing 94 markers, has been developed covering about 1000 cM. The mapping populations were the F_2 populations from two crosses between diverse cultivars. From one cross, CI1303 and Stormont Cirrus, 20 RFLP and 520 RAPD markers were analyzed. Thirteen RFLP and 80 RAPD markers were on the 15 linkage groups, in addition to one sequence-tagged site (STS). Seven polymorphic RAPD markers were found to have unusual segregation patterns. RAPDs were expressed as dominant markers, but for these markers a prevalence of the progeny lacked a band rather than the expected one-fourth ratio. However, these exceptions may be related to the instability of the genome of Stormont Cirrus in which stable and heritable genomic changes can be induced by environmental factors. The current map could be used for the identification of markers linked to loci controlling the ability to generate heritable changes in response to environmental growth conditions, and to develop anchor loci with STSs for a more general application.

Key words RFLPs · RAPDs · Flax · Linkage map

Introduction

The advent of DNA markers has greatly increased the ease and speed with which the genetic map of a species can be obtained. Thus, all of the opportunities afforded by the existence of a genetic map in any species are more readily available. The genetic maps developed using DNA markers have been used to isolate genes, identify quantitative trait loci (Patterson et al. 1988) and aid in marker-assisted breeding (Schoemaker et al. 1992). Two of the most-used DNA markers are restriction fragment length polymorphisms (RFLPs) (Botstein and Lander 1990) and random amplified polymorphic DNAs (RAPDs) (Williams et al. 1990).

A number of genes have been described in flax (Beard and Comstock 1965; Gorman et al. 1993), but no systematic efforts have been made to determine the linkage relationships between them. Linkage relationships between loci for rust resistance have been determined (Flor 1962), and some of these genes have been isolated (Lawrence et al. 1995). However, this data has not been placed onto a more general linkage map. Flax has a small genome in terms of the total DNA content per 2C nucleus, and this DNA is packaged into 15 pairs of small chromosomes, all of which are of about the same size (Schweitzer 1979). The genes for the large ribosomal RNAs have been localized and reside on a single chromosome making up at least half of that chromosome (Creissen and Cullis 1987). The 5S ribosomal RNA genes also have been shown to be distributed among most members of the chromosome complement (Schneeberger et al. 1989).

The primary aim in generating the present map was to identify the genes responsible for controlling the ability of some flax varieties to respond to environmental cues by generating heritable genetic changes (Durrant 1962; Cullis 1976). Therefore one of the pairs of parents employed was (Stormont Cirrus (a responding line) and a stable line CI1303 (Dybing 1970). A secondary aim was to begin the development of a set of markers that could be used on any germplasm without the process of generating a map for each new cultivar or accession being introduced. In particular, a series of anchor loci that appear to be descriptive of a particular chromosome are being developed. Subsequent use of these anchor loci will enable the rapid identification of the linkage group of any

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new gene as well as the identification of any chromosomal rearrangements.

Materials and methods

Plant material and DNA extraction

Four lines were used in the generation of the map. Stormont Cirrus and CI1303 were used as the parents for one cross and Koto and Leona as the parents for the other. At least 50 individuals from each segregating F_2 family were employed in the mapping studies. DNA was extracted as described by Cullis (1976).

Restriction fragment length polymorphism (RFLP) analysis

The detection of RFLPs was done as described elsewhere (Schneeberger and Cullis 1991). Known genes and random *Pst*I clones isolated in the vector pBluescript (Stratagene) were used as probes.

Random amplified polymorphic DNA (RAPD) and linkage analysis

Conditions for the RAPD analysis were modified from original methods (Aldrich and Cullis 1993). For the mapping, DNA from parents and about 60 progenies from the cross between Stormont Cirrus and CI1303 were used as templates at a final concentration of 500 pg per reaction. Five hundred and twenty different 10-mer sequences were used in the initial screening. These are the sets 101–300 and 401–500 from the University of British Columbia, and 20 sequences of each "Kit A" through "Kit K" from Operon Technologies, Inc. The enzymes used for RAPD analysis were either Ampli*Taq* DNA Polymerase, the Stoffel fragment or Klen*Taq*. The amplification program was a cycle of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C, with a final 7 min at 72°C. The linkage analysis was performed by the use of the programs, LINKAGE-1, version 3.50, (Suiter et al. 1983) and LINKEM, version 1.2, (Vowden et al. 1995).

Gel-electrophoresis, Southern transfer and hybridization

The detection of RFLPs (Schneeberger and Cullis 1991) was done with DNAs digested with one of four enzymes, *Eco*RI, *Bam*HI, *Hin*dIII or *Eco*RV. Two to three micrograms of genomic DNA were digested with the appropriate enzyme and the fragments separated on 1.0% vertical agarose gel using Tris-acetate buffer. For RAPD analysis PCR products were electrophoresed in 2.0% agarose gels. The fragments were transferred to Nytran membranes after denaturation and neutralization, and fixed by UV cross-linking. The probes were labeled using the oligolabeling technique (Fineberg and Vogelstein 1984), and hybridized at 65°C in 5×SSC for 18 h. The filters were washed twice in 2×SSC for 15 min and then twice in 0.5×SSC for 15 min at 65°C. The filters were either autoradiographed for 5–15 days or processed with a nonradioactive system (Gene images, Amersham). The filters were stripped by boiling in 10 mM Tris, pH 9.0 for 15 min. Filters were re-used up to a maximum of 15 times.

Results

The *Pst*I clones, as well as known genes, were used to detect polymorphisms. Those currently on the map include the site of the ribosomal RNA genes (rDNA), a specific subset of the 5S rRNA (15s1, 15s2, 15s3) genes, two ubiquitin loci (Vub1, Vub2), four random *Pst*I clones generating six polymorphisms (I108, I109, I137, I941, I943) and one single-copy sequence (I3.8). Three RFLP polymorphisms (I941, I942, I943) are shown in Fig. 1. These three polymorphisms map to two linkage groups (6F and 7F). The RFLPs are located on five of the linkage groups. In addition one sequenced-tagged site (Y6.2) has also been placed on the map. Seven RFLP loci were not placed on the map. The segregation patterns were skewed towards a larger number of progeny containing the polymorphic band and the mapping programs placed them in more than one linkage group. The RAPD patterns for 520 random primers were analyzed. Ninety six polymorphic bands were observed, 80 of which were analyzed to develop the 15 linkage groups. An example of segregating RAPD markers is represented in Fig. 2. Bands for seven of these polymorphisms were skewed towards the absence of the band in an exceptionally high proportion of the F_2 progeny. Because all RAPDs express as dominant markers, it would be expected that the average ratio of presence to absence would be 3:1. In addition nine of the polymorphisms were indistinct and difficult to score, and so have not been included in the data.

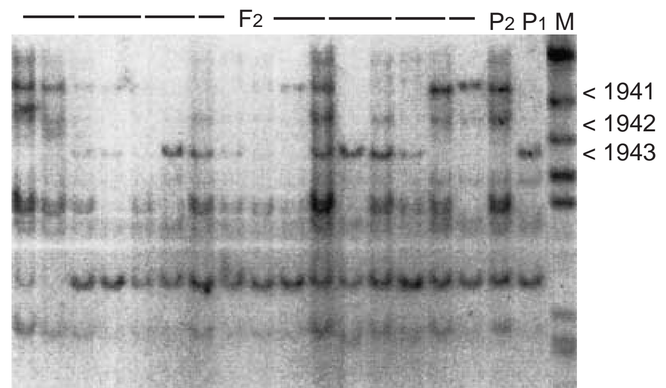

Fig. 1 A Southern blot of I941, I942, and I943 as RFLP markers in the parents and their segregation in 16 F_2s. The first lane (M) from the right is a mixture of lambda DNA digested with *Hin*dIII and the double digest with *Hin*dIII and *Eco*RI. The *arrows* indicate the polymorphic fragments. The I941 and I942 loci are mapped on 6F, and I943 on 7F

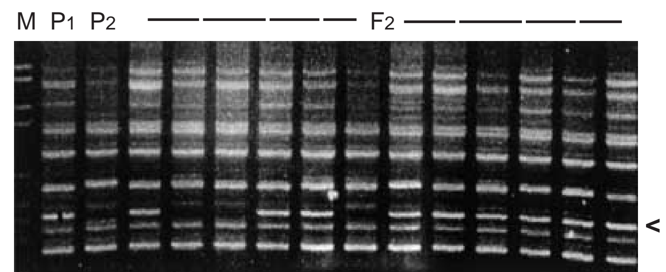

Fig. 2 Polymorphism of a 151 in the parents and its segregation in 12 F_2s. The first lane (*M*) from the left is DNA MWM VI (Boehringer Mannheim). This locus is mapped on linkage group 3F as shown in Fig. 3

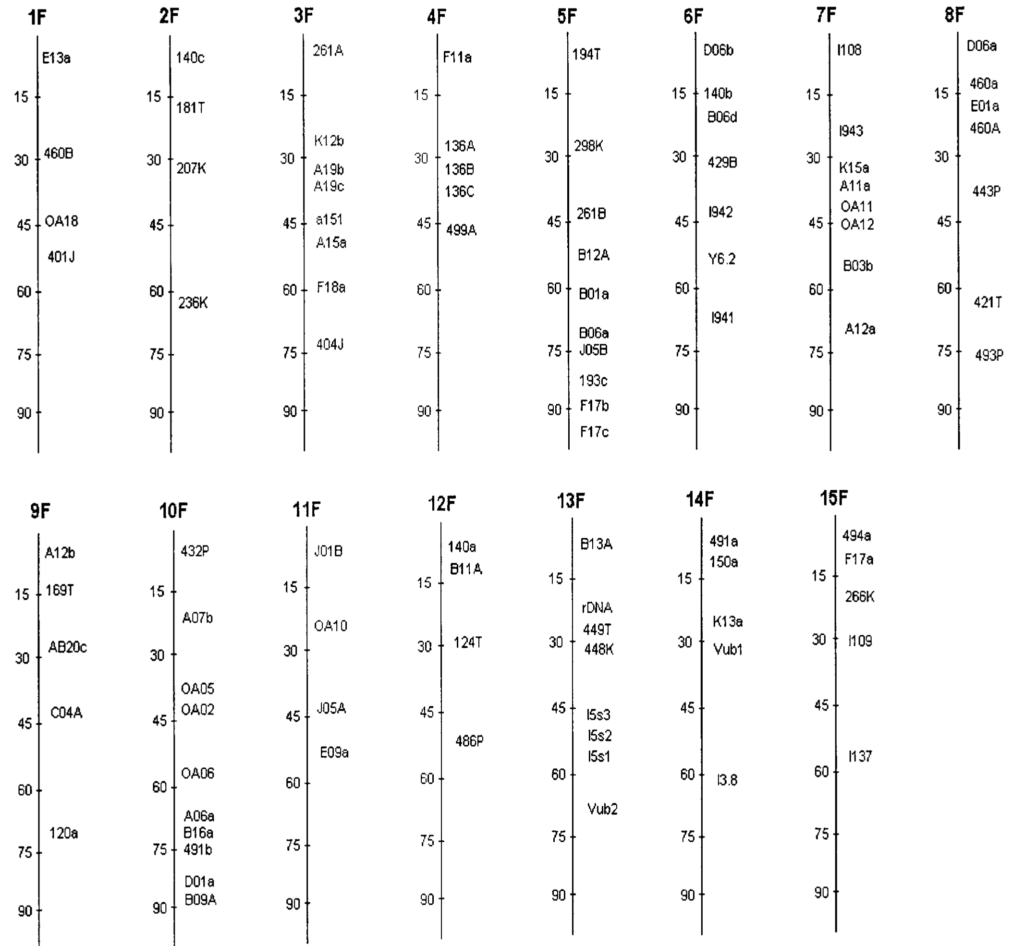

Fig. 3 Composite map of the flax genome. Three types of markers are placed on this map. RFLP markers are designated Ixxx (for polymorphisms detected in *Eco*RI digests), Vxxx (for polymorphisms detected in *Eco*RV digests), and rDNA (for the ribosomal RNA gene locus). The single sequence-tagged site is designated Y6.2. RAPD polymorphisms were designated according to the primer used and the band identified; for example, E13a is the 'a' band identified using Operon primer E13

The 5S ribosomal DNA clone pRS20.7 (Schneeberger and Cullis 1991) was the probe used to generate the group of polymorphisms 15s1, 15s2 and 15s3 mapped to linkage group 13F. This subset of the 5S ribosomal RNA genes appears to be at a single site with about 500 copies per haploid genome. The three polymorphisms are separated by 2.6 cM, and the repeat unit is 706 bp. Therefore, the relationship between map distance and physical distance can be estimated as about 150000 (500×706/2.6) bp per cM. Pulsed-field gel electrophoresis has placed this locus as smaller than 2 Mg (Schneeberger 1992), but it has not been reduced further.

The current map of the 15 linkage groups covering about 1000 cM is shown in Fig. 3.

Discussion

The genetic length of the map reported here is close to 1000 cM. The organization of the flax genome includes about 30% of highly repeated tandemly arrayed sequences. There are two possible effects of these tandem arrays. The genetic distances may be expanded compared with the physical distances by the presence of tandem arrays if recombination rates are increased by their presence. However, if recombination is suppressed across these arrays, the genetic distances will be reduced with respect to the physical distance. The only estimate for the relationship between physical and genetic distance was made from a subset of the 5S ribosomal RNA genes.

The *Pst*I clones were hybridized across a series of 25 flax varieties. Twenty different inserts from the first 100 clones tested demonstrated polymorphisms with one or more restriction enzymes across this germplasm. Twelve of these polymorphisms were segregating in the P1×CI1303 population used in these experiments. The six polymorphic bands that have not been placed on the map may be present in multiple copies and therefore cannot be unambiguously assigned. Since flax appears to be an ancient tetraploid, it is possible that these are loci that have not diverged sufficiently to be distinguished and therefore cannot be mapped at present.

RAPD polymorphisms have been characterized between flax genotrophs (Cullis et al. 1999). These results confirmed the occurrence of DNA variations when stable lines were derived from Stormont Cirrus by growth in particular environments. All RAPD bands that segregated in an anomalous fashion in the Stormont Cirrus×CI1303 F_2 were also shown to be variable when heritable environmentally induced DNA changes were generated in the variety Stormont Cirrus. In every instance, the polymorphism was generated by the presence of a

band in Stormont Cirrus and its absence in a genotroph. Therefore, if the individuals in the mapping population were still susceptible to heritable, environmental variation, then the aberrant segregations could be a result of this process. That is, prior to the isolation of DNA from the F_2 plants, a change in the genome of some of these individuals resulted in the loss of a RAPD band altering the observed ratio from that expected. However, more evidence is necessary to demonstrate that this hypothesis is in fact the basis for the aberrant segregation patterns.

The linkage map described here obviously has limited use for other varieties or crosses because the RAPD polymorphisms are not immediately transferable to other varieties. The use of the map will be expanded by developing a series of sequence tagged sites (STSs) for each of the linkage groups.

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