Inferences of relationships in the genus Hordeum by molecular analysis of repetitive DNA
by Maria del Mar Sanchez Garcia

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University

Abstract:
Five dispersed middle-repetitive elements were selected from a barley (Hordeum vulaare L.) random genomic library. Their genus specificity, nuclear origin, and chromosomal distribution was determined. Elements were characterized by restriction analyses, and sequence data was obtained. The objective of this project was to compare five different families of repeated sequences and to assess the extent of variation in organization of these repetitive DNA families across twenty-three Hordeum species during the evolution of the genus.

The distribution of five repetitive elements in the barley genome and variation in their structure across species of the genus Hordeum was evaluated.

Selected clones were used to probe Hordeum species genomes for the presence of interspecies polymorphic hybridization patterns. Variability in repeated sequence organization was found to be . considerably higher between different sections (according to Bothmer and Jacobsen 1985) than among species of the same section.

Southern blot hybridization data were included in a cladistic analysis that revealed relationships among species that were compatible with the four sections of the genus described by Bothmer and Jacobsen in 1985: section Hordeum. section Anisolepis, section Critesion, and section Stenostachys. A further clustering of H. vulgare and H. bulbosum in a separated subsection within the Hordeum section may be suggested by our results.

Partial sequence data was used to design five different sets of primers that amplified the corresponding sequences in samples of DNA from twenty-three Hordeum species via the polymerase chain reaction. Polymorphic amplification patterns were observed at interspecific and intraspecific levels for different species/primer combinations. Cladograms produced from total PCR data analyses were compared to those produced when only fragments that were monomorphic within species were considered or when alloploid taxa were excluded from the analysis. Monomorphisms proved useful for delineating taxonomic sections and species of alloploid origin were found to increase homoplasy in the analyses.

INFERENCES OF RELATIONSHIPS IN THE GENUS *HORDEUM* BY MOLECULAR ANALYSIS OF REPETITIVE DNA

by

María del Mar Sánchez García

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APPROVAL

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María del Mar Sánchez García

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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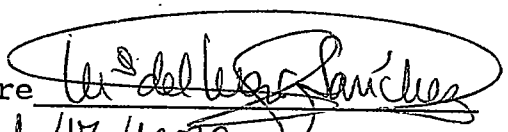
Signature 

Date 11/17/1989

To my husband Antonio Tomás

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CHAPTER 1

INTRODUCTION

Approximately 75% of the barley genome consists of families of repeated DNA sequences. A small proportion of these sequences may include essential coding sequences, but the functions, if any, of most of them remain enigmatic.

The classification of species in the genus Hordeum is still a matter of debate. The purpose of these studies was to elucidate whether or not the reported classification of species in the genus is supported by molecular analyses of dispersed middle repetitive DNA and to provide evidence for the mechanisms by which repetitive DNA becomes reorganized during Hordeum genome evolution.

The study began with the selection of five barley specific repetitive sequences and their characterization by restriction mapping and nucleotide sequencing. Subsequently the study considers the organization and evolution of families of repeated sequences across twenty Hordeum species and the utilization of Southern blot information to construct cladograms of relationships among species. The final part of this study focused on DNA amplification via the polymerase chain reaction.

CHAPTER 2

SELECTION AND CHARACTERIZATION OF REPETITIVE ELEMENTS

Introduction

Repeated sequences constitute much of the chromosomal DNA of higher eukaryotes. A large body of sequence information about repetitive fractions of DNA found in animal genomes is available at present (Jelinek 1982). No single unifying description of their arrangement has been proposed. However, repetitive sequences are found organized either in clusters, for instance satellites, or in a dispersed fashion. A number of possible functions has been suggested for these sequences, including involvement in recombination or replication processes. However, no direct evidence concerning their biological significance has been presented.

Higher plant genomes have been traditionally analyzed by DNA-reassociation kinetics (Sorenson 1984). In all plant species studied so far, reassociation experiments have demonstrated the presence of repetitive fractions which may comprise as much as 70 % of the plant cell DNA.

Amplification of repetitive DNA followed by sequence rearrangements occurs during evolution and enables closely related species to be distinguished on the basis of their repeated sequence DNA complements (Flavell et al. 1977).

The objectives of this part of the study were to select barley-specific nuclear repetitive DNA clones from a barley random genomic DNA library and to characterize them by restriction mapping analysis and DNA sequencing.

Materials and Methods

Selection of Clones

A barley genomic library previously constructed by Dr. Chao in the phage vector EMBL4 (Frischauf et al. 1983) using total DNA from the barley cultivar "Betzes" was utilized. Phage clones were randomly selected and amplified using methods described by Maniatis et al. (1982). DNA from each clone was digested with EcoRI according to manufacturer's instructions and fragments separated by electrophoresis in 0.8% agarose gels at 2V/cm overnight.

Gels were stained with ethidium bromide and photographed under UV light. The DNA was transferred to Zeta-Probe membranes (Reed 1985). Filters were hybridized with total DNA from barley, wheat and rye which had been radioactively labeled by nick-translation (Rigby et al. 1977). Repetitive and mid-repetitive barley DNA inserts were identified as those which bound large amounts of the total barley DNA probe and low or undetectable amounts of the wheat and rye probes (Fig. 1).

Elucidation of the Nature of Cloned Elements

Selected fragments were subcloned into the plasmid vector pBR325, radioactively labeled by nick-translation (Rigby et al. 1977) and utilized as probes against filters containing barley, rye and wheat DNAs digested with BamHI, HindIII, DraI and EcoRV, to verify their barley specificity.

Barley chloroplast and mitochondrial DNAs were isolated (Vedel et al. 1980) and digested with several restriction endonucleases. DNA was separated by gel electrophoresis, Southern transferred and probed with pBR325 clones to determine whether the repetitive elements were nuclear or cytoplasmic in origin.

Restriction Mapping

Repetitive elements were characterized by restriction mapping using about 500 ng of plasmid DNA and 12 different restriction endonucleases either singly or in combination. DNA fragments were separated in 0.8% agarose gels, stained by ethidium bromide, and photographed. The size of each fragment was determined using the HindIII digested fragments of the phage Lambda as standards.

Sequence Analysis

Subfragments of the clones were digested according to their restriction maps and cloned into the sequencing vector pIBI30. Partial nucleotide sequence was obtained using the chain termination method of Sanger et al. (1977).

Results and Discussion

Selection of Repetitive Clones

Approximately 200 genomic clones were screened from the EMBL4 library. Ten were selected as containing barley specific repetitive elements. The selection strategy is illustrated in Fig. 1. Inserts that bound a high amount of barley genomic DNA but low or undetectable amounts of the rye and wheat probes were considered to contain barley-specific repetitive DNA. The goal of this study was to determine whether it was possible to relate taxa at the species level using dispersed repetitive DNA elements. Also, comparative analysis of rapidly evolving repetitive sequences might provide useful information on mechanisms by which repetitive DNA elements have diverged in the course of evolution of the genus Hordeum. For this reason barley-specific repetitive clones were selected for this study.

Selected inserts were subcloned into pBR325 and hybridized to filters containing digested barley, wheat and rye DNA to verify the barley-specificity of the clones. No or very little signal was detected for each of the selected clones against either wheat or rye DNA (Fig. 2). Five subclones were identified as containing nuclear repetitive elements when chloroplast, mitochondrial, and total DNA were extracted from barley and probed with the selected clones. Sizes of inserts ranged from 2.26 kbp to 5.50 kbp.

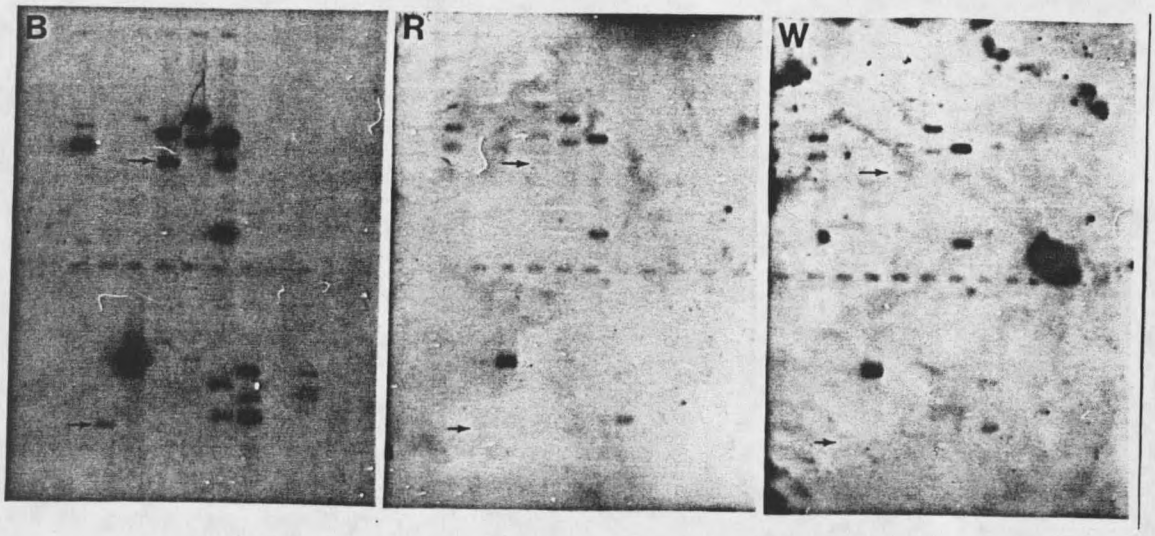

Figure 1. Repetitive element selection strategy. EMBL4 clones containing sequences that bound high amounts of barley total DNA (B) but low or undetectable amounts of either rye (R) or wheat (W) total DNA (arrows) were selected for further subcloning and use as probes.

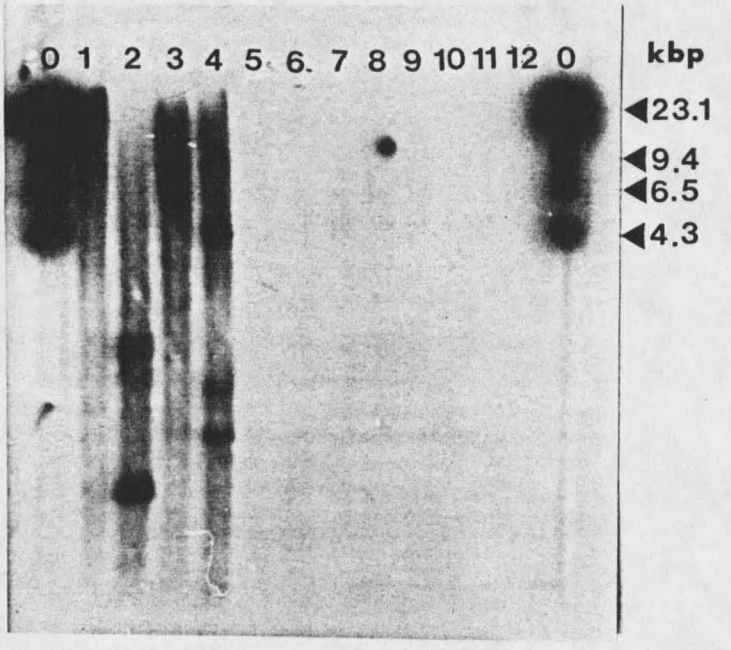

Figure 2. Barley specificity of repetitive elements. Filters contained barley (lanes 1-4), rye (lanes 5-8) and wheat (lanes 9-12) total genomic DNAs digested with BamHI (lanes 1, 5 and 9), HindIII (lanes 2, 6 and 10), DraI (lanes 3, 7 and 11) and EcoRV (lanes 4, 8 and 12) restriction endonucleases, and probed with element #25.

Physical Maps of Repetitive Elements

Physical restriction maps for the five repetitive clones are shown in Figure 3. Fragments smaller than 200 base pairs were not detected on the gels. An apparent lack of internal repetitive structure of restriction sites is observed in the maps of the clones except, perhaps, for clone #44 where HindIII sites seem to be associated with RsaI sites.

Sequence of Subfragments of Repetitive Elements

Sequenced fragments averaged 250 bp. A total of 2550 base pairs were sequenced, representing 13 % of the total sequence of the clones (Table 1). Locations of sequenced fragments are shown by double arrowed lines under the map of each clone (Fig. 3).

Computer analysis of the partial sequence data performed using Genepro (Riverside Sci. Enterprises 1988) did not reveal any simple repetition pattern within the restriction fragments studied. There were, however, several 6 to 10 base pair long direct repeats. Table 2 lists direct repeats at least six bp long where repeated sequences share more than 80 % homology. The two 6 bp long sequences reported here were perfect repeats; the other direct repeats were longer than 6 bp. Nineteen of the 44 short nucleotide sequences listed in Table 2 were found to be repeated in more than one clone.

Short direct repeats could have resulted from the

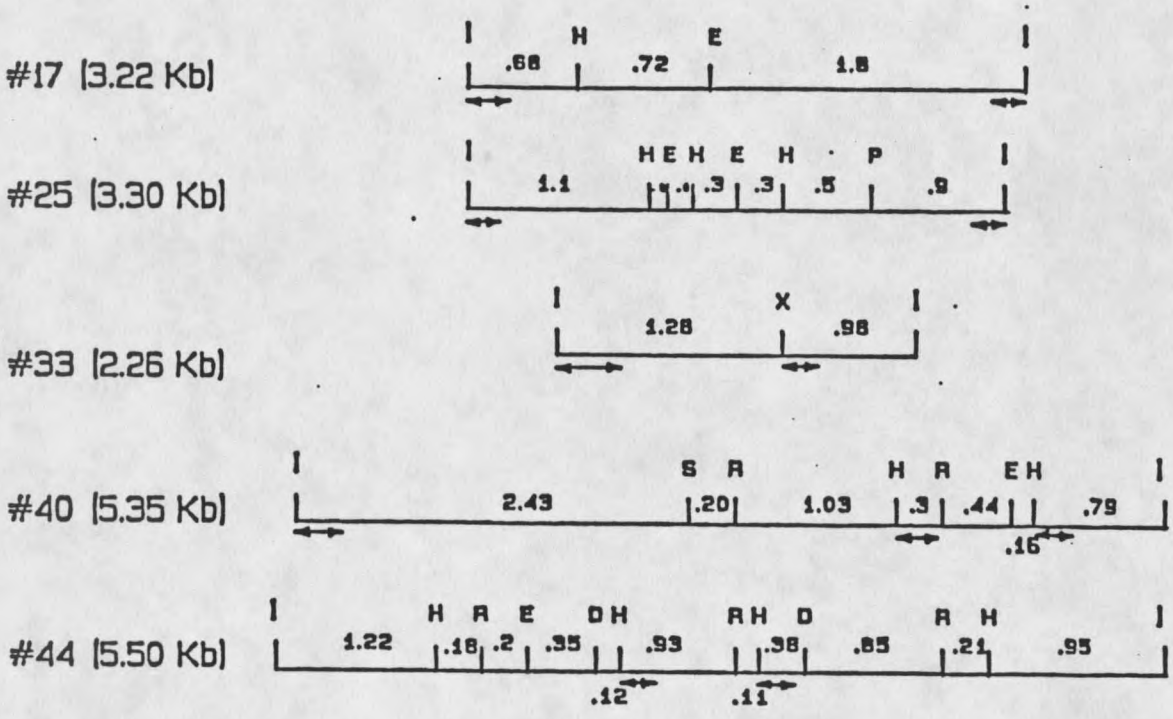

Figure 3. Physical maps of selected repetitive elements. Restriction sites shown are: EcoRI (I), EcoRV (E), HindIII (H), PstI (P), RsaI (R), DraI (D), SstI (S) and XbaI (X). Double arrowed lines represent length of sequence obtained for each fragment. Sizes of restriction fragments are in kilo base pairs.