

# Development-dependent changes in the tight DNA-protein complexes of barley on chromosome and gene level

Tatjana Sjakste(1), Kristina Bielskiene(2), Marion Röder(3), Olga Sugoka(1), Danute Labeikyte(2), Lida Bagdoniene(2), Benediktas Juodka(2), Yegor Vassetzky(4) and Nikolajs Sjakste(5)

1 Genomics and Bioinformatics, Institute of Biology, University of Latvia, Latvia; 2 Department of Biochemistry and Biophysics, Vilnius University, Lithuania; 3 Leibniz Institute of Plant Genetics and Crop Research, Germany; 4 UMR-8126, Institut Gustave Roussy, France; 5 Faculty of Medicine, University of Latvia, Latvia

## Background

The tightly bound to DNA proteins (TBP) is a protein group that remains attached to DNA with covalent or non-covalent bonds after its deproteinisation. The functional role of this group is as yet not completely understood. The main goal of this study was to evaluate tissue specific changes in the TBP distribution in barley genes and chromosomes in different phases of shoot and seed development. We have: 1. investigated the TBP distribution along *Amy32b* and *Bmy1* genes encoding low pI  $\alpha$ -amylase A and endosperm specific  $\beta$ -amylase correspondingly using oligonucleotide DNA arrays; 2. characterized the polypeptide spectrum of TBP and proteins with affinity to TBP-associated DNA; 3. localized the distribution of DNA complexes with TBP (TBP-DNA) on barley 1H and 7H chromosomes using mapped markers; 4. compared the chromosomal distribution of TBP-DNA complexes to the distribution of the nuclear matrix attachment sites.

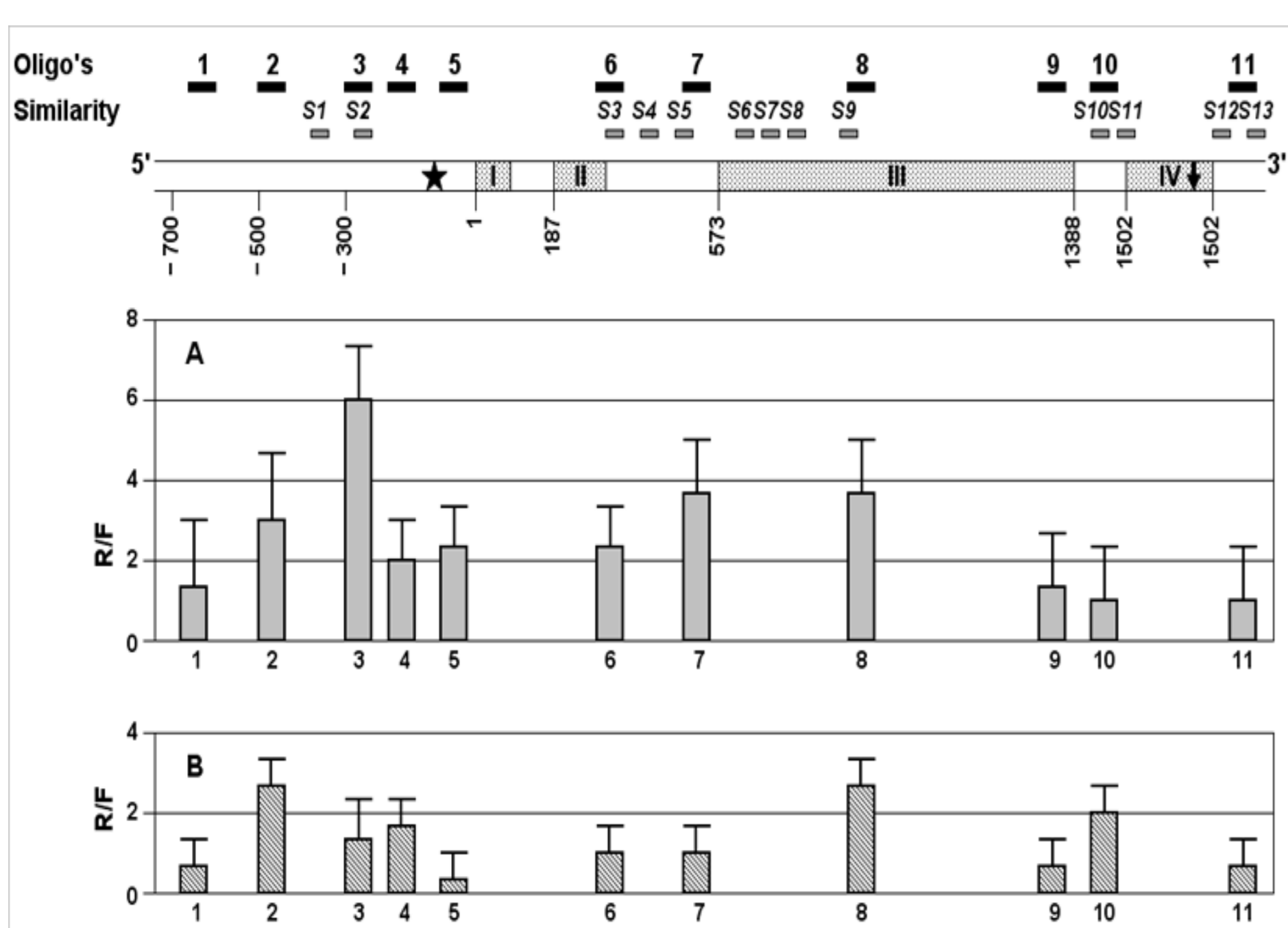
## Results

In the *Amy32b* gene transition from watery ripe to the milky ripeness stage of seed development was followed by the decrease of TBP binding along the whole gene, especially in the promoter region and intron II. Expression of the *Bmy1* gene coupled to ripening was followed by release of the exon III and intron III sequences from complexes with TBPs. Marker analysis revealed changes in the association of chromosome 1H and 7H sites with TBPs between first leaf and coleoptile and at Zadoks 07 and Zadoks 10 stages of barley shoot development. Tight DNA-protein complexes of the nuclear matrix and those detected by NPC-chromatography were revealed as also involved in tissue- and development-dependent transitions, however, in sites different from TBP-DNA interactions. The spectrum of TBPs appeared to be organ and developmental-stage specific. Development of the first leaf and root system (from Zadoks 07 to Zadoks 10 stage) was shown as followed by a drastic increase in the TBP number in contrast to coleoptile, where the TBPs spectrum became poor during senescence. It was demonstrated that a nuclear protein of low molecular weight similar to the described TBPs possessed a high affinity to the DNA involved in TBP-DNA complexes.

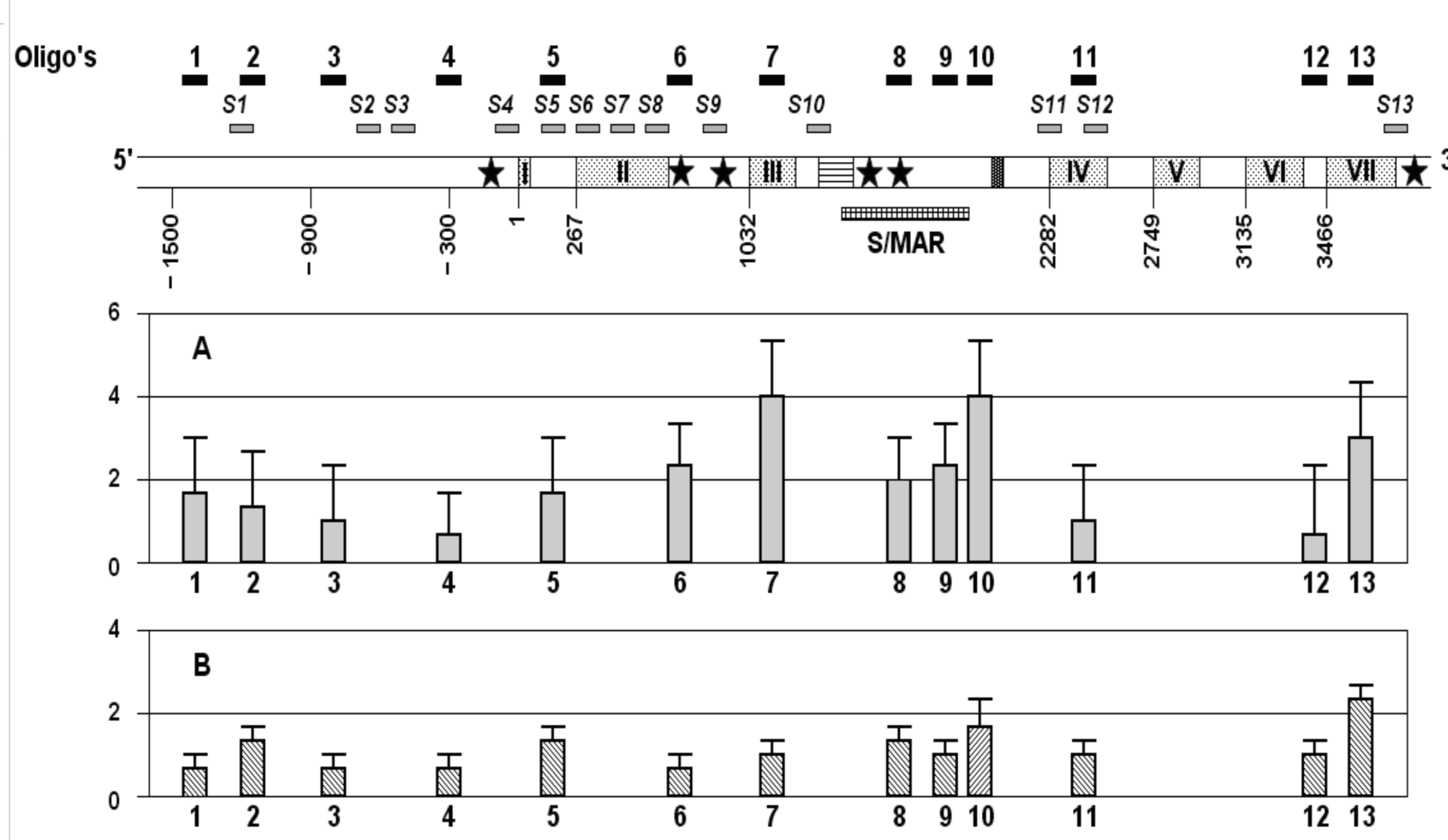
## Conclusions

Plant development is followed by redistribution of TBP along individual genes and chromosomes.

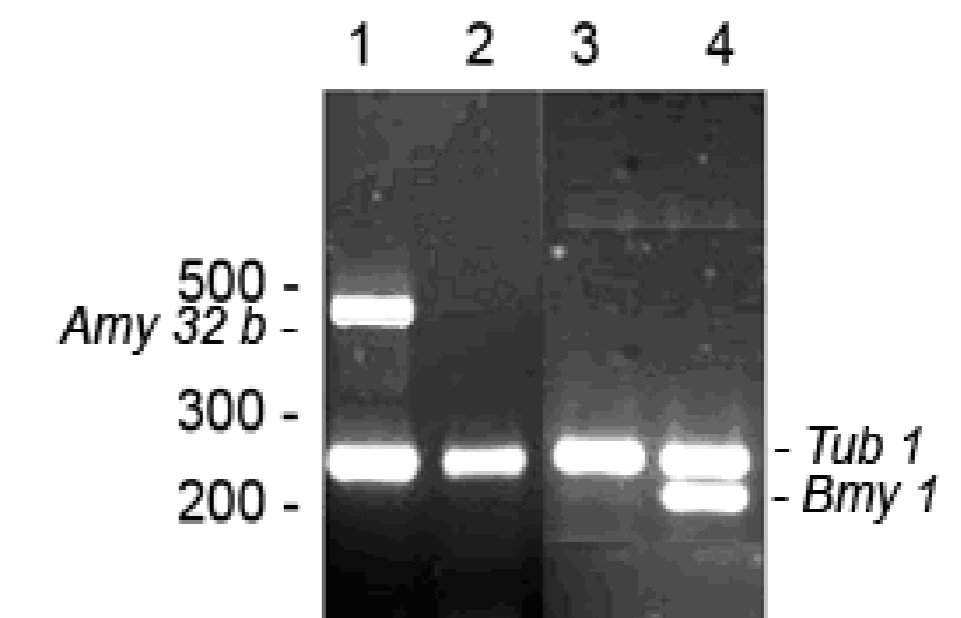
### DNA array based mapping of the TBPs distribution in *Amy32b* structural gene in watery ripe (A) and milky ripe (B) barley seeds



### DNA array based mapping of the TBPs distribution in *Bmy1* structural gene in watery ripe (A) and milky ripe (B) barley seeds



### *Amy32b* (lanes 1, 2), *Bmy1* (lane 3, 4) and alpha tubulin (lanes 1 - 4) RT-PCR products

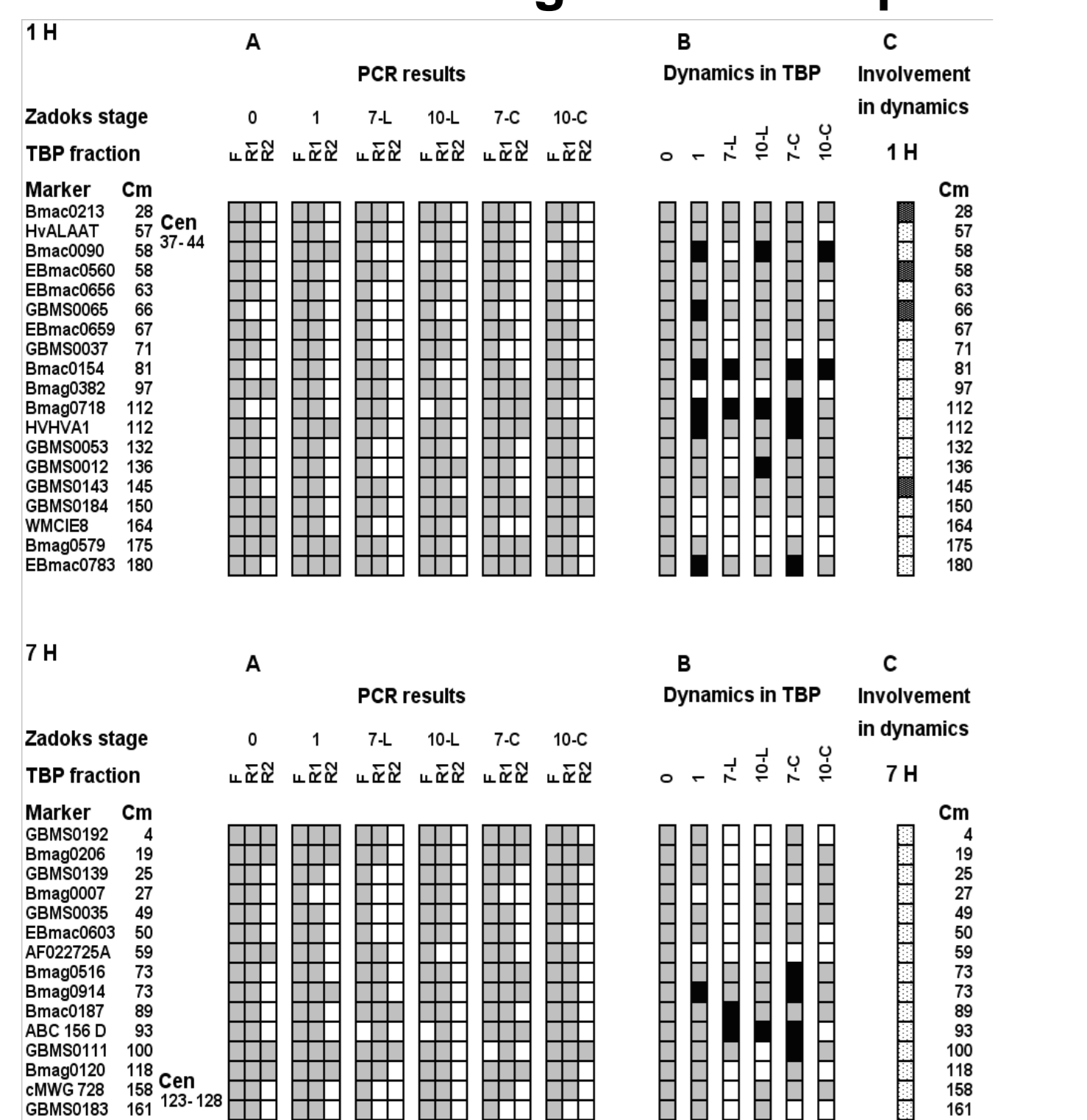


Lanes 1, 3 – RNA from watery ripe seeds; lanes 2, 4 – RNA from milky ripe barley seeds. Positions of the molecular weight markers (bp) are indicated on the right. Positions of RT-PCR products are indicated on both sides of the figure

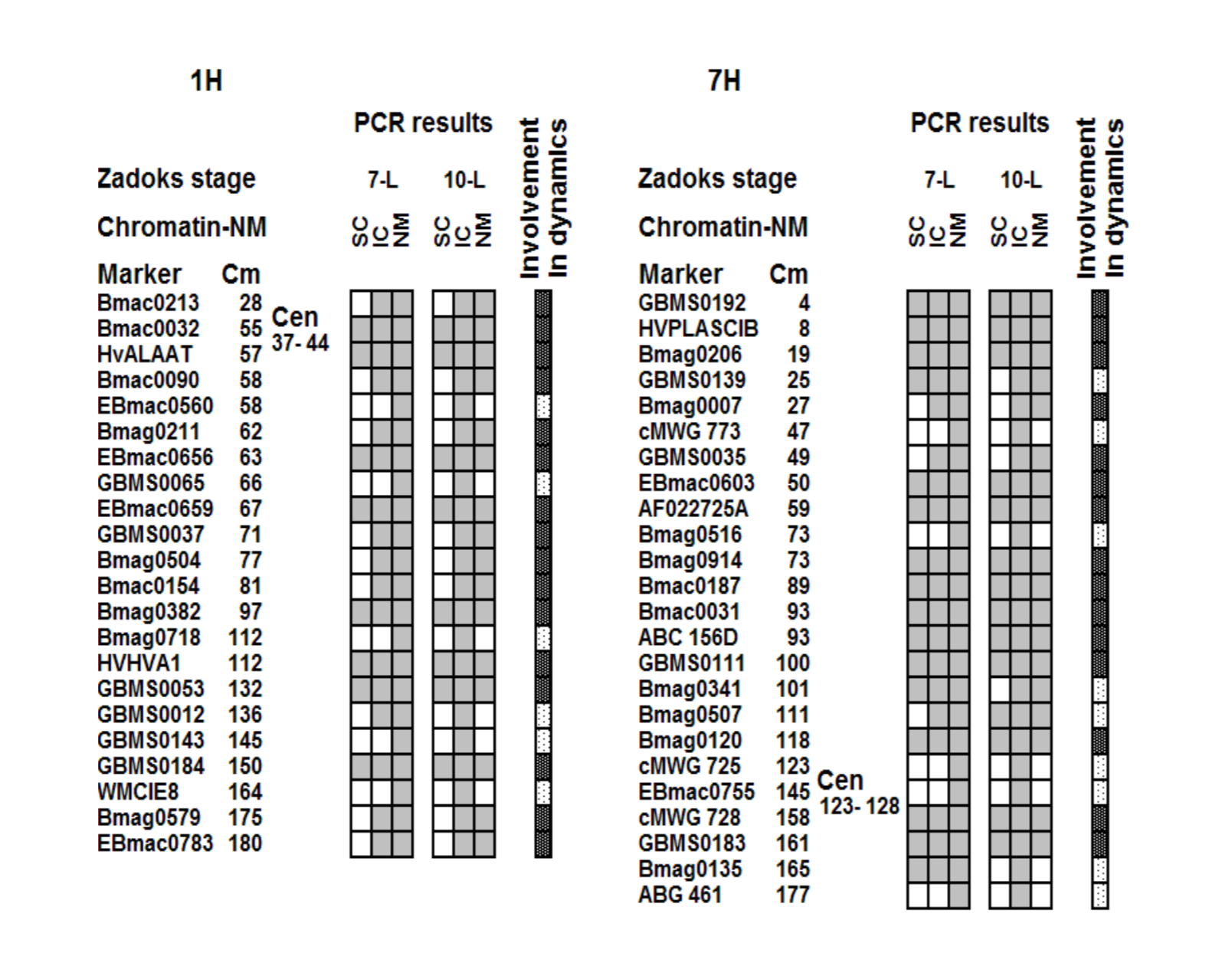
Upper panel presents the gene structure with positions of oligonucleotides of the array and regions of similarity. Stars correspond to the positions of the TATA boxes; black arrow in last exon indicates the stop codon. Black and dashed squares in the *Bmy1* Intron III indicate the positions of the microsatellite and MITE element, correspondingly. The predicted MAR position is indicated by a checked bar. The data on lower panels represent the ratio of hybridization of R vs F DNA fractions scored as an average of three independent experiments (two hybridizations per experiment). Error bars represent standard deviation.

## The distribution of MS sequences along barley chromosomes

### Free DNA and tight DNP complexes



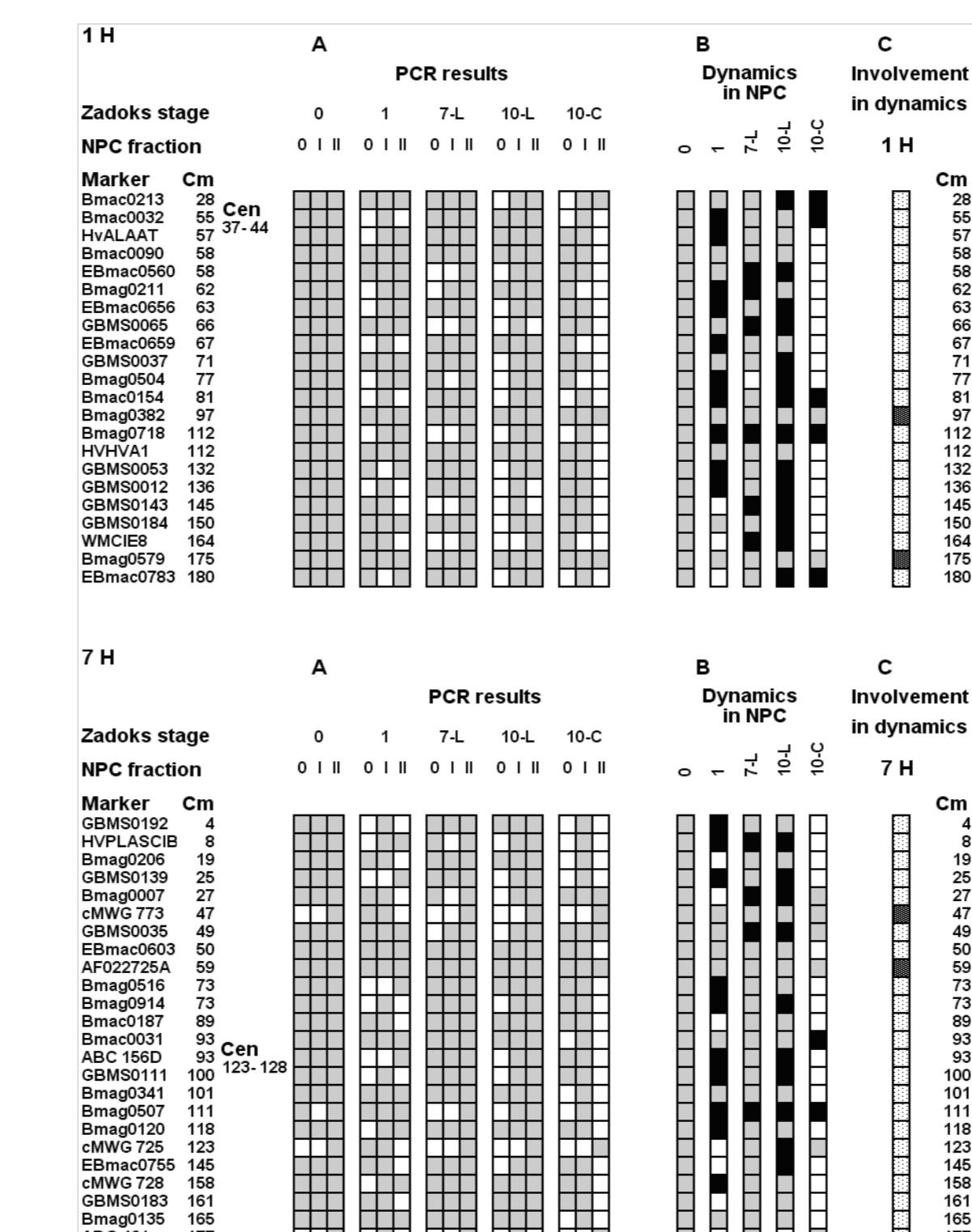
### Chromatin and nuclear matrix-attached DNA



**A.** The distribution of DNA fragments containing given microsatellites in free DNA (F), and tight DNA-protein complexes (R1 and R2). Marker names are given in the left column, following column gives distance from the chromosome extremity in centimorganides. Position of the centromere is indicated separately (Cen). 0 – dry seeds (phase 0), 1 – 20 hours of imbibition (phase 01); 7-L – first leaf on stage 07; 10-L – leaf on stage 10; 7-C – coleoptile on stage 07; 10-C – coleoptile on stage 10. Grey squares – presence of amplification; white squares – absence of amplification. **B.** Summary of the trends of transitions during development of different organs compared with the situation in dry seed. Grey squares – situation in the seed or similar; white squares – decrease of association with TBPs; black squares – increase in TBP-DNA interactions. **C.** General summary of involvement of the studied genomic sites in R-F and adverse transitions. Black points on white background – site involved in transitions; white points on black background – site not involved in transitions.

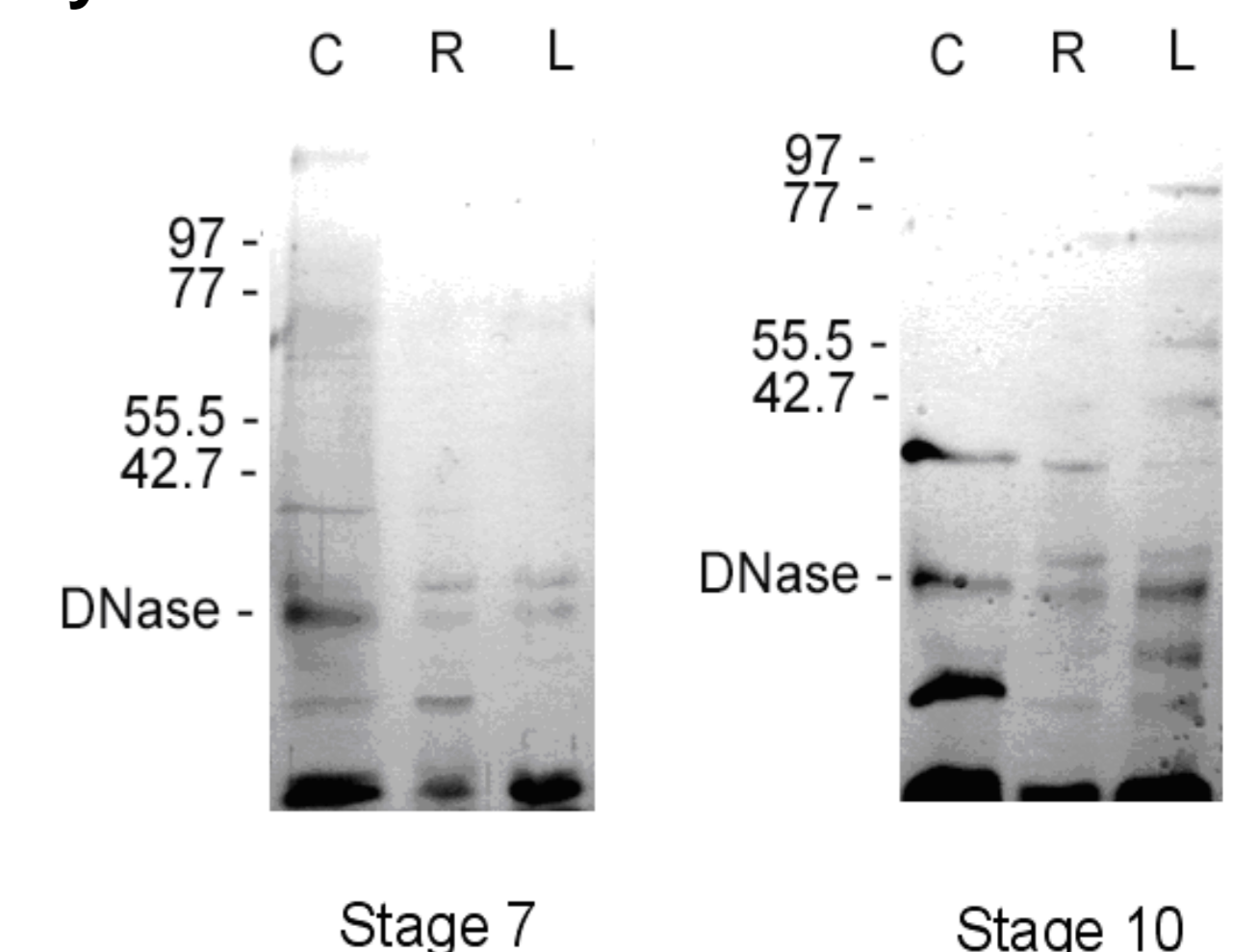
PCR results illustrate the distribution of MS containing DNA fragments in fractions of soluble (SC) and insoluble (IC) chromatin and the nuclear matrix (NM). Panel on the right indicates involvement in association or dissociation of the nuclear matrix. Grey and white squares correspond to the presence and absence of amplification. Black points on white background and white points on black background indicate on the sites involved and not involved in transitions.

### NPC-chromatography fractions



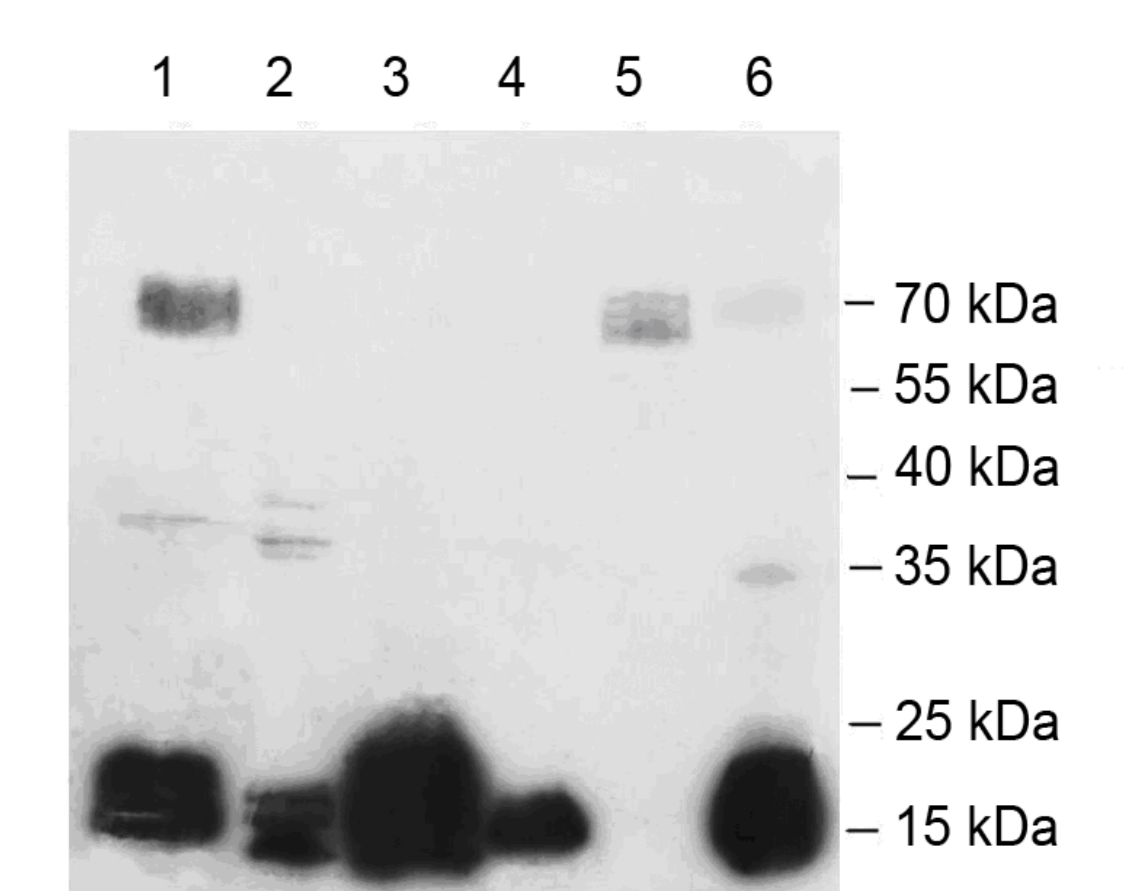
**A.** The distribution of MS containing DNA fragments in unbound to nuclear matrix fraction (eluted in NaCl gradient, DNA 0, column 0), loosely bound to the nuclear matrix (LiCl-urea and temperature gradient below 70°C, DNA I, column I) and tightly bound to the nuclear matrix (temperature gradient at 90°C, DNA II, column II). **B.** Summary of the trends of transitions during development of different organs compared with the situation in dry seed. Grey squares – situation in the seed or similar; white squares – loosening of association with the nuclear matrix; black squares – tightening of interactions. **C.** General summary of involvement of the studied genomic sites in association-dissociation according the NPC chromatography data. Black points on white background – sites involved in transitions; white points on black background – sites not involved in transitions.

### Electropherograms of the tightly bound proteins obtained by DNase I digestion of bulk DNA of leaves (L), roots (R) and coleoptiles (C) of stage 07 and stage 10 barley shoots



Arrows indicate positions of molecular weight markers (kDa). 10% PAAG. Silver staining

### DNA-binding protein blot assay



Assay was performed with electrophoretically fractionated nuclear proteins isolated from the Zadoks 07 (1 – 3) and Zadoks 10 (4 – 6) coleoptiles (1, 4), leaves (2, 5), and roots (3, 6) and incubated with TBP-associated DNA from Zadoks 10 leaves