Insertion Site-Based Polymorphism: A Swiss Army Knife for Wheat Genomics

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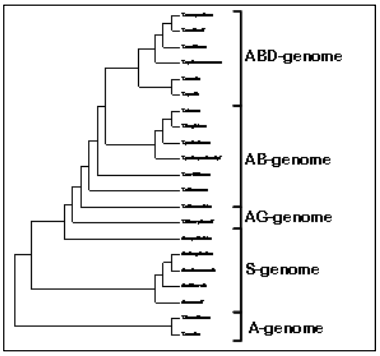
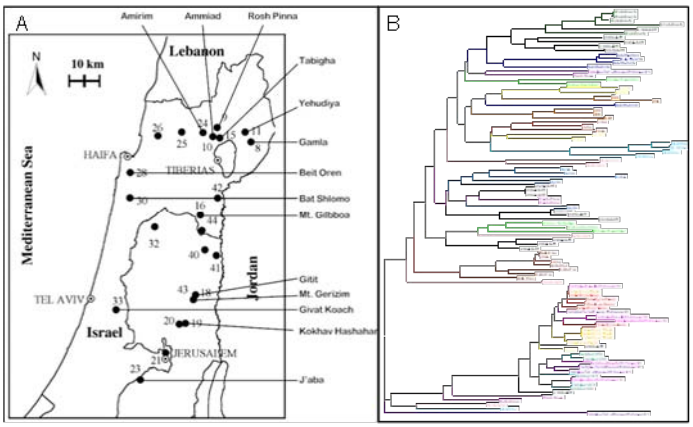
In wheat, transposable elements (TEs) account for more than 70% of the genome and are major drivers of genome evolution. They are ubiquitous, in high-copy number, evenly distributed along chromosomes, in hetero- and euchromatin, and show insertional polymorphism both within and between species. We took advantage of these genetic properties to develop a TE-based genotyping method called Insertion Site-Based Polymorphism (ISBP). ISBP markers are genome-specific, highly polymorphic and adapted to a wide range of detection techniques, including agarose gel electrophoresis, melting curve analysis, TGCE, AS-PCR, SNaPshot... They have been widely used for the construction of the integrated map of the chromosome 3B of hexaploid wheat. Here we go further and confirm the usefulness of ISBP as a new tool for wheat genomics.

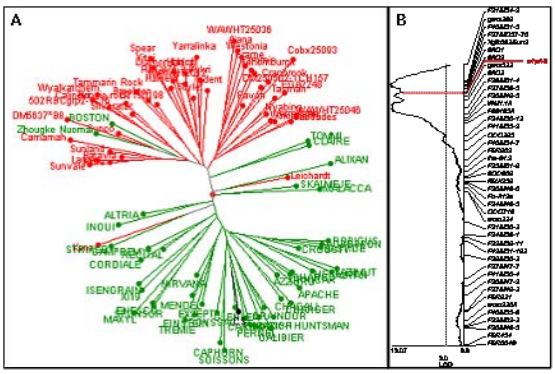
Bread wheat genome evolution is complex and the origin of the B-genome remains controversial. A phylogenetic analysis of 284 accessions consisting of *Aegilops* species of the *Sitopsis* section (S-genome), different subspecies of *T. turgidum* (AB-genome), hexaploid wheat (ABD-genome), *T. timopheevii* (AG-genome) and A-genome accessions with 90 ISBP markers suggested that (1) *Ae. speltoides* does not belong to the *Sitopsis* section; (2) the S-genome of *Ae. speltoides* is closely related to the B- and G-genomes but is likely not the real or only donor; (3) *T. carthlicum* could be an ancestor of hexaploid wheat; (4) the B-genome of *T. petropavlovskyi* could originate from a *T. polonicum* introgression rather than from a classical evolution. These results clearly demonstrate the potential use of ISBP markers for evolutionary studies in wheat.





ISBP markers provide an ideal tool for evaluating to what extent TE-induced genomic variability can be generated under specific environmental conditions in wheat. To this aim, we genotyped a collection of 96 wild emmer wheat (*Triticum dicoccoides*) from 17 populations representing regional patterns as well as contrasting microsites in Israel (A) with 90 ISBPs from chromosome 3B. ISBPs allowed to clearly discriminate between populations (B). Interestingly, we found clear correlations between gene diversity and some environmental factors such as soil type (basalt *vs.* terra rosa), altitude and sun exposure (sun *vs.* shade), strongly suggesting an impact of these factors on TE transposition and subsequent TE-induced genomic variability.

To assess the usefulness of ISBPs for marker-assisted selection, 96 ISBPs from chromosome 3B were used to genotype 92 elite wheat varieties (46 European and 46 Australian). In total, 60% of the markers were polymorphic with 60% of the polymorphism being detected by melting curve analysis and 40% by sequencing. Based on 34 melting curve-polymorphic ISBPs (corresponding to 136 polymorphic alleles), we were able to discriminate between Australian and European lines (A). Polymorphism between lines was used to map one ISBP in the close vicinity of the stem rust resistance locus (*Sr2*) in the Cranbrook x Halberd population. This marker is closer to the *Sr2* gene than any existing microsatellite markers (B).



Considering that ~14 out of the 17 Gb wheat genome is comprised of repetitive DNA, ISBPs represent an almost infinite source of polymorphism in wheat. This potential amount of genome-specific markers is likely to allow saturating genetic maps and subsequently unlock many doors leading to efficient genetic diversity studies, recombination and linkage disequilibrium analyses, association mapping, fine mapping and cloning of QTLs, as well as marker assisted-selection.

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