Development and Use of SSRs of Bread Wheat for Genetic and Physical Mapping and Transferability to the Species of *Triticum-Aegilops* Complex

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Abstract: SSRs were developed from random gDNA sequences and ESTs of bread wheat and used for genetic and physical mapping of wheat genome. The transferability of these SSRs to the other species of *Triticum-Aegilops* complex was also studied. Using random gDNA SSRs, 66 new SSR loci were genetically mapped through IWMMN (International Wheat Microsatellite Mapping Network). Using terminal deletion lines of bread wheat, more than 300 SSR loci were physically mapped to specific chromosome bins of bread wheat; these physical maps were compared with the available genetic maps. Linear order of loci in physical maps largely corresponded with those in the genetic maps except for a few discrepancies. Additional deletion lines of bread wheat using gamma-irradiation are being produced in our laboratory to physically order the SSRs mapped to specific chromosome bins. Both gSSRs and EST-SSRs tested for their transferability to different cultivated and wild species of *Triticum-Aegilops* complex, which comprised seven different genomes and represented three ploidy levels. As expected, the EST-SSRs showed higher level of transferability than the random gSSRs to the target species of *Triticum-Aegilops* complex, although the level of polymorphism detected by the two types of SSRs was comparable. The EST-SSRs also proved to be useful in discriminating different genera, species of a particular genus and the different genomes of the tribe *Triticeae*.

Keywords: SSR; EST-SSR; genetic mapping; physical mapping; deletion lines; transferability; polymorphism; *Triticum-Aegilops* complex species; bread wheat

Bread wheat (*Triticum aestivum* L. em. Thell) is a segmental allohexaploid ($2n = 6x = 42$; A, B, D sub-genomes) species with a large genome of 16×10^9 bp having more than 80% repetitive DNA. This makes bread wheat a difficult material for genome-wide studies. A number of molecular markers have been extensively used in bread wheat for a variety of purposes. In the recent past, the emphasis has shifted towards the use of markers from the expressed portion of the genome such as EST (expressed sequence tag)-SSRs, which has a number of advantages (for a review, see Gupta & Rustgi 2004). In this communication, we have summarized our efforts of the past few years towards the development and use of SSRs for genetic/physical mapping, transferability to species of the *Triticum-Aegilops* complex, and development of new radiation induced deletion stocks for fine physical mapping of SSRs.

MATERIAL AND METHODS

Seed material. (*i*) 70 RILs of ITMI population (ITMI*pop*), (*ii*) eight bread wheat genotypes (Chinese Spring and Rye Selection; HD2329 and SPR8198; WL711 and PH132; Opata85 and W7985), (*iii*) 23 species of *Triticum-Aegilops* complex, (*iv*) 21 nulli-tetrasomic (NT) lines, (*v*) 24 ditelosomic (DT) lines, (*vi*) 164 homozygous overlapping deletion lines, and (*vii*) monosomics for chromosomes 1A, 2A and 3A.

Development and genetic mapping of gSSRs. We developed gSSRs as one of the members of Wheat Microsatellite Consortium (WMC) and mapped them as a member of International Wheat Microsatellite Mapping Initiative (IWMMN) (for details, see Varshney *et al*. 2000; Gupta *et al*. 2003).

Designing of 5′-anchored primers for gSSRs. Utilizing sequences of WMC clones (http://wheat.pw.usda.gov/ggpages/SSR/WMC/) that were earlier considered unsuitable, we designed 105 5′-anchored SSR primers. These primers were used for SSR and MFLP analyses.

In silico ***search for EST-SSRs and designing of primers***. The detailed account of the *in silico* discovery of EST-SSRs of bread wheat are described elsewhere (Gupta *et al*. 2003). Primers sequences for the 78 EST-SSRs that we designed are available at the EST-SSR Coordination web page (http://www.wheat.pw.usda.gov/ITMI/EST-SSR/Gupta2.html).

Physical mapping of SSRs and comparison of genetic and physical maps. For physical mapping, a set of 527 SSR primer pairs (167 wmc, 192 gwm, 34 cfa, 74 cfd and 60 psp) were tried with NT and DT lines, and 164 overlapping deletion lines of bread wheat (Goyal *et al*. 2005). The SSR physical maps were compared with the three genetic maps (for details, see Goyal *et al*. 2005).

Isolation of radiation induced disomic deletion stocks. Seeds of monosomics for chromosomes 1A, 2A and 3A were irradiated with three doses of gamma rays (30 kR, 40 kR and 50 kR). In M_1 generation monosomic plants were identified following cytological analysis. The M_1 monosomic plants carrying deletions in the respective monosomic chromosome were identified by the loss of mapped SSR markers. The M_2 progenies of these plants are currently being analyzed to isolate disomic deletion stocks.

DNA isolation and PCR analysis. The protocols for DNA isolation and PCR analyses used are described elsewhere (Gupta *et al*. 2003).

Polymorphic information content (PIC). Estimation of polymorphic information content was carried out as given in Gupta *et al*. (2003).

RESULTS AND DISCUSSION

Development and genetic mapping of gSSRs

As a member partner of WMC, we designed primer pairs for 16 SSRs. Primers for 15 of the 16 SSRs proved functional. Using 12 of these 15 functional primers, we were able to assign 14 SSR loci on 11 different chromosomes (Varshney *et al*. 2000). In addition, 61 (58%) out of 105 5′-anchored SSR primers designed by us proved functional in SSR analysis and 39 (37.14%) primers detected polymorphism in eight bread wheat genotypes with an average PIC of 0.409. A subset of 45 anchored primers was also used for MFLP analysis with the same eight bread wheat genotypes. In MFLP analysis, 42 of 45 anchored primers used amplified an average of 12.74 polymorphic bands with an average PIC of 0.061.

An international effort IWMMN involving ten different laboratories, including our own laboratory, led to mapping of 66 new SSRs in a framework map of ITMI*pop* having 266 anchor markers (for details, see Gupta *et al*. 2003). The 66 loci were distributed on 20 of the 21 bread wheat chromosomes. Twelve (12) of the 58 SSR primer pairs, amplified more than one locus, and only 50% of these multilocus SSR primers amplified homoeoloci. The remaining 50% multilocus SSRs amplified loci on nonhomoeologous chromosomes. These observations suggested that SSRs may not prove very useful for comparative genomics, although they are very useful for gene tagging and QTL analysis.

SSR physical maps and their comparison with genetic maps

A set of 270 SSRs was used for mapping 313 SSR loci on 21 chromosomes. A maximum of 119 loci (38%) were located on B-sub-genome, and a minimum of 90 loci (29%) mapped on D-sub-genome. Similarly, homoeologous group 7 carried a maximum of 61 loci (19%), and group 4 carried a minimum of 22 loci (7%). The distribution pattern of EST-loci in chromosome bin map of bread wheat in a recent study was no different, with B-sub-genome having significantly higher number of mapped EST-loci than the A and D-sub-genomes (Qi *et al*. 2004).

Comparison of the physical map with three genetic maps (Goyal *et al*. 2005) showed that the linear order of loci in physical maps largely corresponded with those in the genetic maps. Apparently, distances between each of only 26 pairs of loci significantly differed from the corresponding distances on genetic maps. Thirty-nine (14.44%) SSR primers mapped multiple loci including homoeoloci and paralogues (nonhomoeologous duplicates)

and represented 36 duplicate, two triplicate and one quadruplicate loci. A small fraction of SSRs (12 SSR loci, 3.77%) were physically mapped in regions other than those on the corresponding genetic maps. This could be attributed to the occurrence of paralogues and subtle chromosome rearrangements as was also inferred by Weng *et al*. (2000) for group 6 chromosomes of bread wheat. Alternatively, this could be attributed to their separation from other genetically mapped loci through long low recombination regions. This could be true for *wmc*446 and *gwm*397, which were genetically mapped together at a distance of 9 cM near the centromere on 4AL, but were placed together in the telomeric bin in the physical map of 4AL.

Development of new radiation induced deletion lines

A particular disadvantage of the available physical maps is that the loci mapped within individual chromosome bins cannot be ordered within a bin. To overcome this problem, we have initiated a programme for obtaining radiation induced new deletion stocks for three chromosomes 1A, 2A and 3A carrying genes for important agronomic traits. We have already isolated 61 M_1 monosomic plants for chromosome 2A, which carry radiation induced deletions in the monosomic chromosomes. A similar exercise is being carried out to obtain deletions for chromosomes 1A and 3A. Selfed progenies of the above M_1 monosomic plants for chromosome 2A are being analyzed to recover disomic deletion stocks. The deleted segments in each of the above disomic deletion stocks will be characterized using mapped SSRs and stocks representing deletions within the existing chromosome bins will be used for ordering of SSRs within the individual bins.

Transferability and polymorphism of EST-SSRs and gSSRs

Sixty four (64) EST-SSRs and 15 gSSRs of bread wheat were used for study of their transferability, and their ability to detect polymorphism among 18 and 13 cultivated and wild species of *Triticum-Aegilops* complex, respectively. EST-SSRs as well as gSSRs amplified SSR loci not only in species containing A, B (including S and G) and D genomes but also in species that contained other genomes such as M, N, C and U (Sharma *et al*. 2002; Bandopadhyay *et al*. 2004). This suggested that both EST-SSRs and gSSRs were transferable to related cultivated and wild species of *Triticeae*. However, the transferability of EST-SSRs (amplification in 83.59% primer-species combinations) was higher relative to the transferability of gSSRs, (amplification in 68% primer-species combinations), making the EST-SSRs a more useful resource of molecular markers in comparative genomic studies in *Triticeae*. This higher level of transferability of EST-SSRs can be attributed to a higher level of conservation of DNA sequences belonging to the transcribed region of the genome.

All the 15 gSSRs (100%) and a very high proportion (52, i.e. 88.14%) of the EST-SSRs (that amplified products of the expected size) detected polymorphism in the species of *Triticum-Aegilops* complex examined. The average number of alleles per locus (6.8; range of 2 to 14 alleles) detected by 52 EST-SSRs in 18 species of *Triticum-Aegilops* complex was comparable with the average number of alleles per locus (6.6; range of 4 to 11) detected by a much smaller number of 15 gSSRs in a relatively smaller number of 13 *Triticum-Aegilops* species. Polymorphic EST-SSRs and gSSRs were also found to be suitable for discrimination between genera, among species within a genus and between different genomes of the species of *Triticum-Aegilops* complex belonging to tribe *Triticeae*.

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