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# **Czech J. Genet. Plant Breed.**

**Bandopadhyay R.,  
Balyan H.S., Gupta  
P.K.:**

## **EST-SNPs in bread wheat: discovery, validation, genotyping and haplotype structure**

Czech J. Genet. Plant Breed., 45 (2009):  
106-116

The present study involves discovery, validation and use of single-nucleotide polymorphisms (SNPs) in bread wheat utilizing 48 EST-contigs (individual contigs having 20-89 ESTs, derived from 2 to 11 different genotypes). In order to avoid a problem due to homoeologous relationships, the ESTs in each contig were classified into 175 sub-contigs (3.7 sub-contigs/EST-contig) using characteristic homoeologue sequence variants (HSVs), which had a density of 1 HSV every 136.7 bp. *In silico* analysis of

sub-contigs led to the discovery of 230 candidate EST-SNPs with a density of 1 SNP/273.9 bp. Locus specific primers (each primer pair flanking 1– 18 SNPs) were designed utilizing one sub-contig each from 42 EST-contigs that contained SNPs, the remaining 6 contigs having no SNPs. To provide locus specificity to the PCR products, each primer was tagged with an HSV at its 3' end. Only 10 primer pairs, which gave each a characteristic solitary band, were utilized to validate EST-SNPs over 30 diverse bread wheat genotypes; 7 SNPs were validated through resequencing the PCR products. Allele specific primers were designed and utilized for genotyping of 50 diverse bread wheat accessions (including 30 bread wheat genotypes previously used for validation of SNPs), with an aim to test their utility in genotyping and map construction. The allele specific primers allowed the classification of 50 genotypes in two alternative allele groups for each SNP as expected, thus suggesting their utility for genotyping. Of the above 7 validated SNPs, 4 belonged to a solitary locus (PKS37); 7 haplotypes were available at this locus. Altogether, the

results suggested that EST-ORF's constitute an important source of molecular markers for studies on wheat genomics.

## Keywords:

contigs; DNA polymorphism; DNA sequencing; homoeologue sequence variants (HSVs); *Triticum aestivum* L.

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