Expression analysis and molecular mapping of abiotic stress tolerance genes in Mustard

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Abstract

Crop *Brassica* belongs to the family *Brassicaceae*. A substantial yield in this crop is lost due to biotic and abiotic stress, among which abiotic stress is the primary cause of crop loss worldwide. Among abiotic stress drought is the most important. Water deficit stress leads to significant changes in gene expression. Crop *Brassica* belongs to same family as *Arabidopsis* whose small genome has been completely sequenced this is expected to benefit Indian mustard. To study the expression pattern of candidate stress responsive genes from *Arabidopsis*, phenotypic characterization of *Brassica* genotypes to abiotic stress was done with 38 genotypes using agar solidified high osmotic medium and based on the results obtained top five tolerant and susceptible genotype were screened, gene expression profiling was done using 13 *A. thaliana* stress response gene in 3 genotypes of *B. juncea* namely Varuna, BEC-144 and RIM619 and one genotype of *S.alba*. The expression profiling was further validated in real time PCR using leaf tissue of the four genotypes. With the objective of developing candidate based markers primers were designed for PCR amplification of 43 different stress responsive genes. Out of these 43 pairs of primers used in amplification 25 pairs gave successful amplification. Thus polymorphism analysis was carried out between *B. juncea* genotypes Varuna and BEC-144 for these 25 stress responsive candidate genes. For these 25 of the genes there was no polymorphism between Varuna and BEC-144. The amplicons for these genes were digested by 12 different tetra-cutter restriction enzymes. Polymorphism was observed for 12 genes and one candidate gene based on STS marker was identified. So overall we developed 13 (one candidate stress related gene sequence based on STS marker and twelve gene sequence based CAPS marker) which were polymorphic between stress tolerant Varuna and stress susceptible BEC-144. Cloning for four *A. thaliana* candidate stress responsive gene based markers was done. Total RNA was extracted from two

B. juncea namely Varuna and BEC-144. The amplicons of expected product size were purified, ligated, transformed and plasmid DNA was isolated. The plasmid DNA was digested and the restricted fragments were analysed to check the size of release insert. To discover SNPs and InDels in *Brassica* further sequencing was done. A total of 176 bases high quality sequences were compared among Varuna and BEC-144 based on sequence comparison, 16 SNPs and 6 InDels were identified. Transitions were more frequent than transversions. Molecular mapping of the polymorphic genes based markers was done with 12 genes (excluding *LOS4*). A set of 94 RILs from the cross of Varuna and BEC-144 were used for studying their segregation and establishing genetic linkage relationship. Expected Mendelian segregation was observed for four of the genes namely *FCA, HAL3A, HSP101* and *ALDH3* these markers were further used in Map-marker software for establishing genetic linkage. The study of linkage relationship among these CAPS markers assigned four markers to one linkage group of *B. juncea* at a maximum fraction of 0.35 and a minimum LOD of 3.