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NEW SNPs MUTATIONS OF DREB GENES IN DURUM WHEAT IDENTIFIED BY HRM TECHNOLOGY

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DREB (Dehydratation-responsive-element binding factor) is one of the largest families of transcriptional regulators and form an integral part of signalling webs which modulate many plant processes, such as abiotic stresses tolerance. DREB genes are induced by low temperature, salinity and drought, they contain conserved EREBP/AP2 domains and the DRE-motif functions as a DREbinding transcription factor sequences. In the present paper an innovative method has been applied to identify novel alleles in DREB genes family (DREB-1, DREB-2, DREB-3, DREB-4, DREB-5 and DREB-6 gene) in four salt and drought resistance and susceptible Triticum durum lines (Cham-I, Jennah Khetifa, Belikh 2 and Trinakria). This technique involves scanning for sequencing variations in cDNA-derived PCR amplicons using High Resolution Melting (HRM) followed by direct Sanger sequencing of only those amplicons which were predicted to carry nucleotide changes. High Resolution Melting represents a novel advance for the detection of SNPs measuring temperature-induced strand separation of short PCR amplicons. The use of this approach is still limited in the field of plant biology. Here, HRM analysis has been applied to the discovery and genotyping of durum wheat SNPs. Specific primers have been designed, starting at multi-alignment of DREB genes conserved portions. The PCR amplicons, containing SNPs, produce distinctive HRM profiles, and by sequencing the PCR products identified, SNPs have been characterized and validated. The results showed that all the identified SNPs are located on salt tolerant variety J. Ketifa treated with the maximum salt concentration (1.5 M) confirming its value in breeding activities. Moreover, all SNPs mutations correspond to amino acid changes in the conserved portion of DREB genes probably influencing protein activity and function.