

FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology



Contribution ID: 76

Type: Poster

HIGH-RESOLUTION MELTING ANALYSIS FOR THE IDENTIFICATION OF SNP MUTATIONS IN THE bZIP DOMAIN OF HvWRKY38 GENE AMONG BARLEY GENOTYPES

Drought tolerance is a complex trait that is governed by many genes and pathways, where transcription factors (TFs) play an important role as regulator molecules. HvWRKY38 gene is a transcription factor of the WRKY family in barley, with two conserved domains: the leucine zipper and WRKY. It is known for transcriptional reprogramming in response to abiotic stress conditions in plants. High Resolution Melting (HRM) procedure has been applied on DNA samples of barley genotypes that include: the wild type *Hordeum spontaneum* L., M8 mutant lines (HvM 7, HvM 58, HvM 64, HvM 66, and HvM 69) and their *H. vulgare* L. parent variety (Furat 9), together with local and introduced varieties (IC9, Furat 3, Tadmour and Banteng). The Type-it®HRMTM PCR kit was used for amplification and HRM analysis was carried out using the Rotor-Gene Q system. The following forward and reverse primers were used to amplify the bZIP motif of HvWRKY38 (NCBI: AAS48544.1): Hv_bZIP_F (5' - AACTTCATGCCGCTCAAGAA -3') and Hv_bZIP_R (5' - TGACCATGTCCGGT-GAACTGG -3'). HRM analysis showed a distinctive curve pattern of the mutant line HvM7. This variant pattern of normalized melting curve and its corresponding difference curve for the 230 bp amplicon, was verified by Sanger sequencing to contain two SNPs, a nonsynonymous transition at 189T>C, and a synonymous transversion at 213 C>A corresponding to the bZIP motif within the ORF of HvWRKY38 sequence. The identification of functional variation among members of TFs gene families, and further links with physiological and morphological data, will enable a robust assessment and selection process of candidate parents.

Country or International Organization

Atomic Energy Commission of Syria

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Track Classification: New challenges and technologies in plant genomics and breeding