

FUNCTIONAL CHARACTERIZATION OF AN E3 UBIQUITIN LIGASE INVOLVED IN PLANT RESPONSE TO ABIOTIC STRESS

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Protein ubiquitylation is a post-translational modification that targets protein substrates for 26S proteasome-mediated degradation. It is based on the covalent attachment of the 76-amino acid eukaryotic molecule, ubiquitin, to substrate proteins. Protein ubiquitylation plays a key role in a wide variety of cellular processes such as hormone signalling, DNA repair, biotic and abiotic stress response, cell cycle regulation. Ubiquitin conjugation is a multistep reaction, sequentially involving three enzymes referred to as E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase). In *Arabidopsis thaliana* more than one thousand of genes code for E3 ubiquitin enzymes that specifically recognise target proteins. In a previous work we isolated an *E3* ubiquitin ligase early induced during cold/light stress in durum wheat; an ubiquitylation assay was carried out to test its functionality *in vitro*. To identify potential ubiquitylation targets during abiotic stress response, several approaches have been initiated in *Triticum durum* and *Arabidopsis* based on the identified *E3* gene. A wheat cDNA library from cold treated leaves of *Triticum durum* has been produced and screened by two-hybrid system to isolate potential E3 interactors and ubiquitylation targets. Transgenic *Arabidopsis* plants overexpressing the *Arabidopsis* homologous E3 enzyme fused to the TAP tag has been developed to isolate protein complexes containing our E3 ligase and to determine subcellular localization of E3 enzyme. An *Arabidopsis* K.O. line for the same gene has been obtained for future evaluation under various abiotic stress condition. Preliminary results of this work will be shown.