

PRODUCTION AND CHARACTERIZATION OF WHEAT LINES SILENCED IN *ALPHA-AMYLASE/TRYPsin* INHIBITOR GENES INVOLVED IN ADVERSE REACTIONS TO WHEAT

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Although wheat is the most consumed crop worldwide, it is also the main factor triggering different adverse reactions, among which celiac disease, true allergies and Non Celiac Wheat Sensitivity (NCWS). Among allergies, the so called “baker's asthma”, is the most common professional asthma in Europe and is caused mainly by proteins present in the soluble fraction, especially alpha-amylase/trypsin inhibitors (ATI). Recent findings indicate in this class of proteins also the main factor triggering NCWS, that at present affects people with a frequency around 1:80, higher than celiac disease (1:100), but this is still a matter of debate. On this basis, we have produced RNAi wheat plants (both durum and bread wheat) in which different *ATI* genes have been silenced, to be used as a proof of concept, in order to test if they have a minor impact on adverse reactions, by using *in vitro* tests. We have silenced *CM3*, *CM16* and *0.28* genes and have now available several lines in T₄ generation. ELISA tests and immunoblotting analysis, by using a monoclonal antibody against ATI proteins, have shown that RNAi silenced wheat kernels present a lower amount of ATI proteins. Moreover, we are characterizing these lines in relation to respiratory allergies. Protein extracts from silenced plants are being tested by using human sera of allergic patients in order to verify if a lower amount of immunogenic polypeptides is recognized in comparison to wild type untransformed plants. If this is the case, the realization of new wheat genotypes expressing a lower amount of ATI proteins can be a realistic target to be reached by classical breeding procedures.