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# SILENCING OF *ATI* GENES INVOLVED IN ADVERSE REACTIONS TO WHEAT BY RNAi AND CRISPR-Cas9 TECHNOLOGIES

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Although wheat is consumed worldwide as a staple food, it can give rise to different adverse reactions, some of which have not been deeply characterized. They are caused mainly by wheat proteins, both gluten and non-gluten proteins. Structural and metabolic proteins, like α-amylase/trypsin inhibitors (ATI) are involved in the onset of wheat allergies (bakers' asthma) and probably non-coeliac wheat sensitivity (NCWS). The ATI are encoded by a multigene family dispersed over several. Notably, WTAI-CM3 and WTAI-CM16 subunits are involved in the onset of bakers' asthma and are likely to contribute to NCWS.

In this study we report the RNAi silencing of WTAI-CM3, WTAI-CM16 and WMAI-0.28 genes in the bread wheat cultivar Bobwhite and the CRISPR/Cas9 mediated gene knockout of WTAI-CM3 and WTAI-CM16 in the durum wheat cultivar Svevo.

We have obtained different RNAi transgenic lines showing an effective decrease in the expression in the targeted genes. These lines do not show differences in terms of yield, but have unintended effects on the accumulation of the high molecular weight glutenin subunits which play a crucial role in the technological performances of wheat flour.

Furthermore, the editing of WTAI-CM3 and WTAI-CM16 genes was obtained through a CRISPR-Cas9 multiplexing strategy in the Italian durum wheat cultivar Svevo with a marker-free approach. The regeneration of plants without selection agents allowed $T_0$ homozygous mutant plants to be obtained without the integration in the wheat genome of CRISPR/Cas9 vectors, demonstrating the capability of CRISPR technology to produce wheat lines in a reduced time compared to conventional breeding approaches.

The possibility to develop new wheat genotypes accumulating a lower amount of proteins effectively involved in such pathologies, not only offers the possibility to use them as a basis for the creation of wheat varieties with a lower impact on adverse reactions, but also to test if these proteins are actually implicated in those pathologies for which the triggering factor has yet to be established.