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CRISPR-CAS9 MULTIPLEX EDITING OF DURUM WHEAT CULTIVAR SVEVO FOR REDUCING THE LEVEL OF ALLERGENS

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The α-amylase/trypsin inhibitors (ATIs) represent a protein family that belongs to the albumins and globulins fractions of wheat endosperm proteins. ATI's family includes a combination of monomeric, dimeric and tetrameric forms with molecular weight ranging between 12,000 and 16,000 Da. ATIs are encoded by genes highly conserved and dispersed over several chromosomes. Three groups of ATIs have been described in common wheat:

1. 12 kDa monomeric inhibitors (WMAI), referred to as 0.28 proteins, encoded on the short arms of the group 6 chromosomes (WMAI-1 on 6D and WMAI-2 on 6B);
2. 24 kDa homodimeric inhibitors (WDAI) encoded by genes on the short arms of the group 6 chromosomes, referred to as 0.19 (WDAI-2on 3D) and 0.53 (WDAI-1on 3B);
3. 60 kDa heterotetrameric inhibitors, referred to as CM proteins because of their solubility in chloroform/methanol. They are composed of one copy of CM1 or CM2 (encoded on chromosomes 7D or 7B respectively), plus one copy of CM16 or CM17 (encoded on the chromosomes 4B or 4D respectively), plus two copies of CM3 encoded on chromosomes 4B and 4D.

The ATIs are mainly involved in plant defense mechanisms and they represent an important reserve for seedlings and for human nutrition, because they have a balanced amino acid compositions compared to gluten protein. Recently, they have been considered the major allergens in patients with baker's asthma, food allergies, non-Coeliac Wheat Sensitivity (NCWS). Here, with the aim to reduce the amount of allergens in durum wheat grain, a multiplex genome editing approach based on CRISPR-Cas9 was used to knock out the genes encoding CM16 and CM3 subunits in the durum wheat cv Svevo. Two rounds of biolistic transformation (about 340 embryos), were carried out without using a selection marker in order to produce transgene-free wheat mutants into T_0 generation.

The regenerated plants were selected through HRM (high resolution melting) and confirmed by sequencing.