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ENGINEERING THE COELIAC DISEASE GENES IN DURUM AND BREAD WHEAT BY CRISPR/CAS9

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Wheat is the most important food grain in the world. Due to the unique biomechanical properties of wheat dough it is used in the production of staple foods such as bread and pasta, providing more than 20% of the calories and protein in human nutrition. However, more than 10% of the Western population suffers from several gluten-related disorders caused by the intake of wheat, barley and rye gluten proteins. The α-gliadins contain three highly stimulatory peptides, being the 33-mer the main immunodominant as it contains sixoverlapping copies of three tandemly-organized epitopes. The high number of α-gliadin genes (between 25 and 100) in bread wheat makes the genome editing highly challenging. We had modified the α-gliadin gene array in one durum wheat and two bread wheat cultivars, using two sgRNAs targeting conserved regions adjacent to the 33-mer peptide. Up to 35 of the 45 possible target genes identified in BW208 (bread wheat) and 29 of 43 in Don Pedro (durum wheat) showed InDels, and all mutant lines showed strong reduction in the α-gliadins content. One of the sgRNAs was more effective inducing InDels, and for some lines up to 75% of the reads were mutated. DNA analysis of progeny showed heritability of the mutations and no off-target. Transgene-free lines were obtained by segregation. Immunoreactivity was reduced up to 85% in comparison to that of the wild type. The results demonstrate that high mutation frequency and specificity can be achieved using CRISPR/Cas9 to modify complex genomic loci, providing lines with reduced immunoreactivity. Although these lines are not suitable for coeliac sufferers, they can be used to produce low gluten foodstuff for those who want to reduce the gluten intake. In addition, they can serve as source material in plant breeding programs to introgress this trait into elite wheat varieties.