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EDITING OF THE DURUM WHEAT PDIL5-1 GENE TO INCREASE RESISTANCE TO SOIL-BORNE BYMOVIRUSES

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Durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.)) can be considered a major cereal crop for food production and agricultural income in specific geographical regions such as the Mediterranean basin, including Italy, despite it accounts for about 7% of the total wheat produced in the world.

However, its production is often affected by several factors, including viral diseases. Among them, the wheat soil-borne bymoviruses, such as the Wheat Yellow Mosaic Virus (WYMV) and the Wheat Spindle Streak Mosaic Virus (WSSMV), are transmitted to plants by the soil-inhabiting plasmodiophorid *Polymyxa graminis*, which infected spores can survive long in the soil. The lack of reliable methods that can counter the spread of the disease, the persistence of the virus in the soil, and the potentially devastating effect of infections make it necessary to develop new strategies based on the development of resistant wheat varieties.

In barley, the gene disulfide isomerase like 5-1 (*HvPDIL5-1*) encodes for an endoplasmic reticulum-localized protein involved in protein folding. Loss-of-function of *HvPDIL5-1* has been previously identified as the cause of the naturally occurring resistance to multiple strains of bymoviruses in barley.

Given that orthologues genes of *HvPDIL5-1* are highly conserved among species, we speculated that the orthologues of *HvPDIL5-1* could represent a susceptibility factor for bymovirus infection also in durum wheat. This

hypothesis is further supported by a recent study showing that in hexaploid wheat the triple editing of the three *TaPDIL5-1* homeoalleles confers resistance to WYMV. To test this hypothesis in durum wheat, the CRISPR/Cas9 technology has been used to inactivate the orthologues of *HvPDIL5-1* in two Italian winter varieties: Svevo and Ofanto. A BLAST search for orthologues of *HvPDIL5-1* in Svevo genome revealed two homoeologous genes on subgenomes 4A and 4B: TRITD4Av1G050720 and TRITD4Bv1G125370. The high level of identity of their nucleotide sequences allowed us to design single guides RNAs for simultaneous editing of these two genes via *Agrobacterium*-mediated transformation. To promote and accelerate the regeneration of transformed plants, we took advantage of a recently developed vector that expresses the fusion protein of the wheat GROWTH-REGULATING FACTOR 4 and its cofactor GRF-INTERACTING FACTOR 1.

Durum wheat plants, that were regenerated from transformed immature embryos, revealed the presence of the construct in their genome. Further molecular analyses of the T1 generations allowed the detection of genomic editing events that are currently under investigation.

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