Proceedings of the LXV SIGA Annual Congress
Piacenza, 6/9 September, 2022
ISBN: **978-88-944843-3-5**

Poster Communication Abstract – 5.49

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

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genome editing, HvPDIL5-1 gene, durum wheat, soil-borne viruses

Durum wheat, although it accounts for about 7% of the total wheat produced in the world, can be considered as a main cereal crop for food production and agricultural income in confined geographical regions. For instance, Italy is the main producer in the European Union. However, the production is often affected by several diseases, including viral diseases. Among them, the wheat soil-borne bymoviruses, such as the W*heat yellow mosaic virus* and the W*heat spindle streak mosaic virus* (WSSMV), are transmitted to plants by the soil-inhabiting plasmodio-phorid *Polymyxa graminis*, which infected spores can survive long in the soil. The lack of reliable methods able to counteract 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 the protein folding process. Loss-of-function of *HvPDIL5-1* was identified as the cause of the naturally occurring resistance to multiple strains of bymoviruses. Given that orthologues genes of *HvPDIL5-1* are highly conserved among species, we speculated that the wheat orthologues of *HvPDIL5-1* could represent a susceptibility factor for bymovirus infection also in durum wheat. To test this hypothesis, the CRISPR/Cas9 technology has been used to inactivate the wheat orthologues of *HvPDIL5-1*.

A BLAST search for orthologues of *HvPDIL5-1* in tetraploid wheat Svevo genome revealed two homoeologous genes on subgenomes 4A and 4B: *TRITD4Av1G050720* and *TRITD4Bv1G125370*. The high level of nucleotide

sequence identity of the two genes allowed us to design different single guides RNAs for their simultaneous editing via *Agrobacterium*-mediated transformation of immature embryos of two Italian winter varieties: Svevo and Ofanto. 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. Experiments of transformation are currently under way, and edited plants will be further analyzed to test their response to WSSMV, for which important yield losses have been recorded in Italy.

This work is supported by Regione Lombardia, d.d.s. n. 4403 28/03/2018, grant n° 42 - SURF.