

ENHANCING DROUGHT TOLERANCE IN DURUM WHEAT TARGETING EPICUTICULAR WAXES CONTENT THROUGH GENOME EDITING

TAFURI A.*, PALOMBIERI S.***, SANTINO A.*, MITA G.*, SESTILI F.***,
BOTTICELLA E.*

*) Institute of Sciences of Food Production (ISPA), National Research
Council (CNR), Lecce, Italy

**) Department of Agriculture and Forest Sciences, University of Tuscia,
Viterbo, Italy

durum wheat, epicuticular waxes, drought stress, genome editing

Climate change poses a significant threat to agricultural harvests, increasing the risk of food insecurity. A rise of 1°C in global temperature has already led to a 3-8% reduction in the global mean yield of major crops. To tackle this challenge, breeding climate-resilient crops with genes that confer tolerance to extreme environmental conditions has emerged as a promising approach. Understanding the mechanisms through which plants develop resistance to abiotic stress is crucial for designing crops capable of thriving in changing climatic conditions. One such mechanism employed by plants to enhance stress tolerance is the production of cuticular wax, a protective layer covering the surface of most plant organs. Cuticular waxes are composed of Very-Long-Chain Fatty acids and their derivatives, including aldehydes, alcohols, alkanes, ketones, and wax esters. The biosynthesis of cuticular waxes typically involves two major pathways: alkane-forming and alcohol-forming pathways.

In Triticeae, an additional class of waxes known as β -diketones contributes to the development of glaucousness in wheat, which enhances resistance to abiotic stresses. The appearance of the β -diketone phenotype in durum wheat is influenced by two major loci, *W1* and *Iw1*. However, the biosynthetic pathway responsible for this class of compounds has yet to be fully elucidated. While enriched wax phenotypes have been generated in model and non-model crops by targeting genes encoding enzymes involved in wax production, our understanding of the regulatory mechanisms governing

cuticular wax synthesis in response to environmental cues remains limited.

In this study, we aim to enhance our understanding of the role played by cuticular waxes in drought and heat tolerance in durum wheat, a major Mediterranean crop. We will investigate three different regulatory mechanisms, focusing on key genes previously studied in model crops using a comparative genomics approach. These mechanisms include a transcription factor belonging to the AP2 family, which has been associated with the negative regulation of wax biosynthesis in *Arabidopsis*; an E3 ubiquitin ligase that interacts with a positive regulator of wax biosynthetic genes; and a Gγ protein involved in calcium signalling perception, which is associated with a QTL for wax content and thermotolerance in rice. To target these three genes, their sequences were isolated in silico from the durum wheat genome (cv Svevo), confirmed by sequencing, and compared for synteny with genomic regions using the NCBI Genomic Bank. We will employ a Genome Editing strategy based on *Agrobacterium tumefaciens*-mediated transformation using embryos isolated from both green and mature seeds in two durum wheat varieties. This communication provides an overview of the research's main objectives and strategies, along with preliminary results related to the design and preparation of vectors for wheat transformation.