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Poster Communication Abstract – 3.10

A STUDY ON VOCS RECEPTORS IN PLANTS: ODORANT BINDING PROTEINS AND THE ODORANT RECEPTORS EDITING

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The importance of the Volatile Organic Compounds (VOCs) in plant signalling and communication is well-known, and many studies documented how plant responses to stress can be elicited by the plant exposition to specific VOCs. In insects, VOCs are detected by Odorant Binding Proteins (OBPs) and Odorant Receptors (ORs) which trigger the downstream transduction cellular metabolic response. However, genes encoding for these receptors are generally unknown in plant. The aim of this work is to investigate how the odorous signals are sensed in plants, by targeting putative OBPs and ORs. By means of bioinformatic tools, firstly an analysis on the known OBPs and ORs in insects and other organisms has been performed. The results were compared with databases of viridiplantae proteins, and a list of about 150.000 plant proteins with structural features which resemble OBPs and ORs was generated. Thus, by applying an *e*-value as similarity threshold, the list was reduced, screened, and compared with proteins of the plant model, *Solanum lycopersicum*. Four proteins were finally selected belonging to families of disease resistance protein, ionotropic glutamate-like receptor, channels and a dehydration-induced 19 transcription factor. To test their function and their involvement to VOC signalling, we decided to use gene editing approach to knock out the identified genes. To purse this aim, we used CRISPR/Cas9 based technology and three specific *Guide* sequences for each gene were designed using bioinformatic tools and inserted in the sgRNA sequence mould. Two couple of sg-RNA/*Guide* were inserted into the final expression vector, to allow the simultaneous cut in two sites of each target gene and maximize the efficiency of gene silencing. The constructs

have been assembled using the Golden Gate cloning method and second-generation restriction enzymes (IIS). To evaluate the functionality of the CRISPR/Cas9 construct and the efficiency of the different combinations of *Guides*, a preliminary step, involving *Agrobacterium rhizogenes*, was carried out, before stable transformation *via A. tumefaciens*. Moreover, with transformed hairy roots it is possible to highlight the effects of the gene silencing on the different mutants and to collect preliminary data. *A. rhizogenes* cells were transformed by electroporation, and cotyledons of *S. lycopersicum* cv Red setter transformed and co-cultivated. Starting from these preliminary results, other studies will be carried out for a deeper mutants morphological and molecular characterization. In particular, the experimental design will consider the exposition of mutants to different stressors and specific blends of VOCs frequently emitted by stressed plants, (e.g. limonene, α-pinene etc.) for the evaluation of differences in molecular and morphological responses.