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Poster Communication Abstract – 3.25

APPLICATION OF FORWARD AND REVERSE GENETIC APPROACHES ON DIPLOTAXIS TENUIFOLIA

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In the large Brassicaceae family, Rocket or Rucola is a leafy vegetable crop whose cultivation is now widespread and particularly appreciated for fresh consumption thanks to the bitter flavor and strong aroma of its leaves. The most common cultivated rocket species is *Diplotaxis tenuifolia*, which is largely cultivated in Italy enough to be one of the major exporters in Europe. Rocket suffers from some undesired agronomic traits such as early flowering in summer and susceptibility to pathogens such as *Fusarium oxysporum*, *Xanthomonas campestris* and the oomycete *Hyaloperonospora* . Summer early flowering and diseases imply a reduction of leaf number and therefore affect yields.

The aim of the project is to breed new lines of rocket using both forward and reverse genetic approaches. These strategies are applied after chemical mutagenesis which was induced on the *Diplotaxis* population using ethyl methanesulfonate (EMS). A M1 population of 200 families was generated and the M2 population is growing during summer to identify lines with a delay in floral transition in inductive conditions and resilient to pathogens. In parallel, for the reverse genetics approach, the mutagenized population undergoes a genotypic analysis to identify mutations in genes known to promote floral transition such as *FLOWERING LOCUS T (FT)* and *CONSTANS (CO)* and the ones involved in resistance to pathogens like *DOWNY MILDEW RESISTANT 1 (DMR1)* and *DMR6*. Furthermore, the *Fusarium redolens* pathogen was isolated and identified from rocket root explants taken directly from the farm. Infection tests were performed on two different genotypes: commercial rocket and one obtained from the IPK germplasm database (DIPLO). The commercial line is more resistant to *F. redolens*

compared to the DIPLO line, showing that seeds commercially available are already in a way resistant to pathogens.

One limitation of rocket breeding is the gene content. The genome of *D. tenuifolia* has almost double the number of genes of *A. thaliana* with many orthologs. Therefore, it is very difficult to determine which gene is involved in a specific process. In order to discriminate between genes involved in a function and not, we are developing a transformation protocol using *A. tumefaciens*. Four independent lines were obtained and the presence of the construct was confirmed by PCR in the progeny. The transformed plants have been, additionally, analyzed with confocal microscopy to confirm the expression of the transgene. The preliminary results open to the possibility of genome editing in rocket for precise mutagenesis.