Proceedings of the LXVI SIGA Annual Congress
Bari, 5/8 September, 2023
ISBN: **978-88-944843-4-2**

Poster Communication Abstract – 3.08

MODIFYING PHYLLOTAXIS IN BRASSICA SEED CROP SPECIES FOR YIELD IMPROVEMENT

FERRARIO C. C.*, CASELLI F.*, DAVLATBOEVA S.*, GREGIS V.*, KATER M.*

*) Università degli Studi di Milano

Brassica napus, yield, editing, phyllotaxis

To feed the growing world population and not devolve further land to agriculture, it is desirable to develop plant genotypes of increased yield. In our lab, in the context of studying genes involved in the inflorescence architecture, *Arabidopsis* mutants in *PHY1*, *PHY2* and *PHY3* were developed. They display phenotypes worthy to be transferred to crops: indeed, they have a different phyllotactic pattern with respect to the wild type, associated with an increased number of siliques and a reduced plastochron. Since canola (*Brassica napus*) is evolutionary close to *Arabidopsis* and one of the main oil crops in the world, it was selected as species where to develop similar mutants.

The putative homologous genes in *Brassica napus* were identified by bioinformatic tools, resulting in a 10 genes pool of which some are placed in linkage. The number of genes is coherent with the genome evolution and hybridization that happened after *Arabidopsis-Brassica* split and *napus* species' birth. The genes expression was studied by real-time PCR and *in situ hybridization*, and it shows a pattern resembling the *Arabidopsis* one. They are expressed in the inflorescence and floral meristems and in floral reproductive organs of different stages. The functional conservation of some of the candidate genes was studied by a complementation test and Y2H assay. Indeed, it is known that in *Arabidopsis* PHY2 can interact with itself and PHY1. While the homologs' expression pattern is similar to the *Arabidopsis* one, the protein interactions differ, showing different homo- and hetero-dimers formation both in intraspecific and interspecific combinations. For the complementation test, the selected genes were cloned under the 35S constitutive promoter and 3 lines expressing the construct at different levels were used. The same 35S overexpression of the *Arabidopsis*

genes themselves was used as a control. The phyllotactic pattern of the lines was considered for the analysis. The genes homologous to PHY2, but not to PHY1, show a partial complementation.

As a final goal we want to create mutants in *Brassica napus* that could recall the phenotype observed in Arabidopsis. 3 gRNAs were designed for each candidate gene, preferring gRNAs targeting more than one gene at the same time. The efficiency of the 21 gRNAs designed was tested by a protoplast destructive assay.

For developing the mutants, we are optimizing several transformation protocols for *B. napus*. We are testing both protoplast transient transformation by Cas9-gRNA constructs as well as *Agrobacterium*-mediated transformation by floral dip and in vitro protocols. The faster and more efficient approach will be selected.